Calcified
Tissue
International

31st EUROPEAN SYMPOSIUM ON CALCIFIED TISSUES

Nice, France, 5-9 June 2004

PROGRAMME AND ABSTRACTS

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Please note that a meeting programme book will be available at the registration desk. The programme book will include more detailed information on the City of Nice, Venue floor plans and maps, Public transport, Social events and tours and Exhibitors and Sponsors.

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European Calcified Tissue Society

The European Calcified Tissue Society is the major organisation in Europe for researchers and clinicians working in the field of calcified tissues and related fields. The Society acts as a forum for the dissemination of high quality research through its annual meeting and workshops. Benefits of membership include reduced subscription to the journals Calcified Tissue International and Osteoporosis International. Membership is open to anyone working in the field at whatever stage in their career and from anywhere in the world.

Annual General Meeting
The Annual General Meeting of the European Calcified Tissue Society will take place in the Athena Theatre at the Acropolis Convention Centre on Monday 7 June 2004 at 11.00.

ECTS Future Meeting Dates

2nd Joint meeting of the European Calcified Tissue Society and International Bone and Mineral Society
Geneva, Switzerland, 25–29 June 2005
33rd European Symposium on Calcified Tissues
Prague, Czech Republic, 10–14 May 2006

For more information about the ECTS please contact:

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Programme Overview

Time	Saturday 5 June	Sunday 6 June	Monday 7 June
7.15		**Satellite Symposium Supported by AMGEN** Impacting Bone Disease via the RANK/RANKL/OPG System	
8.00		**Posters**	**Posters**
8.30			
8.45			
9.00		**Symposium 1** Bone Formation	**Symposium 3** Bone Resorption
10.00	**Training Course** Bone Turnover Markers	**Oral Presentations** Osteoblasts and Bone Formation	**Oral Presentations** Osteoclasts and Bone Resorption
11.00		**Coffee & Posters**	**Coffee & Posters** / **ECTS Annual General Meeting**
12.00			
12.30		Lunch	Lunch
12.45			
13.00	**Lunch**	**Workshop 1** Glucocorticoid Induced Osteoporosis **Workshop 2** Biochemical Markers	**Workshop 3** Tissue Engineering **Workshop 4** Rare Bone Diseases
13.45			
14.00	**Training Course** Bone Turnover Markers	**Symposium 2** Bone Development & Bone Quality	**Symposium 4** Metabolic Bone Diseases
15.00	**Break**		
15.15		**Oral Presentations** Osteoblasts and Bone Formation	**Oral Presentations** Metabolic Bone Diseases
15.15	**Training Course** Bone Turnover Markers		
16.00		**Coffee**	**Coffee**
16.30	**Satellite Symposium Supported by Eli Lilly** Evolving Perspectives in Osteoporosis Treatment: From Bone Properties to Fracture Efficacy	**Oral Presentations** Osteoporosis and Fractures	**Oral Presentations** Management of Osteoporosis
17.30			
18.00		**Satellite Symposium Supported by the Alliance for Better Bone Health** Bone Strength and Fracture Resistance In Osteoporosis	**Satellite Symposium Supported by Nycomed** Calcium and Vitamin D in Osteoporosis Management
18.15			
18.30	**Opening Ceremony** *G. Karsenty (Houston)* Central Control of Bone Mass Biology and Medical Implications		
19.30			
20.00	**Welcome Reception and presentation of the Alliance for Better Bone Health Iain T. Boyle Award**		**ECTS Football Cup**
20.30			

Saturday: 8.30 - 20.00 Registration Open

Sunday: 8.00 - 19.30 Registration Open

Monday: 8.00 - 19.30 Registration Open

Tuesday 8 June   Wednesday 9 June

	Tuesday 8 June			Wednesday 9 June	Time
					7.15
	Posters			Posters	8.00
					8.30
					8.45
	Symposium 5 Oestrogen			Symposium 7 Prevention and Treatment of Osteoporosis	9.00
	Oral Presentations Hormones			Coffee & Posters	10.00
	Coffee & Posters			Oral Presentations Hot Topics	11.00
		Lunch			12.00
					12.30
					12.45
	Workshop 5 Biomineralization Workshop 6 Case Presentations			Closing Ceremony and Presentation of Awards	13.00
					13.45
					14.00
	Symposium 6 Genetics of Osteoporosis				
	Oral Presentations Genetics				15.00
					15.15
	Coffee				16.00
	Oral Presentations PTH				16.30
	Satellite Symposium Supported by Merck Sharp & Dohme Achieving the Goal of Osteoporosis Therapy; Fracture Reduction				17.30
					18.00
					18.15
					18.30
					19.30
					20.00
					20.30
	Gala Dinner at Juan les Pins				

Registration Open 8.00 - 19.30 (Tuesday column left margin)

Registration Open 8.00 - 12.45 (Wednesday column left margin)

Symposia

1. Bone Formation
1. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist - *C. Löwik (Leiden)*
2. Regulation of bone formation by the FGF2 pathway - *M.M. Hurley (Farmington)*

2. Bone Development & Bone Quality
1. Hox genes and limb development - *D. Duboule (Geneva)*
2. Why some bones fracture and others don't: a biomechanical perspective - *J. Currey (York)*

3. Bone Resorption
1. Chloride channels and proton transport: CLC-7 regulates bone density - *U. Kornak (Berlin)*
2. Bone lining cells and the regulation of bone resorption - *V. Everts (Amsterdam)*

4. Metabolic Bone Diseases
1. Molecular mechanisms of phosphate transport in kidney and bone: recent advances - *H. Tenenhouse (Montreal)*
2. Pathogenesis and treatment of osteoporosis in men - *J.-M. Kaufman (Ghent)*

5. Oestrogen
1. Estrogens, androgens and the regulation of bone remodeling - *R. Baron (New Haven)*
2. Clinical role for estrogen replacement and SERMS after Women's Health Initiative - *J. Stevenson (London)*

6. Genetics of Osteoporosis
1. Progress in detecting osteoporosis susceptibility genes - *T. Spector (London)*
2 Quantitating the effects of Polygenes by meta-analysis - *J. Ioannidis (Ioannina)*

7. Prevention and Treatment of Osteoporosis
1. Targeting treatment by fracture risk rather than BMD - *O. Johnell (Malmo)*
2. Osteoporosis treatments: past, present and future - *J. Compston (Cambridge)*

Workshops

1. Glucocorticoid Induced Osteoporosis
1. Epidemiology of glucocorticoid-induced osteoporosis - *T.P. Van Staa (Staines)*
2. Mechanisms of osteoblast inhibition by steroids - *P. Hulley (Stellenbosch)*
3. Prevention and management of glucocorticoid-induced osteoporosis - *D. M. Reid (Aberdeen)*

2. Biochemical Markers
1. Biology of matrix proteins - *D. Heinegard (Lund)*
2. New biochemical markers of bone turnover in osteoporosis - *P. Garnero (Lyon)*
3. Clinical use of biochemical markers - *R. Eastell (Sheffield)*

3. Tissue Engineering
1. Tissue engineering of cartilaginous tissues: gene enhancement of chondrogenic cells - *T.E. Hardingham (Manchester)*
2. Pluripotency of adult stem cells: instructions for osteogenic lineage direction - *J.B. Lian (Worcester MA)*
3. Engineering new bone with BMP's - *J.M. Wozney (Cambridge)*

4. Rare Bone Diseases
1. Medical treatment of fibrous Dysplasia of bone - *P. Meunier (Lyon)*
2. Osteogenesis imperfecta & juvenile osteoporosis - *F. Glorieux (Montreal)*
3. Juvenile Paget's disease - *T. Cundy (Auckland)*

5. Biomineralization
1. Analysis of bone mineral by qBEI - *P. Roschger (Vienna)*
2. Vibrational spectroscopic characterization of bone mineral and collagen cross-links - *A. Boskey (New York)*
3. Microscopy for mineralised tissues in bones - *A. Boyde (London)*

6. Case Presentations (Tbc)

Programme Day-by-Day

Saturday 5 June 2004

08.30–20.00 Registration Open

ECTS TRAINING COURSE

09.00–18.00 Training course   **Hermès Theatre**
Bone Turnover Markers

CONGRESS OPENING

16.15–18.15 Satellite Symposium   **Athena Theatre**
Evolving Perspectives in Osteoporosis Treatment:
From Bone Properties to Fracture Efficacy
Supported by Eli Lilly & Co
Chair: **J. Reeve (Cambridge, UK)**

- Welcome and opening remarks   **J. Reeve (Cambridge, UK)**
- Bone quality: shifting the paradigm   **R. P. Heaney (Omaha, USA)**
- Unique effects of Raloxifene: implications for long-term efficacy   **P. Delmas (Lyon, France)**
- Teriparatide: an innovative solution for established osteoporosis   **E. Eriksen (Indianapolis, USA)**
- Optimizing osteoporosis management: prevent, treat, and maintain   **J.-Y. Reginster (Liège, Belgium)**
- Closing remarks   **J. Reeve (Cambridge, UK)**

18.30   Opening Ceremony   **Apollo Theatre**
Chair: **P. Marie (Paris, France)**
S. Ralston (Aberdeen, UK)

I001   • Central control of bone mass: biology and medical implications   **G. Karsenty (Houston, USA)**

Welcome Reception   **Acropolis Terrace**
and presentation of the Alliance
for Better Bone Health
Iain T. Boyle Award

Sunday 6 June 2004

08.00–19.30	**Registration Open**	
07.15–08.45	**Satellite Symposium** **Impacting Bone Disease via the RANK/RANKL/OPG System** *Supported by AMGEN* Chair: **J.-J. Body (Brussels, Belgium)**	**Athena Theatre**
	• The role of the RANK/RANKL/OPG system in bone remodeling and resorption	**T. J. Chambers (London, UK)**
	• Osteoporosis and the RANK/RANKL/OPG system: considerations for therapy	**L. C. Hofbauer (Marburg, Germany)**
	• Future directions in the treatment of cancer-related bone disease: targeting RANKL	**J.-J. Body (Brussels, Belgium)**
08.00–09.00	**Posters**	**Hall Agora 2**
09.00–10.00	**Symposium 1** **Bone Formation** Chair: **S. Roman-Roman (Romainville, France)** **T. M. Skerry (London, UK)**	**Athena Theatre**
I002	• Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist	**C. Löwik (Leiden, Netherlands)**
I003	• Regulation of bone formation by the FGF2 pathway	**M. M. Hurley (Farmington, USA)**
10.00–11.00	**Oral Presentations 1** **Osteoblasts and Bone Formation** Chair: **S. Roman-Roman (Romainville, France)** **T. M. Skerry (London, UK)**	**Athena Theatre**
OP001	• Mice lacking Fra-2 are severely osteopenic due to osteoclast and osteoblast defects	**A. Hoebertz (Vienna, Austria)**
OP002	• Enhanced trabecular bone mass and architecture in mice over-expressing ATG1-to-TTG1 mutant histone H4 transgene	**B. Frenkel (Los Angeles, USA)**
OP003	• Identification of novel RUNX2 target genes	**A. Barski (Los Angeles, USA)**
OP004	• Wnt-signaling regulates human osteoblast differentiation and mineralization in a steroid-dependent manner	**M. Eijken (Rotterdam, Netherlands)**
OP005	• Zoledronate up-regulates bone sialoprotein expression in SAOS-2 cells through RhoA inhibition	**M. Chaplet (Liège, Belgium)**
11.00–12.45	**Coffee and Posters**	**Hall Agora 2**
12.00–12.45	**Lunch**	
12.45–14.00	**Workshop 1** **Glucocorticoid-Induced Osteoporosis** Chair: **N. Franchimont (Liège, Belgium)** **L. Euller-Ziegler (Nice, France)**	**Athena Theatre**
I004	• Epidemiology of glucocorticoid-induced osteoporosis	**T. P. Van Staa (Staines, UK)**
I005	• Mechanisms of osteoblast inhibition by steroids	**P. Hulley (Stellenbosch, South Africa)**
I006	• Prevention and management of glucocorticoid-induced osteoporosis	**D. M. Reid (Aberdeen, UK)**

| 12.45–14.00 | **Workshop 2** | **Hermès Theatre** |

Biochemical Markers
Chair: **P. Delmas (Lyon, France)**
G. Russell (Oxford, UK)

I007	• Biology of matrix proteins	**D. Heinegard (Lund, Sweden)**
I008	• New biochemical markers of bone turnover in osteoporosis	**P. Garnero (Lyon, France)**
I009	• Clinical use of biochemical markers	**R. Eastell (Sheffield, UK)**

Sunday 6 June 2004

| 14.00–15.00 | **Symposium 2** | **Athena Theatre** |

Bone Development & Bone Quality
Chair: **G. Karsenty (Houston, USA)**
M. Kassem (Odense, Denmark)

| I010 | • Hox genes and limb development | **D. Duboule (Geneva, Switzerland)** |
| I011 | • Why some bones fracture and others don't: a biomechanical perspective | **J.Currey (York, UK)** |

| 15.00–16.00 | **Oral Presentations 2** | **Athena Theatre** |

Osteoblasts and Bone Formation
Chair: **G. Karsenty (Houston, USA)**
M. Kassem (Odense, Denmark)

OP006	• Defective bone mineralization and osteopenia in young adult FGFR3−/− mice	**J. Henderson (Montreal, Canada)**
OP007	• Accelerated bone aging in DNA repair deficient trichothiodystrophy mice	**K. Diderich (Rotterdam, Netherlands)**
OP008	• Genetic deficiency in PERK cause severe osteopenia and neonatal growth retardation	**D. Cavener (University Park, USA)**
OP009	• Prostaglandins-dependent activation of ERK mediates cell proliferation induced by TGFß in MC3T3-E1 osteoblastic cells	**J. Caverzasio (Geneva, Switzerland)**
OP010	• 11ß-hydroxysteroid dehydrogenase expression and glucocorticoid synthesis is directed by a molecular switch during osteoblast differentiation	**M. Eijken (Rotterdam, Netherlands)**

| 16.00–16.30 | **Coffee Break** | |

| 16.30–17.30 | **Oral Presentations 3** | **Athena Theatre** |

Osteoporosis and Fractures
Chair: **P. Garnero (Lyon, France)**
H. Pols (Rotterdam, Netherlands)

OP011	• Cumulative risks of fracture in patients using oral glucocorticoids	**T. Van Staa (Egham, UK)**
OP012	• Contrasting effects of teriparatide and alendronate on bone turnover assessed by bone histomorphometric parameters in women with osteoporosis	**P. Meunier (Lyon, France)**
OP013	• Biochemical markers of bone formation are positively correlated with volumetric BMD following teriparatide therapy	**P. Miller (Denver, USA)**
OP014	• Urine osteocalcin-a novel marker of bone metabolism and prediction of fractures	**K. Ivaska (Turku, Finland)**
OP015	• Fracture prediction using markers of bone turnover in the general population: the OPUS study	**A. Blumsohn (Sheffield, UK)**

17.30–19.30	**Satellite Symposium** **Bone Strength and Fracture Resistance In Osteoporosis** **Supported by the Alliance for Better Bone Health** Chair: **R. Rizzoli (Geneva, Switzerland)**	**Hermès Theatre**
	• Factors influencing bone strength	**D. Felsenberg (Berlin, Germany)**
	• The role of bone quality in fracture risk reduction	**R. Lindsay (West Haverstraw, USA)**
	• Clinical perspectives on differences in bisphosphonate pharmacology	**P. Delmas (Lyon, France)**
	• Concluding remarks	**R. Rizzoli (Geneva, Switzerland)**

Monday 7 June 2004

08.00–19.30	**Registration Open**	

08.00–09.00	**Posters**	**Hall Agora 2**

09.00–10.00	**Symposium 3** **Bone Resorption** Chair: **P. Jurdic (Lyon, France)** **A.Teti (L'Aquila, Italy)**	**Athena Theatre**
I012	• Chloride channels and proton transport: CLC-7 regulates bone density	**U. Kornak (Berlin, Germany)**
I013	• Bone lining cells and the regulation of bone resorption	**V. Everts (Amsterdam, Netherlands)**

10.00–11.00	**Oral Presentations 4** **Osteoclasts and Bone Resorption** Chair: **P. Jurdic (Lyon, France)** **A. Teti (L'Aquila, Italy)**	**Athena Theatre**
OP016	• Cannabinoid receptor antagonists inhibit osteoclastic bone resorption in vitro and prevent ovariectomy induced bone loss in vivo	**A. Idris (Aberdeen, UK)**
OP017	• Role of inositol polyphosphate 4-phosphatase type II in osteoclast cytoskeletal organization and activity	**M. Ferron (Montreal, Canada)**
OP018	• Osteoclast alphaVbeta3 integrin-induced ERK1/2 activation is intracellular $Ca2+$/c-Src-dependent, Shc/Ras/Raf-1-independent, and is triggered by engagement of PKCalpha	**N. Rucci (L'Aquila, Italy)**
OP019	• Transdifferentiation of human dendritic cells towards osteoclasts	**M. Mazzorana (Lyon, France)**
OP020	• ABD56 a potent inhibitor of bone resorption, induces osteoclast apoptosis via NFKB and ERK inhibition in vitro	**A. Idris (Aberdeen, UK)**

11.00–12.00	**ECTS Annual General Meeting**	**Athena Theatre**

11.00–12.45	**Coffee and Posters**	**Hall Agora 2**

12.00–12.45	**Lunch**	

12.45–14.00	**Workshop 3** **Tissue Engineering** Chair: **B. Rossi (Nice, France)** **E. Wagner (Vienna, Austria)**	**Hermès Theatre**
I014	• Tissue engineering of cartilaginous tissues: gene enhancement of chondrogenic cells	**T. E. Hardingham (Manchester, UK)**
I015	• Pluripotency of adult stem cells: instructions for osteogenic lineage direction	**J. B. Lian (Worcester, USA)**
I016	• Engineering new bone with BMP's	**J. M. Wozney (Cambridge, USA)**

	Workshop 4 **Rare Bone Diseases** Chair: **M.-C. de Vernejoul (Paris, France)** **W. van Hul (Antwerpen, Belgium)**	**Athena Theatre**
I017	• Medical treatment of fibrous Dysplasia of bone	**P. Meunier (Lyon, France)**
I018	• Osteogenesis imperfecta & juvenile osteoporosis	**F. Glorieux (Montreal, Canada)**
I019	• Juvenile Paget's disease	**T. Cundy (Auckland, New Zealand)**

Monday 7 June 2004

14.00–15.00	**Symposium 4** **Metabolic Bone Diseases** Chair: **B.-L. Langdahl (Aarhus, Denmark)** **U. Kornak (Berlin, Germany)**	**Athena Theatre**
I020	• Molecular mechanisms of phosphate transport in kidney and bone: recent advances	**H. Tenenhouse (Montreal, Canada)**
I021	• Pathogenesis and treatment of osteoporosis in men	**J.-M. Kaufman (Ghent, Belgium)**

15.00–16.00	**Oral Presentations 5** **Metabolic Bone Diseases** Chair: **B.-L. Langdahl (Aarhus, Denmark)** **U. Kornak (Berlin, Germany)**	**Athena Theatre**
OP021	• RAS signaling regulates osteoblast differentiation and extracellular matrix mineralization. Implications for the bone manifestations of neurofibromatosis type I	**F. Elefteriou (Houston, USA)**
OP022	• FGFR2 downregulation results from C-CBL-dependent FGFR2 proteasome degradation and contributes to increased osteoblast differentiation in apert syndrome	**K. Kaabeche (Paris, France)**
OP023	• Sequestosome 1 gene mutations lead to disregulated NFκB signaling in Paget's disease of bone	**A. Daroszewska (Aberdeen, UK)**
OP024	• Twist haploinsufficiency in the Saethre-Chotzen syndrome regulates osteoblast gene expression independently of FGFR2signaling	**H. Guénou (Paris, France)**
OP025	• Alterations in transcriptional activity of the 24-Hydroxylase gene due to a novel promoter polymorphism	**N. Schuetze (Wuerzburg, Germany)**

16.00–16.30	**Coffee Break**

16.30–17.30	**Oral Presentations 6** **Management of Osteoporosis** Chair: **P. Meunier (Lyon, France)** **R. Eastell (Sheffield, UK)**	**Athena Theatre**
OP026	• Head-to-head comparison of Risedronate and Alendronate	**C. Christiansen (Ballerup, Denmark)**
OP027	• Sustained effect of Risedronate on trabecular architecture and mineralization over 5 years of treatment: triple biopsy studies by micro-CT	**B. Borah (Mason, USA)**
OP028	• Teriparatide (rhPTH [1–34]) treatment improves the structure of the proximal femur in women with osteoporosis: results from the fracture prevention trial	**E. F. Eriksen (Indianapolis, USA)**
OP029	• Strontium ranelate reduces the risk of vertebral and non-vertebral fractures in caucasian women with post-menopausal osteoporosis	**S. Adami (Valeggio sul Mincio, Italy)**
OP030	• Effects of 10 years of estradiol on fracture risk and other outcomes in early postmenopausal women–the Danish osteoporosis prevention study	**L. Mosekilde (Aarhus, Denmark)**

Monday 7 June 2004

17.30–19.30	**Satellite Symposium** **Calcium and Vitamin D in Osteoporosis Management** *Supported by Nycomed* Chair: **P. Meunier (Lyon, France)** **J.-Y. Reginster (Liège, Belgium)**	**Hermès Theatre**
S001	• Osteoporosis: underestimated, under diagnosed, and under treated	**S. Boonen (Leuven, Belgium)**
S002	• The effect of calcium and Vitamin D supplementation on secondary hyperparathyroidism and bone resorption	**P. Lips (Amsterdam, Netherlands)**
S003	• Effect of Vitamin D and calcium in improving muscle strength and reducing the risk of falls in older persons: a systematic review	**H. A. Bischoff-Ferrari (Boston, USA)**
S004	• Efficacy of calcium and Vitamin D in reducing fracture risk	**C. Cooper (Southampton, UK)**
S005	• The importance of compliance within osteoporosis in general and with calcium and Vitamin D in particular	**Ö. Ljunggren (Uppsala, Sweden)**

20.00	**ECTS Football Cup**	**Stade St Roch**

Tuesday 8 June 2004

08.00–19.30	**Registration Open**	

08.00–09.00	**Posters**	**Hall Agora 2**

09.00–10.00	**Symposium 5** **Oestrogen** Chair: **J. Compston (Cambridge, UK)** **H. van Leeuwen (Rotterdam, Netherlands)**	**Athena Theatre**
I022	• Estrogens, androgens and the regulation of bone remodeling	**R. Baron (New Haven, USA)**
I023	• Clinical role for estrogen replacement and SERMS after Women's Health Initiative	**J. Stevenson (London, UK)**

10.00–11.00	**Oral Presentations 7** **Hormones** Chair: **J. Compston (Cambridge, UK)** **H. van Leeuwen (Rotterdam, Netherlands)**	**Athena Theatre**
OP031	• Gene therapy with growth hormone alone normalizes bone mass and bone structure in hypophysectomized MICE	**N. Ditzel (Odense, Denmark)**
OP032	• DLK1/PREF-1 (EGF-like homeotic protein) inhibits gene expression of insulin-like growth factor (IGF) – binding proteins during osteoblast differentiation of human bone marrow stromal cells	**B. Abdallah (Odense, Denmark)**
OP033	• Ghrelin is expressed in human bone cells and enhances the proliferation of osteoblasts	**B. Van der Eerden (Rotterdam, Netherlands)**
OP034	• Novel insights into the mechanism of action of vitamin D metabolites in bone: evidence for 1,25-dihydroxyvitamin D3 synthesis by osteoblasts and direct stimulation of mineralization	**M. Van Driel (Rotterdam, Netherlands)**
OP035	• An upstream region of mouse osteoprotegerin binds estrogen receptor and contributes to estrogen responsiveness	**M. Rumpler (Vienna, Austria)**

11.00–12.45	**Coffee and Posters**	**Hall Agora 2**

12.00–12.45	**Lunch**	

12.45–14.00	**Workshop 5** **Biomineralization** Chair: **G. Boivin (Lyon, France)** **K. Klaushofer (Vienna, Austria)**	**Athena Theatre**
I024	• Analysis of bone mineral by qBEI	**P. Roschger (Vienna, Austria)**
I025	• Vibrational spectroscopic characterization of bone mineral and collagen cross-links	**A. Boskey (New York, USA)**
I026	• Microscopy for mineralised tissues in bones	**A. Boyde (London, UK)**
	Workshop 6 **Case Presentations**	**Hermès Theatre**

14.00–15.00	**Symposium 6** **Genetics of Osteoporosis** Chair: **G. Carle (Nice, France)** **A. Uitterlinden (Rotterdam, Netherlands)**	**Athena Theatre**
I027	• Progress in detecting osteoporosis susceptibility genes	**T. Spector (London, UK)**
I028	• Quantitating the effects of polygenes by meta-analysis	**J. Ioannidis (Ioannina, Greece)**

Tuesday 8 June 2004

15.00–16.00	**Oral Presentations 8** **Genetics** Chair: **G. Carle (Nice, France)** **A. Uitterlinden (Rotterdam, Netherlands)**	**Athena Theatre**
OP036	• Loci for regulation of BMD in men and women: the Famos study	**S. Ralston (Aberdeen, UK)**
OP037	• Ethnic variation in haplotype structure of the complete VDR gene and association with fracture risk	**Yue Fang (Rotterdam, Netherlands)**
OP038	• Polymorphisms of oestrogen receptor alpha predict bone loss in perimenopausal women	**U. Pettersson (Aberdeen, UK)**
OP039	• Gene interaction between estrogen receptor alpha and IGF-I determines hip bone strength in elderly men	**F. Rivadeneira (Rotterdam, Netherlands)**
OP040	• LDL-receptor related protein 5 gene polymorphisms influence the normal variation of bone mineral density	**A. Koay (Oxford, UK)**

16.00–16.30	**Coffee Break**

16.30–17.30	**Oral Presentations 9** **PTH** Chair: **C. Silve (Paris, France)** **J. Reeve (Cambridge, UK)**	**Athena Theatre**
OP041	• Resistance to the bone anabolic effect of PTH(1-34) on an isocaloric low protein diet	**P. Ammann (Geneva, Switzerland)**
OP042	• Arrestins regulate bone remodeling induced by ovariectomy and intermittent PTH treatment	**S. L. Ferrari (Geneva, Switzerland)**
OP043	• Role of osteoblastic matrix metalloproteinases (MMP's) on the anabolic action of parathyroid hormone in vivo	**V. Geoffroy (Paris, France)**
OP044	• Anabolic actions of PTH are associated with an early inhibition of mineralization in vivo	**G. Pettway (Ann Arbor, USA)**
OP045	• High-impact exercise and bone mineral density in randomly selected population of premenopausal women	**A. Vainionpää (Oulu, Finland)**

17.30–19.30	**Satellite Symposium** **Achieving the Goal of Osteoporosis Therapy; Fracture Reduction** *Supported by Merck Sharp & Dohme*	**Hermès Theatre**

20.30	**Gala Dinner**	**Restaurant *Les Pêcheurs* Juan Les Pins**

Wednesday 9 June 2004

08.00–12.45	**Registration Open**	
08.00–09.00	**Posters**	**Hall Agora 2**
09.00–10.00	**Symposium 7** **Prevention and Treatment of Osteoporosis** Chair: **R. Rizzoli (Geneva, Switzerland)** **C. Ribot (Toulouse, France)**	**Athena Theatre**
I029	• Targeting treatment by fracture risk rather than BMD	**O. Johnell (Malmo, Sweden)**
I030	• Osteoporosis treatments: past, present and future	**J. Compston (Cambridge, UK)**
10.00–11.00	**Coffee and Posters**	**Athena Theatre**
11.00–12.30	**Oral Presentations** **Hot Topics** Chair: **R. Baron (New Haven, USA)** **S. Ralston (Aberdeen, UK)**	**Athena Theatre**
OP046	• Inhibition of bone resorption and increase in bone formation contribute to the high bone mass of mice deficient in β2-adrenergic receptor, a receptor downstream of leptin for its antiosteogenic function	**F. Elefteriou (Houston, USA)**
OP047	• Severely diminished bone resorption as well as reduced bone thickness in mice lacking the transient receptor potential channel TRPV5	**B. Van der Eerden (Rotterdam, Netherlands)**
OP048	• Inhibition of GSK3β, a key regulator of the wnt signaling pathway, reverses the osteoporotic phenotype in LRP5 knock-out mice	**G. Rawadi (Romainville, France)**
OP049	• Association of Aromatase (CYP19) gene polymorphisms with estradiol and estrone levels in postmenopausal women	**S. Schuit (Rotterdam, Netherlands)**
OP050	• NE-10790, a phosphonocarboxylate analogue of the bisphosphonate Risedronate, exhibits direct antitumor activity in vivo	**P. Fournier (Lyon, France)**
OP051	• Serum concentrations of AMG 162 were detectable and bone resorption suppressed for 6 to 9 months following a single dose in postmenopausal women	**S. W. Martin (Thousand Oaks, USA)**
OP052	• Kyphoplasty - results of the first prospective controlled trial	**C. Kasperk (Heidelberg, Germany)**
12.30–14.00	**Closing Ceremony and Presentation of Awards**	

ABSTRACTS S001 TO S005

ORAL PRESENTATIONS, SATELLITE SYMPOSIA

Nycomed

S001

OSTEOPOROSIS: UNDERESTIMATED, UNDERDIAGONISED, AND UNDERTREATED

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Osteoporosis with its associated morbidity and mortality is a serious problem that will dramatically increase in the near future, with substantial health-economic implications. In Europe alone, 75 million people have osteoporosis, with a staggering 40% of postmenopausal womenat risk of osteoporosis-related fractures during their lifetime. Early detection and prompt treatment of osteoporosis are key to the prevention and reduction of fragility fractures. Yet, overwhelming evidence shows that the disease remains largely underdiagnosed and undertreated. With advancing age, nonvertebral fractures (particularly hip fractures) contribute increasingly to the burden associated with osteoporosis. However, vertebral fractures are the most common type of osteoporotic fracture. Among women who have suffered a vertebral fracture, nearly 20% will experience a further fracture within 1 year. These data change the paradigm for osteoporosis, which has long been considered a slowly progressing disease. Vertebral fractures can be particularly problematic, as they may remain undiagnosed until symptoms become severe. Moreover, physicians are not using existing radiologic information to identify prevalent fractures among their patients. Similarly, they underuse densitometry equipment to identify women with low bone density who would benefit from intervention. Effective treatments to reduce osteoporotic fracture risk are available, including calcium and vitamin D, alendronate, raloxifene, and risedronate. Calcium and vitamin D are an essential component of an integrated management strategy for the prevention and treatment of osteoporosis in patients with dietary insufficiencies, although maximal benefit in terms of fracture prevention requires the addition of antiresorptive therapy. Nevertheless, surveys reveal that awareness of the efficacy of calcium and vitamin D is particularly low among physicians, patients and women at risk; measures are urgently needed to address this lack of awareness. Furthermore, given the serious consequences and widespread prevalence of osteoporosis, calcium and vitamin D should continue to be classified as medicinal products so that physicians and other healthcare professionals are able to provide guidance for the optimal use of these agents. In summary, widespread and substantial deficiencies in the awareness and management of osteoporosis persist. Changes in attitudes towards osteoporosis and its diagnosis and treatment are urgently required.

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S002

THE EFFECT OF CALCIUM AND VITAMIN D SUPPLEMENTATION ON SECONDARY HYPERPARATHYROIDISM AND BONE RESORPTION

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Vitamin D deficiency causes secondary hyperparathyroidism and increased bone turnover, leading to bone loss and fractures. Both vitamin D and calcium have been used to suppress parathyroid function and to prevent bone loss and fractures, but findings have proven ambiguous. Vitamin D deficiency (due for example to low sunshine exposure or an insufficient diet) can be readily suspected but it is important to recognise other causes of secondary hyperparathyroidism such as renal insufficiency, loop diuretics and low calcium intake. Consensus on the threshold level of serum 25-hydroxyvitamin D (25(OH)D) that defines vitamin D deficiency is lacking. Some cross-sectional studies have proposed a threshold serum 25(OH)D level indicating mild vitamin D deficiency or insufficiency of approximately 30 nmol/L, whereas other studies have suggested higher levels (up to 100 nmol/L). Such discrepancies could be explained by the assay method used, as well as differences in dietary calcium intake. Vitamin D supplementation (400–800 IU/day) decreases serum parathyroid hormone (PTH) by at least 10–30% within 3 months, generally with a concomitant decrease in bone turnover markers. These decreases of serum PTH are dependent upon baseline serum 25(OH)D, with the greatest decrements in patients with severe vitamin D deficiency. Calcium supplementation can decrease serum PTH within 1–2 hours, and doses of 1000–1500 mg/day may lower serum PTH for 24 hours. Intervention studies generally show that combined vitamin D and calcium supplementation has a greater effect on serum PTH and bone turnover than vitamin D supplementation alone. Combined calcium and vitamin D may lower serum PTH and bone resorption markers (eg, urinary N- or C-telopeptide) by 50% compared with controls. However, the effect of calcium and/or vitamin D supplementation on serum PTH or bone resorption markers does not always predict the anti-fracture effect. Vitamin D and calcium supplements should be continued long-term; however, the latter have been associated with gastrointestinal side effects that could reduce patient compliance. In conclusion, the effect of combined calcium and vitamin D supplementation on parathyroid function is greater than the effect of either administered alone. Moreover, the effect is greater when vitamin D deficiency is severe and calcium intake poor. Furthermore, issues with patient compliance must be addressed.

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S003

EFFECT OF VITAMIN D AND CALCIUM IN IMPROVING MUSCLE STRENGTH AND REDUCING THE RISK OF FALLS IN OLDER PERSONS: A SYSTEMATIC REVIEW

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Falls are common in the elderly (> 65 years), increasing in incidence with age, and represent a considerable burden on both the individual and society. The role of vitamin D and calcium in the prevention of falls in the elderly has yet to be fully established; however, if proven effective, these supplements may offer a simple and well-tolerated intervention. Our current understanding in this area has focused upon the direct effect of vitamin D on muscle strength. It has been proposed that highly specific receptors for 1,25-dihydroxyvitamin D are expressed in human muscle tissue and that these promote protein synthesis eventually leading to improved strength. We conducted a review of current findings regarding the effectiveness of vitamin D and vitamin D analogues in the improvement of muscle strength, body sway, and the prevention of falls in the elderly. Randomised controlled trials (RCTs) on the effect of vitamin D with calcium supplementation suggest a beneficial effect on musculoskeletal function, including body sway and lower extremity strength. A positive effect of vitamin D on strength is supported by several cross-sectional observations and one population-based survey documenting a positive association between lower extremity strength and 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D levels in both institutionalised and ambulatory older persons. The beneficial effects of vitamin D on muscle strength and function appear to translate into a reduced risk of falling. We performed a meta-analysis of five RCTs in elderly persons in a steady health state (n = 1237; two trials with cholecalciferol plus calcium, one trial with cholecalciferol, one trial with 1-alpha-hydroxy-calcidiol, and one trial with calcitriol). Based on a random effects model, the overall pooled odds ratio in persons receiving any form of vitamin D suggested a 31% reduction in the risk of falling (pooled odds ratio = 0.69; 95% CI [0.53, 0.88]) compared to persons receiving calcium alone or placebo. Similar percentage reductions in falls were observed for cholecalciferol given in a daily dose of 800 IU plus 1200 mg calcium and active vitamin D. We conclude that in RCTs with an a priori definition of a fall, vitamin D reduces the fall risk by one-third in ambulatory or institutionalised elderly. The few RCTs with musculoskeletal function as the outcome suggest a significant improvement in body sway and lower extremity strength by vitamin D plus calcium supplementation.

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S004

EFFICACY OF CALCIUM AND VITAMIN D IN REDUCING FRACTURE RISK

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Elderly people frequently have inadequate dietary intakes of calcium and vitamin D, both essential for bone health. Vitamin D insufficiency leads to reduced intestinal calcium absorption and secondary hyperparathyroidism, increasing bone turnover particularly in cortical bone, thus accelerating bone loss and predisposing the patient to osteoporotic fractures. Supplementation with vitamin D alone has been shown to reduce bone loss from the femoral neck in postmenopausal women; however, efficacy in terms of fracture reduction has been equivocal. For example, Lips and colleagues reported that vitamin D (400 IU/day) given without calcium did not reduce the risk of hip fractures among elderly Dutch men and women, whereas in the recent study of Trivedi and colleagues, vitamin D 100,000 IU every 4 months did reduce fracture incidence. Similarly, annual intramuscular injections of high doses of vitamin D reduced arm fracture rates among elderly Finnish subjects. There is a strong rationale for the use of calcium and vitamin D in the prevention of osteoporotic fractures and there is evidence from a number of studies that combined therapy has beneficial effects on bone mineral density in the spine, femoral neck and whole body in postmenopausal women. Additionally, calcium and vitamin D combination therapy significantly reduces fracture rates in both institutionalised and free-living elderly populations. In the study of Chapuy and colleagues, the number of hip and non-vertebral fractures were reduced by 43% and 32%, respectively, after 18 months, treatment with calcium and vitamin D. A concomitant increase in serum 25-hydroxyvitamin D and decrease in serum parathyroid hormone level was observed, providing a plausible mechanism for the reduced fracture risk. These findings have subsequently been confirmed in a follow-up-study (Decalyos II). The Medical Research Council of Great Britain has recently initiated a randomised, double-blind, factorially designed trial (the RECORD trial) to evaluate the effectiveness of daily oral calcium, daily oral vitamin D, combined calcium and vitamin D, and double placebo among 5292 men and women aged 70 years and over with previous low trauma fracture. Data collection for this trial will be completed during 2004; the principal outcome measure is all new low trauma fractures. The study has 80% power to detect a difference in fracture incidence of 30% between any of the intervention groups and placebo.

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S005

THE IMPORTANCE OF COMPLIANCE WITHIN OSTEOPOROSIS IN GENERAL AND WITH CALCIUM AND VITAMIN D IN PARTICULAR

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Randomised clinical trials have demonstrated the benefits of drugs and hip protectors in preventing osteoporotic fractures and form the basis for current clinical guidelines and guide assessments of cost-effectiveness. The issue of compliance in the clinic is vital in order to generate valid health-economic data; unfortunately Phase III trials of osteoporosis treatments have rarely reported on compliance, for example in terms of non-use and early drug termination, and other published data addressing this issue are also limited. The data that are available suggest poor acceptance of hip protectors; one study reported that 38% of patients accepted these when recommended and that of these only 50% continued to use them after 1 year. In the case of HRT, route of administration (eg, oral versus transdermal) seems to have no influence on long-term adherence. Compliance with bisphosphonates appears to be similar to that with HRT, with approximately 70% of patients remaining on treatment after 1 year. The use of biochemical markers to monitor antiresorptive effects, and thereby improve patient awareness, has been proposed but the efficacy and relative importance of such an intervention has proven controversial. Initially, oral bisphosphonates were administered once daily; however, these formulations were associated with problems with patient compliance. Consequently, new formulations were tested head-to-head in short-term patient preference trials, with strong patient preference (86%) shown for the once-weekly formulation. Calcium and vitamin D supplementation is the basis of osteoporosis treatment, and is often recommended as a life-long treatment. A randomised crossover study of 'Calcichew' D3 Forte and 'Adcal-D3' in elderly patients indicated a strong preference (80%) for 'Calcichew' D3 Forte. Moreover, a recent study of calcium and vitamin D supplementation conducted at University Hospital Uppsala demonstrated that a chewable tablet is preferred (60%) to a tablet that needs to be swallowed. These short-term studies on patient preference and acceptability suggest that the formulation of calcium and vitamin D is pivotal to achieving long-term patient compliance with consequent important health economic implications.

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ABSTRACTS I001 TO I030

ORAL PRESENTATIONS, INVITED SPEAKERS

I001

CENTRAL CONTROL OF BONE MASS: BIOLOGY AND MEDICAL IMPLICATIONS

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Based on numerous clinical information and on the analyses of several mutant mouse strains, some of them generated in our laboratory, we hypothesized that bone mass, body weight and reproduction may be regulated by the same hormones. Given the hypothalamic nature of the control of body weight and reproduction, this hypothesis, if correct, implied that bone mass could be controlled centrally as well. This hypothesis led to study the function that leptin, a hormone regulating body weight and reproduction, could have in the control of bone mass. To our surprise, mice deficient in leptin signaling (*ob/ob* and *db/db* mice) have an increase in bone mass due to an increase in bone formation parameters and despite their hypogonadism. The circumstances in which leptin antiostegenic functions were revealed, i.e hypogonadism, showed immediately how important was this novel regulatory loop in bone remodeling and how important this function of leptin was. The high bone mass observed in absence of leptin signaling was not due to obesity and could be corrected in ob/ob mice, by central infusion of leptin at doses that did not result in the presence of leptin in blood. Thus, in agreement with our initial hypothesis, there is a central control of bone mass, body weight and reproduction. To better understand how this central control of bone mass occurs we subsequently showed that modulating leptin serum level did affect bone mass and identified neuronal network located in the ventromedial hypothalamus nuclei as being a central antiosteogenic center (CAC). Further experiments showed that the mediator of the CAC function was of neuronal nature and more precisely the sympathetic nervous system. Novel development about the regulation of bone mass by the sympathetic nervous system will be presented at the meeting.

I002

SCLEROSTIN IS AN OSTEOCYTE-EXPRESSED NEGATIVE REGULATOR OF BONE FORMATION, BUT NOT A CLASSICAL BMP ANTAGONIST

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Sclerosteosis, a skeletal disorder characterized by high bone mass due to increased osteoblast activity, is caused by loss of the SOST gene product, sclerostin. The localization in bone and the mechanism of action of sclerostin are not yet known, but it has been hypothesized that it may act as a bone morphogenetic protein (BMP) antagonist. We show here that SOST/sclerostin is expressed exclusively by osteocytes in mouse and human bone and inhibits the differentiation and mineralization of murine pre-osteoblastic cells (KS483). Although sclerostin shares some of the actions of the BMP antagonist noggin, we show here that it has also actions distinctly different from it. Sclerostin, in contrast to noggin, did not inhibit basal ALP activity in KS483 cells neither did it antagonize BMP-stimulated alkaline phosphatase activity in mouse C2C12 cells. In addition, sclerostin had no effect on BMP-stimulated Smad phosphorylation and direct transcriptional activation of MSX-2 and BMP response element (BRE) reporter constructs in KS483 cells. Its unique localization and action on osteoblasts suggest that sclerostin may be the previously proposed osteocyte-derived factor that is transported to osteoblasts at the bone surface and inhibits bone formation. These observations suggest that inactivation of sclerostin by small molecules or humanized neutralizing antibodies may induce a positive bone balance, an effect that may have therapeutic implications for patients with osteoporosis.

I003

REGULATION OF BONE FORMATION BY THE FGF-2 PATHWAY

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Fibroblast growth factor-2 (FGF2) is an important stimulator of osteoblast replication, bone resorption and osteoclast formation. Adult mice with disruption of the fibroblast growth factor-2 gene developed osteopenia manifested by decreased trabecular bone volume, mineral apposition and bone formation rates in femoral bones. In this study, we characterized the time course of bone loss in vertebral bones from Fgf2−/− mice and the effect of Fgf2 haplo-insufficiency (Fgf2+/−) on vertebral bone mass. DEXA analysis revealed that vertebral bone mineral density (BMD) was similar in Fgf2+/+ and Fgf2−/− mice at 2–3 months of age. Fgf2+/+ mice attained peak bone mass at 8–9 months of age. In contrast BMD was reduced by 23% in 8–9 month old Fgf2−/− mice. Interestingly, BMD was also significantly reduced by 13% in vertebrae from 8–9 month old Fgf2+/− mice. Tibiae were harvested from adult male Fgf2+/+ and Fgf2−/− mice to assess basal expression of mRNA for FGF receptors (FGFRs), type 1 collagen (COL1A1), osteocalcin (OC), bone morphogenetic protein 2 (BMP-2) which is important in osteoblast precursor differentiation and Runx2 which is necessary for both osteoblast differentiation and bone formation. There was a 40% reduction in FGFR2 mRNA, a 68% reduction in COL1A1 and a 44% reduction in OC mRNA in tibiae from Fgf2−/− mice compared with Fgf2+/+ mice. In addition, Runx2 mRNA was reduced by 40% and BMP-2 mRNA was decreased by 78% in tibiae from Fgf2−/− mice. Marrow stromal cells from adult Fgf2+/+, Fgf2+/− and Fgf2−/− mice were cultured for 7 days. Northern and Western blotting revealed a marked reduction in FGFR2 mRNA and protein in FGF2+/− and Fgf2−/− mice. There was a 68 and 84% reduction in BMP-2 mRNA in stromal cells from Fgf2+/− and Fgf2−/− mice, respectively, compared to Fgf2+/+ mice. We next examined colony formation in marrow stromal cells from male Fgf2+/+ and Fgf2−/− mice that were cultured in the absence or presence FGF2 (0.1 nM) in differentiation medium. After 16 days of culture, the number of mineralized colonies was significantly decreased in vehicle treated cultures from Fgf2+/− and Fgf2−/− mice compared with Fgf2+/+. Exogenous FGF-2 markedly increased mineralized nodule formation in cultures from all 3 genotypes. We conclude that FGF2 plays an important role in bone formation and propose that the reduction of bone formation in Fgf2−/− mice may correlate with impaired signaling by FGFR2 and suppression of BMP-2 and Runx2 gene expression.

I004

EPIDEMIOLOGY OF GLUCOCORTICOID-INDUCED OSTEOPOROSIS

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Oral glucocorticoids (GC), also known as oral corticosteroids, are widely used for the treatment of a variety of inflammatory and allergic disorders. Estimates for the USA suggest that 1–3% of men and women aged more than 50 years are using long-term GC therapy.

It is now well recognized that therapy with oral GCs can lead to rapid loss of bone mineral density (BMD) and to an increased risk of fracture, particularly at the hip and spine. Several epidemiological studies have reported that this increase in fracture risk occurs rapidly after the start of GC therapy, and that this is strongly correlated to daily GC dose. This increased risk was independent of underlying disease, age and gender. It appears to be substantially reversible after discontinuation of oral GC therapy.

The issue of BMD threshold for fracture in oral GC users has received considerable attention, but published data on presence of prevalent fracture and BMD level are inconsistent. Recently, it was found that oral GC users have considerably higher risks of incident fractures than non-users, at similar levels of baseline BMD. Also, a meta-analysis reported that the actual increases in fracture risk in oral GC users were considerable larger than expected on the basis of BMD changes.

With respect to users of inhaled GCs, dose-related increases in hip fracture risk have been reported in several studies. Also, a meta-analysis found that inhaled GC users have, on average, lower BMD than expected. But the aetiology of this increased risk of fracture and osteoporosis remains controversial: Is it related to the adverse systemic effects of inhaled GCs? Or is it related to the underlying disease? Two recent cross-sectional studies conducted in the general population both found that patients with airflow obstruction were more likely to have osteoporosis, independent of GC use.

It is suggested that any preventive measures against GC-induced osteoporosis should be given in parallel to the GC therapy.

I005

MECHANISMS OF OSTEOBLAST INHIBITION BY STEROIDS

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Glucocorticoids (GC) have anti-mitogenic, pro-apoptotic and pro-diabetic side- effects *in vivo*, involving disruptions of multiple cell signalling pathways. Vanadium, an inhibitor of the family of protein tyrosine phosphatases (PTPs), reverses many of these effects *in vivo* and *in vitro* via its unique ability to inhibit PTPs at sub-micromolar concentrations. Physiological effects of vanadium are seen when a PTP acts as a "gateway" to a particular cellular response. For instance, vanadium improves blood glucose handling and insulin resistance in mice and humans primarily by inhibiting PTP-1B, which acts as an off-switch for insulin receptor and IRS signalling in muscle and liver. In bone, vanadium acts as an *in vitro* mitogen, and prevents GC- induced apoptosis of osteoblasts. Furthermore, vanadium opposes the transcription- dependent negative effects of GC on osteoblast proliferation, and *in vivo*, vanadium supplementation prevents the bone formation deficits associated with steroid osteoporosis in the rat. Several PTPs are likely to be involved, but the inducible, dual-specificity phosphatase, MKP-1, is strongly upregulated by GC in osteoblasts and accounts for the anti-proliferative effects of high dose GC treatment *in vitro*. MKP-1 dephosphorylates and inactivates the ERK axis, the central mitogenic effector pathway in osteoblasts. ERK plays a role, not only in control of proliferation, but also differentiation, adhesion to and secretion of bone matrix proteins and mineralisation. It controls the expression of the transcription factors c-fos, Fra-1 and delta-FosB, overexpression of which cause excessive osteoblast proliferation and/or mineralizing function. ERK acts as a molecular switch, activating osteoblast differentiation-associated transcription factors such as cbf-a1, while repressing adipocytic transcription factors such as PPAR-gamma. Since adipocytes and osteoblasts in the bone marrow are recruited from adult mesenchymal stem cells, disruption of ERK signalling contributes to adipocyte differentiation at the expense of osteoblast numbers. Vanadium has the potential to influence all ERK-mediated pathways in osteoblasts and therefore PTP inhibitors should be considered as powerful tools for investigating the molecular basis of bone disease.

I006

PREVENTION AND MANAGEMENT OF GLUCOCORTICOID-INDUCED OSTEOPOROSIS

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Glucocorticoids are commonly used to manage inflammatory conditions with at any one time approximately 1% of the adult population in the UK using therapy rising to 2.5% of individuals aged 70–79. It is clear that the relative risk of vertebral and other fractures rise very shortly after commencing oral glucocorticoid treatment and fracture risk is primarily daily-dose dependent. Fracture rates fall almost as rapidly as they rise on discontinuation of the drugs. One clear method of preventing the adverse effects therefore is not to use the drugs at all or to withdraw therapy as soon as possible after initiation of therapy. However this is not feasible for most patients and in the UK evidence-based guidelines have been recently introduced which should lead to substantial improvements in management of the condition. Available online at www.rcplondon.ac.uk, they recommend that for men and women over the age of 65 or those with prior fragility fracture who are to be exposed to oral glucocorticoids for 3 months or more, bone-protective therapy should be started at the time of starting glucocorticoids. For men & women less than 65, measurement of spine and hip BMD is recommended and if the T score is < −1.5 bone-protective therapy should be commenced. In the USA a T-score cut-off of −1.0 for treatment is recommended due to the evolving evidence that fractures occur at higher bone density levels in glucocorticoid users than non-users. A number of potential bone-protective therapies have been used in the prevention or reversal of bone loss but the most effective and best studied of the currently available therapies are the bisphosphonates, etidronate, alendronate and risedronate. These drugs prevent bone loss in those commencing glucocorticoids, in part reverse bone loss in those established on steroid therapy and almost certainly reduce at least vertebral fractures. Newer therapies with parenteral bisphosphonates or PTH may be an additional therapeutic option in the near future.

I007

BIOLOGY OF MATRIX PROTEINS

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The properties of connective tissues depend on the extracellular matrix. This matrix in tissues like cartilage, bone and tendon is made up of a few basic functional units composed of specific molecular assemblies. These constitutes the collagen 1 or 2 fibril assemblies, the collagen 6 based network and the aggrecan network and in bone also the mineral deposits.

The assembly of these networks is governed by a number of matrix proteins that in several cases remain bound at the surface of the fibers modulating their properties.

Among these proteins the extracellular Leucine Rich Repeat proteins with some ten repeats include the decorin subfamily of three proteins, the fibromodulin subfamily of five proteins and the chondroadherin forming a separate subfamily. It has been shown for most of these proteins that they bind tightly to a number of quite different collagens that have different functions. Several members of this family of proteins also bind to cell surface receptors and trigger signaling, thereby having the potential to provide feedback to the cells on the extracellular matrix.

Other proteins in the extracellular matrix, e.g. COMP, also have roles in catalyzing fibril formation and in modulating collagen surface properties.

Many of these proteins are cleaved and removed from the matrix in pathology. This appears to occur before collagenous core of the fibrils is affected.

Mechanisms appear to involve new production of proteinases, e.g. certain matrix metalloproteinases. Following their activation they will cleave proteins in the extracellular matrix. By identifying such cleavage sites, new indicators for assessing the activity of the proteinase and the tissue breakdown can be developed in the form of antibodies only recognizing the new termini formed. We have used the occurrence of specific cleavages of matrix proteins in search of the nature of the enzyme involved.

I008

NEW BIOCHEMICAL MARKERS OF BONE TURNOVER IN OSTEOPOROSIS

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Increasingly specific biochemical markers for bone remodelling have been identified in recent years. At present, the most sensitive markers for bone formation are serum osteocalcin (OC), bone alkaline phosphatase (bone ALP), and procollagen type I N-terminal propeptide (PINP). Immunological assays for deoxypyridinoline (DPD) in urine and for C-terminal and N-terminal type I collagen peptides (CTX and NTX, respectively) in serum or urine are currently the most sensitive resorption markers.

In osteoporosis, the main cause for concern is the increase in the risk of fractures. An important issue is whether combined use of bone markers and bone mineral density (BMD) measurements improves the accuracy of fracture risk evaluation. Several prospective studies have shown that an increased bone resorption evaluated by urinary free DPD, urinary or serum CTX was associated with increased risk of the hip, spine and non-vertebral fractures independently of BMD. The use of bone markers in individual patients may be appropriate in some situations, especially in women who are not detected at risk by BMD measurements. In the OFELY study including 668 postmenopausal women followed prospectively over 9 years, we found that among the 115 incident fractures, 54 (47%) actually occurred in non-osteoporotic women. Among these women, the combination of bone markers and history of previous fracture was highly predictive of fracture and allowed the detection of 59% of women with incident fracture. In the FIT trial, we found that higher baseline levels of bone turnover was associated with a greater efficacy of alendronate to reduce fracture risk in non-osteoporotic women, suggesting that identification of women with no osteoporosis but high bone turnover would be relevant for treatment decision with antitresorptive therapy. Thus, bone markers may be used in the assessment of fracture risk in selected cases in which BMD and clinical risk factors are not enough to take a treatment decision. In patients given bone resorption inhibitors, such as estrogens, selective estrogen response modifiers, or bisphosphonates, the changes in BMD are small as compared to the long-term reproducibility of this parameter. In addition, it has been shown that BMD changes account for a small part of the efficacy of treatment on fracture risk. It was found that the short term changes of OC and bone ALP with raloxifene treatment were associated with the subsequent risk of vertebral fractures in a large subgroup of osteoporotic women enrolled in the MORE study, while changes in hip BMD were not predictive. In the VERT study with risedronate it has been shown that changes of urinary CTX and NTX after 3 to 6 months predicted the risk of subsequent incident vertebral fractures after both 1 and 3 years, these changes explaining 50 to 70% of the effect of risedronate on fracture risk. A significant association between changes of bone ALP and ver-

tebral, hip and non spine fracture was also found in women treated with alendronate participating in the FIT trial. Advances in our knowledge of bone matrix biochemistry, most notably of post-translational modifications in type I collagen, may allow identification of biochemical markers that reflect changes in the material property of bone, which is an important determinant of bone strength. In postmenopausal women of the OFELY study, we found that changes of the degree of isomerization of type I collagen as reflected by the urinary ratio of native (α) to isomerised (β) CTX was associated with fracture risk independently of BMD and of bone turnover. *In vitro* experiments have also demonstrated that changes in the extent of post-translational modifications of type I collagen (e.g. intermolecular crosslinking such as pyridinoline and pentosidine and CTX isomerization) play a role in determining the mechanical competence of cortical bone, independently of BMD. Further studies in osteoporosis should explore the changes in these biochemical parameters of bone matrix in response to treatment, as they may represent a key component of bone quality.

I009

CLINICAL USE OF BIOCHEMICAL MARKERS

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The development of improved assays for the measurement of bone turnover markers makes them widely accessible. These markers may have a number of uses, particularly in the field of osteoporosis. They may be used for the assessment of risk of future fractures. In several studies, high level of bone turnover markers may be associated with an increase in the risk of fracture. The WHO working group is attempting to include such measures in its approach to estimate 10-year fracture risk. They may be used for the identification of secondary osteoporosis. High levels may prompt the search for endocrine diseases, malabsorption syndrome or malignant diseases that might have caused the osteoporosis. The most appropriate use for bone turnover markers is their use in monitoring. The changes in bone turnover marker in response to anti-resorptive and anabolic treatment is rapid and so early information may be obtained about response. A poor response is most commonly caused by poor patient adherence to the therapy, but can be caused by the co-existence of secondary osteoporosis. There is evidence that feeding back the result of the bone turnover marker may improve long term compliance with osteoporosis medications.

I010

HOX GENES AND LIMB DEVELOPMENT

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Abstract not available

I011

WHY SOME BONES FRACTURE AND OTHERS DON'T: A BIOMECHANICAL PERSPECTIVE

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Since bone is obviously in some way adapted to the loads falling on it, and since fracture is usually the type of failure of mechanical competence that is of main clinical importance, it is often thought that bones are adapted to resist fracture. This may not be the case. There are two major compromises that must be made: between the strength, and stiffness, of the whole bone and its mass, and between stiffness of the bone material and its toughness.

First, an increase in the 'strength' and stiffness of a bone can be bought by making it fatter and more robust. However this increased strength is bought at the cost of having a heavier, more expensive bone, which takes longer to develop. This increased mass will have been selected against. Safety factors have been adapted, over evolutionary time, to produce some optimum compromise for a host of different design constraints. The greatly differing fracture incidences in different bones seen in pre-senile human adults, and in horses, suggest that the compromise is different for different bones. For instance there is a tendency for distal long bones to fracture more frequently than more proximal ones, and there is a fairly simple mechanical explanation for this difference in safety factors.

In general, bone material that is stiff is less tough than bone material that is flexible. The reduction in toughness as humans mature is a reflection of the increased need for locomotory efficiency that our ancestors faced as they neared adulthood. Similar changes are found in deer.

Some bones may be designed to be very stiff, and therefore highly mineralized and therefore brittle. An example is the bones associated with the ear. Bones may also potentially fail in completely contrasting modes, and therefore their design has to be a compromise which resists neither mode completely successfully. An example is the radius of teenagers which, to resist impact fracture of the metaphysis should (counterintuitively) be more flexible than it actually is, but to resist Euler buckling should be less flexible than it actually is.

Finally, the process of adaptation occurring during life may result in bones being adapted to normal loads, but not to the characteristic loads occurring in falls. An example is the femoral neck.

I012

CHLORIDE CHANNELS AND PROTON TRANSPORT: CLC-7 REGULATES BONE DENSITY

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Voltage-gated chloride channels of the CLC family fulfill numerous functions in a variety of species from bacteria to humans. Many of these physiological functions are related to proton transport processes. ClC-7 is a ubiquitously expressed chloride channel that is mainly localized in late endosomes and lysosomes. Clcn7−/−-mice show a severe osteopetrosis (marble bone disease) that becomes apparent shortly after birth. Although osteoclasts are present in normal numbers, they fail to resorb bone. In osteoclasts, ClC-7 is highly expressed in the ruffled membrane that is formed by the fusion of $H+$-ATPase containing late endosomal vesicles. Chloride ions conducted by ClC-7 provide the necessary countercharge to allow the $H+$-ATPase to efficiently pump large amounts of protons into the resorption lacuna. Independent of the skeletal changes the retina of Clcn7−/−-mice undergoes rapid degeneration, thereby causing visual loss as demonstrated by electroretinogram measurements. The murine phenotype closely resembles human infantile malignant osteopetrosis. A screening of the human gene, CLCN7, revealed several mutations that lead to a partial or complete loss of ClC-7 expression. Recently, mutations in CLCN7 were found to cause also the much milder autosomal dominant form of osteopetrosis (ADOII). Surprisingly, expression levels and subcellular localization of ClC-7 are not detectably altered in primary osteoclasts from several ADOII patients. Nevertheless, their resorptive activity is diminished. This implies that dominant mutations most likely perturb the electrophysiological function of ClC-7 and that changes in the chloride conductance of the ruffled membrane are able to regulate bone resorption and bone density.

I013

BONE LINING CELLS AND THE REGULATION OF BONE RESORPTION

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Bone modelling depends on the activity of osteoblasts for bone deposition and on osteoclasts to resorb this tissue. In addition to these cells, a large fraction of the bone surface is covered by the alleged bone lining cells (or surface osteocytes or resting osteoblasts). Since bone lining cells are generally considered to be relatively inactive, they received little attention thus far. However, bone lining cells are present not only at surfaces characterized by a very low modelling activity (resting surface) but also at sites where osteoclasts are active. At the latter sites we noted their presence in Howship's lacunae that were vacated by osteoclasts. It appeared that bone lining cells engulf and subsequently digest demineralised bone collagen fibrils protruding from the bottom of the resorption lacunae. In this way they clean the bottom of the pit of non-digested bone matrix left by the osteoclast. The cleaning is followed by the deposition of a thin layer of proteins along the bottom of the pit. After this, osteoblasts enter the site and deposit a new layer of bone matrix. Cleaning of the bottom of the pit proved to be crucial for subsequent bone formation; without the cleaning activity bone deposition did not occur. Recently, we found pre-osteoclasts to strongly attach to bone lining cells. This interaction induced withdrawal of the bone lining cells after which the pre-osteoclasts populated the areas freed of cells and then fused to form osteoclasts. We propose a dual role played by bone lining cells in the sequence of events leading to bone resorption and subsequent bone apposition.

First, these cells attract and direct (pre-) osteoclasts to the site of bone where resorption has to occur, and second, following the resorption of bone by osteoclasts, the bone lining cells clean the bottom of the pit and prepare this site for bone apposition by osteoblasts. Thus it appears that bone lining cells fulfil a hitherto unrecognised important role in coupling bone resorption to bone formation.

I014

TISSUE ENGINEERING OF CARTILAGINOUS TISSUES : GENE ENHANCEMENT OF CHONDROGENIC CELLS

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Tissue engineering, regenerative medicine and research into the understanding the molecular mechanisms in chronic diseases provide a number of strategies to address major health care needs and priorities of the next 20 years. This theme is a recognised priority of governments and funding agencies in many countries and new initiatives have sprung up worldwide to address these issues. The strategy underlying our tissue engineering programme at the Universities of Manchester and Liverpool is based on our belief that through good basic molecular and cellular research our scientific understanding of how living cells function will enable us to gain control and direct their activity to promote the repair of damaged and diseased tissues. This research programme was established in 2001 funded by UK Government Research Councils. It contains clinical foci in areas of skin/wound healing, cartilage/intervertebral disc repair and vascular biology and it is complemented by work on bioreactors, haemodynamics, biocompatibility, angiogenesis and a significant programme in gene transfer.

Gene transfer offers great potential in tissue engineering for short or long-term enhancement of cell function. We have investigated gene transfer of the transcription factor SOX9 into passaged adult human articular chondrocytes to reinitiate chondrogenic matrix production. These cells were from osteoarthritic joints and when grown in monolayer culture become fibroblastic and lose the expression of major cartilage matrix genes. We have shown that by increasing the rate of proliferation by specific growth factors there was efficient retroviral transduction and sustained SOX9 expression. In monolayer cultures the transduced cells had increased collagen type II expression. This was increased further by transfer of the cells to alginate or pellet culture and maximum matrix production was achieved in the presence of additional growth factors. The results showed that SOX9 transduced chondrocytes from OA joints, even after extensive passage in monolayer culture (up to 1 million expansion), showed enhanced response to chondrogenic culture conditions and growth factors and reinitiated cartilage matrix production.

I015

PLURIPOTENCY OF ADULT STEM CELLS: INSTRUCTIONS FOR OSTEOGENIC LINEAGE DIRECTION

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Stem cells isolated from various tissues, hematopoietic stem cells and the mesenchymal stromal cells of the bone marrow represent opportunities for correction of genetic and malignant disorders, tissue repair and regeneration. Of particular interest is the replacement of bone lost as a consequence of hormonal deficiencies and the aging skeleton. Challenges in isolating and defining the properties of stem cell populations and maintaining competency for engraftment to support tissue regeneration are being overcome through understanding of characteristics of stem cells. Cell-type specific transcription factors have been identified that control programs of gene expression; for example, differentiation of chondrocytes, osteoblasts, adipocytes, nerve, muscle or hematopoietic cells. These findings will be discussed within the context of new insights for ensuring commitment of stem cells to the skeletal lineage, expansion of osteoprogenitors and differentiation to osteoblasts that produce a functional tissue. The Runx/ Cbfa transcription factors serve has a model for defining how pluripotent cells limit phenotypic options, and mediate osteogenic differentiation. During mitosis, Runx factors remain associated with the chromosomes for phenotype specific control. Runx2 is a target gene of bone morphogenetic proteins, interacts with other tissue related transcription factors and has the ability to integrate the activity of signaling pathways (Smads, Src, Wnt) for transcriptional control of gene expression through the formation of multimeric complexes that reside in specific subnuclear domains. Proof of principle experiments will be presented that demonstrate intranuclear targeting of Runx is obligatory for regulatory activity and provides a molecular mechanism for controlling the program of

bone formation. The temporal-spatial parameters of Runx mediated transcription supports activation, repression and physiological responsiveness of phenotypic target genes that change during the stages of osteoblast maturation derived from the stem cell. This insight will contribute to progress in harnessing adult stem cell plasticity for clinical applications in bone biology.

I016

ENGINEERING NEW BONE WITH BMPS

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Tissue engineering requires the interplay between three components, the cells that create the tissue, biologic signaling molecules to direct the cells to form the desired tissue, and a matrix to hold these components and create the physical form of the regenerated tissue. Members of the BMP family of molecules, such as BMP-2, are potent osteoinductive agents. When combined with a matrix/ carrier system, recombinant human BMP-2 (rhBMP-2) directs mesenchymal cells present in the host to differentiate into bone and cartilage cells. The ability of rhBMP-2 to regenerate bone suggests a wide range of potential applications in bone repair and augmentation. Large clinical studies have now been performed using rhBMP-2 combined with an implantable absorbable collagen sponge (ACS) matrix. One series of studies has shown the ability of rhBMP-2/ ACS to regenerate and augment bone in the alveolar ridge, allowing placement and loading of dental implants. A second series has shown its ability to induce bone when placed in titanium spinal fusion cages, and thus create successful spinal arthrodesis. In this setting, it replaces the use of bone graft harvested from the iliac crest, therefore decreasing operative time, blood loss, and pain associated with autogenous bone graft harvest. Finally, a large study has been conducted that demonstrates the acceleration and assurance of healing of difficult tibial fractures by rhBMP-2/ACS. In this study, the proportion of patients healed was significantly increased at all time points. In addition, patients treated with rhBMP-2/ACS had fewer infections at the fracture site and demonstrated accelerated soft tissue wound healing.

Additional carrier systems are being evaluated for clinical settings where the use of ACS is inappropriate or not optimal. An injectable formulation of rhBMP-2 that could be administered percutaneously, for example, could be used to accelerate closed fracture repair. We have developed a calcium phosphate matrix (CPM), formulated to disperse around the fracture site. rhBMP-2/ CPM has been evaluated in a series of preclinical fracture models in rabbits, dogs, and non-human primates. In the latter studies, rapid (2–4 wks) bone induction was visible. Radiographic and biomechanic evaluations indicated a 40–50% acceleration in fracture repair by this formulation of rhBMP-2. These results suggest that rhBMP-2, with appropriate matrices, can be successfully used in a variety of clinical indications.

I017

MEDICAL TREATMENT OF FIBROUS DYSPLASIA OF BONE

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Caused by somatic mutations of the GNAS1 gene inducing an overproduction of cAMP in osteogenic cells which deposit abnormal bone, fibrous dysplasia (FD) is a rare congenital bone disease characterized by a focal proliferation of fibrous tissue in the bone marrow, leading to osteolytic lesions, bone pain and fractures. Until mid 90's the only treatment of FD was orthopaedic. An increased osteoclastogenesis, often combined with the fibrosis and the osteoblastic dysfunction and favouring the expansion of the lesions, led us to propose the use of bisphosphonates as a medical therapy of FD. Intravenous (IV) pamidronate, oral or IV alendronate have been now used for a decade in several open studies. In our center 28 men and 30 women (median age 28; 5–63) treated with IV pamidronate (180 mg administered in 3 days every 6 months in 41 adults; 1 mg/kg/day for 3 days in 17 children or adolescents) were followed-up for an average 50 months (1 up to 11 years). Daily supplements of 1000 mg of calcium and 400 −1200 iu of vitamin D were added to pamidronate. Among the 80% of patients who had bone pain at baseline we observed a 70% decrease in pain intensity after the first course of IV pamidronate. This relief was maintained throughout follow up, with 60% of patients classified as complete responders. 45% of patients had a decrease of at least 30% in serum total alkaline phosphatase after one year of treatment and this proportion was 64% for urinary CTX. 54% of patients were radiographic responders (filling of osteolytic lesions or substantial thickening of cortices). There was no association between radiographic response and clinical or biochemical responses. In 12 patients having femoral lesions and measured by DXA, a mean increase of 17% in femoral bone mineral density was noted after treatment. Intravenous pamidronate was well tolerated. A renal phosphate wasting was identified in 22% of our patients and may be responsible for a

mineralization defect. These patients received oral phosphate and calcitriol in addition to pamidronate. The use of bisphosphonates very likely represents an advance in the medical management of FD, but their clinical and radiographic efficacy, based for the time being on observational studies, remains to be confirmed by a randomized, placebo-controlled trial. Such a study has been recently undertaken, involving several European centers. It is aimed at evaluating the effects of oral risedronate (PROFIDYS study).

I018

OSTEOGENESIS IMPERFECTA AND JUVENILE OSTEOPOROSIS

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Osteoporosis is increasingly recognized as an important medical problem affecting pediatric patients either because of an intrinsic skeletal disorder (primary osteoporosis) or as the result of other diseases and/or their treatment (secondary osteoporosis). This presentation will focus on the primary forms of the disease that are divided into two main groups: the heritable disorders of connective tissue (including osteogenesis imperfecta, OI), and idiopathic juvenile osteoporosis (IJO). The latter is a rare (∼100 cases in the literature) self-limiting disorder which affects cancellous rather than cortical bone. IJO may represent a failure to adapt to the increasing mechanical challenges that occur during growth. Treatment attempts are virtually impossible to assess because of the spontaneous improvement that usually occurs.

OI is a heritable disorder where the hallmarks are bone fragility and low bone mass. Incidence is estimated at 1:15,000 live births. At least seven different forms have been identified, with a large proportion of them associated to mutations in one of the two genes encoding type I collagen. Bone abnormalities in OI affect matrix organization, modeling, formation and thickening of secondary trabeculae, as well as cortical thickening. A higher than normal degree of mineralization, consequence of the matrix abnormalities, contributes to the brittleness of OI bone.

OI has attracted a lot of attention in the last few years as it became the first documented indication for the use of bisphosphonates (BP) in the pediatric age group. Effects of BP in OI include significant gain in bone mass and density, and muscle force, suppression of chronic pain, and reduction of fracture incidence. Growth rate is enhanced, but delayed fracture and osteotomy healing may occur in the long term. The best modalities for the use of BP in OI in terms of dose, route of administration, and duration of therapy remain to be precisely determined. It is already clear however that, in the current absence of a cure for OI, the use of BP has brought about remarkable gains in terms of quality of life, and control of the clinical expression of this heterogeneous condition.

I019

JUVENILE PAGET'S DISEASE

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Juvenile Paget's Disease/Familial Hyperphosphatasia is an autosomal recessive bone disorder characterized by greatly accelerated bone turnover. Affected children are normal at birth but generalized skeletal disease becomes evident in infancy or childhood. The clinical features include short stature, deafness (from skull involvement) spinal osteoporosis, diaphyseal widening, fracture and progressive deformity. Many of the factors regulating bone turnover are mediated through the RANK-RANK-L-OPG pathway. JPD is genetically heterogeneous, but the majority of cases result from mutations in TNFRSF11B, the gene encoding osteoprotegerin (OPG), that result in OPG deficiency. Nearly all cases reported to date have had homozygous mutations. Major gene deletions and mutations affecting cysteine residues in the ligand-binding domain are associated with a particularly severe phenotype, presenting in the first year of life. Such children may never walk. Within families the phenotype is similar, but two (unrelated) subjects with the same mutation but quite different phenotypes have been described, so there are clearly modifying genes. In the short term antiresorptive treatment with calcitonin or bisphosphonates reduces bone turnover, but there is no long-term data on effectiveness. We have recently documented the outcome of intravenous ibandronate treatment in an 11 year old with OPG deficiency. Very high doses were required to suppress bone turnover to age-appropriate levels, but over 3 years there was no progression of deformity, an improvement in deafness and radiographic evidence of diaphyseal normalization. She remains ambulant and mobile at an age when her two affected older siblings were wheelchair-bound. Replacement of the defective protein should be the ideal treatment, and the older siblings are currently participating in a trial using recombinant OPG (kindly donated by Amgen). Preliminary results indicate that both subjects are exquisitely sensitive to recombinant OPG - bone resorption being inhibited at doses an order of magnitude lower than in normal subjects.

I020

MOLECULAR MECHANISMS OF PHOSPHATE TRANSPORT IN KIDNEY AND BONE: RECENT ADVANCES

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Inorganic phosphate (Pi) is fundamental to cellular metabolism and, in vertebrates, to skeletal mineralization. To accomplish these vital functions, transport systems have evolved to permit the efficient transfer of negatively charged Pi ions across hydrophobic membrane barriers. Ingested Pi is absorbed by the small intestine, deposited in bone, and filtered by the kidney where it is reabsorbed and excreted in amounts that are determined by the specific requirements of the organism. Although several distinct Na-dependent Pi transporters have been identified in renal, bone and intestinal cells, the most significant advances have been made in our understanding of the molecular mechanisms involved in renal tubular Pi transport and its regulation by PTH and dietary Pi intake, both major determinants of Pi homeostasis. Npt2a (type IIa) is the most abundant of the renal Na/Pi cotransporters and is expressed in the brush border membrane of proximal tubular cells where the bulk of filtered Pi is reabsorbed. Studies in mice in which the Npt2a gene was knocked out by homologous recombination underscored the crucial role of Npt2a in the maintenance of Pi homeostasis. Npt2a-deficient mice exhibit a ∼70% reduction in renal brush border membrane Na/Pi cotransport, hypophosphatemia, an adaptive increase in the renal production and the serum concentration of 1,25-dihydroxyvitamin D, intestinal calcium hyperabsorption, hypercalcemia, hypercalciuria, renal calcification and an age-dependent skeletal phenotype. Moreover, renal brush border membrane Na/Pi cotransport in Npt2a-deficient mice is not responsive to PTH or to changes in dietary Pi intake, indicating that in wild-type mice Npt2a is the target for regulation. The latter is achieved primarily by PTH- and dietary Pi-induced changes in the abundance of Npt2a protein in the renal brush border membrane. Several proteins essential to brush border membrane insertion, retrieval and degradation of Npt2a have recently been described. In addition, two novel Pi regulating genes, PHEX and FGF23, which are mutated in patients with Mendelian Pi wasting disorders, have been identified. Future studies are necessary to uncover additional Na/Pi cotransporters in kidney, bone and intestine that play an important role in determining Pi economy in vivo and to define the precise mechanism whereby PHEX and FGF-23 regulate Pi homeostasis.

I021

PATHOGENESIS AND TREATMENT OF OSTEOPOROSIS IN MEN

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The risk of osteoporotic fractures in men is lower than in women, which is explained at least in part by the achievement of a larger size of the bones during growth, by the lack of increased bone turnover with its attendant deterioration of trabecular architecture and accelerated bone loss at middle-age, and by more continuous subperiosteal bone apposition during aging. Nevertheless, the age-specific incidences of vertebral and hip fracture in men still amount to half that in women, and the consequences of fractures in men tend to be more severe. Although there are quantitative differences, qualitatively the risk factors for fracture in men are similar to those in women, with age, low bone mineral density (BMD) and prevalent fracture being the cardinal elements for individual risk assessment. There is presently no consensus on an operational definition of osteoporosis based on BMD, but prospective observational studies reveal an identical risk for fracture in men and women with a same absolute areal BMD as assessed by dual-energy X-ray absorptiometry. Glucocorticoid excess, hypogonadism, alcohol abuse and various causes of calcium malabsorption are major secondary causes of osteoporosis in men. Deficient acquisition of bone mass and size, with a gender-specific genetic determination and predominant expression at the axial skeleton, appears to underlie idiopathic osteoporosis in younger men (<65y), although high turnover bone loss may be involved in a subgroup of men with multiple vertebral fractures. Higher rates of senile bone loss are associated with higher bone turnover and lower serum levels of bioavailable estradiol and with a polymorphism of the CYP 19 aromatase gene. The evidence base for pharmacological intervention to reduce fracture risk in osteoporotic men is still narrow, with the best available evidence suggesting that the treatment effects of bisphosphonates, in particular alendronate, and of teriparatide (1–34 PTH) on BMD and fracture risk are of similar magnitude as previously demonstrated in postmenopausal women, and are largely independent of factors such as pretreatment serum levels of testosterone or biochemical markers of bone turnover.

I022

ESTROGENS, ANDROGENS AND THE REGULATION OF BONE REMODELING

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Although the role of estradiol (E2) in maintaining bone mass is well established, the relative importance of ERα, ERβ, and the androgen receptor (AR), is still controversial. Similarly, the respective roles of transcriptional activation (TRACT) and membrane initiated signal tranduction (MIST) in mediating these effects remains unclear. To determine the contribution of each ER on bone mass, the effects of deletion of each or both ERs and the effects of gonadectomy (GDX) and E2 treatment were studied in single and double ER KO mice. E2 treatment of OVX female ERαβ$^{-/-}$ mice failed to prevent bone loss, precluding any effect of E2 on bone through the AR. In contrast, E2 prevented OVX-induced bone loss and increased bone mass at high doses in female ERβ$^{-/-}$ mice, as in wild type males and females, indicating that ERα is the major mediator of E2 effects in bone. Importantly, no response of bone to E2 was detected in ORX male ERα$^{-/-}$ mice, demonstrating that ERα is the sole regulator of bone response to E2 in males, and suggesting that E2 cannot protect bone mass via the AR *in vivo*. In contrast, female ERα$^{-/-}$ mice responded to E2 with partial protection against OVX-induced bone loss, confirming that ERβ can mediate E2 effects in bone, but only in females and with a lower efficacy than ERα*fnfzf*nparnparticularly on inhibition of bone resorption. Thus, ER? is the main effector of E2 protective function in bone in both male and female mice and, in its absence, AR is not sufficient to mediate bone response to E2 *in vivo*. Following a report suggesting that Estrens acts on both AR and ERs and only via MIST (Kousteni et al., Science, 2003), we then compared the ability of E2, a SERM and 4-estren3α-17β-diol (Estren) to prevent bone loss in GDX male and female mice as well as their respective impact on reproductive organs. In this model, E2, DHT and SERM not only prevented GDX-induced bone loss, but also elicited an anabolic increase in bone mass compared to sham. In contrast, Estren, at a dose more than 150X that of the SERM, only maintained bone mass at control levels. Unexpectedly, whereas the SERM induced moderate increases in seminal vesicle and uterus weights compared to GDX controls, Estrens increased both the uterus and seminal vesicles, as did E2 and DHT. Relative binding affinities of Estren showed that it actually is a ligand for both ERs and for AR. Estren is a weak agonist of both ERs and can stimulate transcription from several E2-responsive promoters, albeit at much higher concentrations than E2 (1000X), whereas the SERM displayed limited activity. Finally, and again in contrast to SERM or E2, Estren displayed full agonist activity on the AR, and stimulated ARE-dependent transcription. In conclusion, 1/ERα is the main effector of E2 in bone 2/E2 cannot protect bone via the AR and 3/Estrens, which bind to both the ERs and AR and may activate only MIST, display, in contrast to SERMs, a non-selective effect on bone, reproductive organs and breast cancer cell lines.

I023

CLINICAL ROLE FOR ESTROGEN REPLACEMENT AND SERMS AFTER WOMEN'S HEALTH INITIATIVE

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Observational studies have shown that postmenopausal hormone replacement therapy (HRT) is associated with a reduction in osteoporotic fracture incidence. Randomised clinical trials, using bone density as a surrogate outcome, have clearly shown that HRT prevents, and to some extent reverses, postmenopausal bone loss at all clinically relevant sites. This effect is does-dependent, with older women requiring lower doses of estrogen. The beneficial effect of HRT on osteoporotic fracture reduction was confirmed by a large randomised clinical trial, the Women's Health Initiative (WHI). However, this study of over 16,600 postmenopausal women showed primarily that giving one HRT regimen to healthy women resulted in neither benefit nor harm in more than 99%. The most surprising clinical outcome was that a small number of women experienced cardiovascular harm early in the study. In contrast, observational studies have consistently shown that postmenopausal hormone replacement therapy (HRT) reduces the incidence of cardiovascular disease by approximately 40–50%, but the women start on HRT at a younger age than those in the randomised trials. The biological plausibility for a cardiovascular benefit of oestrogen is overwhelming, and is supported by results from several randomised trials of surrogate markers. However, it is possible that HRT could produce vascular harm under certain circumstances. Adverse effects of estrogen on thrombogenesis and vas-

cular remodelling are dose-dependent, so starting with an inappropriately high dose for the age of the women could have such effects. A recent pilot study (WHISP) of secondary prevention using a different low-dose HRT regimen suggested a reduction in events. Cardiovascular effects of HRT may depend critically on dosage and type of hormones. WHI also confirmed a small increase in breast cancer development, but not mortality, with HRT use, similar to that seen with alcohol consumption but less than that seen with obesity. The effects of alternatives to HRT, such as tibolone or selective estradiol receptor modulators (SERMs), need to be evaluated. SERMs do not appear to increase breast cancer incidence, but have adverse effects on thrombogenesis, and have no benefit for hip fracture reduction. HRT is the cheapest and most effective therapy for prevention of postmenopausal osteoporosis. Given appropriately, HRT or its alternatives still have a major role to play in improving and ensuring female health.

I024

ANANLYSIS OF BONE MINERAL BY QBEI

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The amount of mineral particles within the collagenous bone matrix, their shape, size and arrangement play a pivotal role in the biomechanical properties of bone. Diseases and treatments, which affect the bone material properties can alter bone strength significantly in these patients. The usage and development of suitable methods to evaluate the material quality of bone is therefore of great importance to medicine, as well as for materials science and biomimetics. Quantitative backscattered electron imaging (qBEI) is a valuable research tool to, nondestructively, assess the mineral concentration in bone tissue areas accessed by sectioning of plastic embedded biopsies. The technique is based on the ratio of electrons backscattered from the sample in the scanning electron microscope (SEM), whereby the ratio is positively correlated with the calcium weight percentage (Ca wt%) of the analyzed sample location. Maps of Ca wt% and frequency histograms of Ca wt%, designated as bone mineralization density distributions (BMDD) are generated. Bone material is not uniformly mineralized due to the ongoing resorption of old and the formation of new bone matrix, which is subsequently mineralized in a specific time course. The BMDD therefore sensitively indicates all changes in bone turnover as well as the time course of mineralization. With respect to material science qBEI can be combined with other nondestructive scanning methods providing complementary data from the collagen and mineral nanocomposite of bone. For instance, the combination of qBEI and small angle x-ray scattering (SAXS) enables the determination of mineral particle growth and thickness in individual bone packets. Further, sequential analysis of identical bone areas by qBEI and nanoindentation-AFM allows to establish the correlation between mineral particle content and E-modulus. Employing fluorescence labeling of bone tissue, the combination of qBEI and confocal scanning laser light microscopy (CSLM) gives information of both tissue age and degree of mineralization. In conclusion quantitative analysis of the bone mineral content by qBEI provides important information to evaluate the material quality of bone as well as to target therapeutically the bone mineralization density in patients properly.

I025

VIBRATIONAL SPECTROSCOPIC CHARACTERIZATION OF BONE MINERAL AND COLLAGEN CROSS-LINKS

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Vibrational spectroscopy (Raman and Infrared (IR)) has been widely used for the analysis of bone mineral content, composition, and crystallinity. The coupling of an optical microscope with IR or Raman spectrometers has recently enabled the acquisition of spectra at particular sample sites, with a spatial resolution approaching the diffraction limit. The availability of array detectors has allowed the rapid acquisition of thousands of spectra thus permitting the efficient analysis of large tissue sections. Both IR and Raman require no special specimen staining and are thus non-destructive. Each technique has advantages and disadvantages. Raman spectroscopy does not require thin sections, does not require dehydration of the tissue (water has a strong IR signal), has a 1 um spatial resolution, has relatively weaker but narrower bands than IR, can be performed on microscope slides and permits data acquisition in a confocal manner. In contrast, IR spectra are inherently

stronger, can provide information on larger sample areas, and for mineralized tissue analysis, provides more detail on phosphate structure/environment. The parameters used to characterize mineralized tissues are mineral-to-matrix ratio (area ratio of the phosphate v_1, v_3, contour (900–1200 cm^{-1}) in the IR or v_1 (960 cm^{-1}) in the Raman to the amide I at 1585–1720 cm^{-1} IR or in Raman, the hydroxyproline band (859 cm^{-1}). Mixtures of apatite crystals (of predetermined size and composition) with type I collagen analyzed by FTIR and gravimetric determination of ash weight, BSE, and micro-CT density analyses have been used to validate the spectroscopic mineral-to-matrix measurement. Analysis of the phosphate vibrations in IR are used to determine crystal size and perfection (as validated by x-ray diffraction line-broadening analysis of synthetic apatites). The Amide I contour shows changes with tissue age in both health and disease. Ratios (IR) of subbands at 1660/1690 cm^{-1} based on analyses of peptides containing variable amounts of stable (non-reducible) and reducible collagen cross-links have been related to collagen maturity. The power of this approach is illustrated by analysis of bone mineral and matrix changes during bone aging, in diseases such as osteoporosis, osteopetrosis, and osteomalacia, and in transgenic and knockout mice.

I026

MICROSCOPY FOR MINERALISED TISSUES IN BONES

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What we look at should be intact. Entire bones can be imaged with MRI or x-ray microtomography, but with limited resolution. Whole explants of thin, platelike bones can be imaged with light, live, at sub-cellular resolution. However, better resolution structural studies will require contrived samples. The mineralised tissues found in bones are tough to section, and microtomed sections are shattered. The general solution is therefore to remove surface layers from a bulk sample, carefully, and to study what remains. Except for ground sections for light microscopy and microradiography and ion- or fast atom beam thinned sections for TEM, preferred methods for block surface microscopy will involve some kind of epi-illumination, as in reflected and confocal LM and SEM.

Preparing a 'block' in which both hard and soft elements are to be studied in the correct context is greatly simplified if it is embedded in a hard resin - this also improves transparency and the depth to which confocal fluorescence (CSLM) methods may be applied. If the block is finished flat (> 0.1 µm relief) and carbon coated, the backscattered electron (BSE) signal in SEM can be used to study variations in mineral concentration, but, to avoid problems due to electron channeling contrast, standard materials used for calibration must have no long range crystallographic order. Scanning both the electron beam and the sample permits imaging of the largest bones at high resolution, the time cost being the limiting factor. As both SEM and CSLM images are digital, they can be exactly matched to enhance local information content, matching cell characters and fluorochrome labelling to calcified tissue structure and composition.

Much interest in bones is simply in looking at bone tissue, its osteoid surface, mineralising, resting and resorbing or resorbed surfaces, to which end all cells and/or all unmineralised matrix are digested, after polishing any cut surfaces. SEM is again one choice method. Today, we eschew heavy metal (e.g. gold) coating to give electrical conductivity and a high secondary electron (SE) yield, but use the several attendant advantages of BSE imaging of carbon coated samples. With appropriate stage automation, we generate many projections of the same sample volume. Played in rapid sequence to give motion parallax, such SEM tomography allows excellent 3D visualisation.

Improvements in the depth of field of SEM imagery and from the use of multiple detectors will also be shown.

I027

PROGRESS IN DETECTING OSTEOPOROSIS SUSCEPTIBILITY GENES

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Genetic factors play an important role in osteoporosis. Twin and family studies have shown that bone mineral density is highly heritable, as are other key risk factors for osteoporotic fractures such as bone ultrasound, femoral neck geometry and bone turnover. Current evidence suggests that susceptibility to osteoporosis is mediated by the effects of many genes, although some diseases related to osteoporosis can rarely occur as the result of mutations in a single gene – such as osteogenesis imperfecta and osteoporosis-pseudoglioma syndrome. Many approaches are being pursued to identify the genes responsible including linkage studies in man and experimental animals and candidate gene studies. Linkage studies have identified multiple quantitative trait loci (QTL) for regulation of BMD and have indicated that the effects of these loci are site dependent and sex-specific. For the most part, the genes responsible for BMD regulation in these QTL have not been identified but allelic variations in at least two positional

candidate genes BMP2 and TNFRSF1B have been identified that explain some of the linkage signal in QTL on chromosomes 20p12 and 1p36.

Most studies on the genetics of osteoporosis have used the candidate gene approach. The vitamin D receptor gene (VDR), the collagen type I alpha I gene (COLIA1) and estrogen receptor gene alpha (ER) have been most widely studied. There is evidence to suggest that allelic variation in all three genes plays a role in regulating BMD, but the effects are modest and together, probably account for less than 5% of the heritable contribution to BMD. Genes may influence only certain phenotypes and not all genes that influence BMD will be important in fracture. Understanding the different genetic determinants of osteoporosis has major clinical implications and is likely to lead to both novel molecular targets for drug design and a battery of diagnostic and prognostic tests.

I028

QUANTITATING THE EFFECTS OF POLYGENES BY META-ANALYSIS

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Osteoporosis and fracture risk is largely under genetic control and genetic effects are probably mediated by a considerable number of different genes and polymorphisms thereof. Each genetic variant probably contributes only a small portion of the overall attributable risk and genetic effects are small or modest in size. Thus single studies, unless very large, are likely to miss the presence of genuine associations due to type II error. At the same time, the vast number of possible genetic markers to be screened for their putative association with osteoporosis risk augments the problem of type I errors. Single studies in the field may also suffer from publication bias, i.e. the preferential publication of research based on the presence or not of statistical significance for its findings. Meta-analysis may offer a solution to these problems by combining quantitatively information across all teams of investigators working on a specific genetic association for one or several distinct polymorphisms. By performing meta-analysis one can estimate the presence of genetic heterogeneity and test for the presence of biases, such as the occurrence of different results in small vs. larger studies and in early published research vs. subsequent validation investigations. Empirical data suggest that it is very common among genetic association research to observe exaggerated genetic effects in the first published study on a postulated association than in subsequent research. A similar bias has been described in the results of smaller vs. larger studies on the same association. Meta-analysis can be either retrospective or prospective and can be the optimal approach to foster international collaboration between scientists working on the same genetics field. Some of the potential main advantages include the standardization of definitions and analyses (both genotypic and statistical) and the accumulation of large sample sizes. Meta-analysis may also provide also sufficient sample size to address gene-gene interactions and gene-environment interactions that would be extremely precarious to address with single studies. We will discuss several examples of applications of retrospective and prospective meta-analyses addressing the modulation of osteoporosis and fracture risk by genetic polymorphisms, including the international collaborative meta-analyses currently conducted under the GENOMOS project.

I029

TARGETING TREATMENT BY FRACTURE RISK RATHER THAN BMD

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The diagnosis of osteoporosis is defined by a WHO group as BMD value (T score of −2.5 SD or less) with DXA technique. T scores have different prognostic significance at different ages and for different measuring sites. Thus, diagnostic thresholds are not equivalent to intervention thresholds due to the fact that the risk varies markedly at any given BMD. There are many risk factors in addition to BMD, age and gender that can provide information on fracture risk over and above that provided by BMD alone. The absolute fracture risk that the patient has should be the guideline for intervention. The objective of risk assessment is to provide information that can be used for decision-making in the management of patients. There are three decisions that can be based on fracture probability: no further assessment or treatment required, further assessment indicated, e.g. diagnostic assessment, and treatment indicated irrespective of any diagnostic assessment. To further analyse these three decision cut offs, 12 cohorts worldwide have been used and cost effectiveness indication cut off. In the group where treatment is indicated irrespective of any diagnostic assessment the individuals had a very high risk and mainly fall in those who with a BMD measurement would have had the diagnosis of osteoporosis. Besides, there are some intervention studies that have not used BMD as inclusion criterion, such as a baseline vertebral fracture, glucocorticoid use, indicating that treatment is possible in this

group. Thus, a BMD measurement is needed among fracture probabilities that are calculated from risk factors alone when the absolute risk is close to the intervention threshold.

I030

OSTEOPOROSIS TREATMENTS: PAST, PRESENT AND FUTURE

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The association between oestrogen deficiency and osteoporosis, made by Fuller Albright in 1941, was followed by the demonstration that oestrogen replacement (HRT) prevented bone loss in postmenopausal women and subsequently this became the gold standard treatment for osteoporosis. Other compounds have since been approved for osteoporosis and HRT has become a second line option because its adverse risk benefit ratio.

A number of important lessons have been learnt from recent clinical trials. First, therapeutically induced increases in bone mineral density explain only a small proportion of the associated fracture reduction. Secondly, it cannot be assumed that an agent with proven efficacy at one site will be effective against all osteoporotic fractures. Thirdly, the rate of onset of action of many interventions appears to be rapid; the offset of treatment effect is also quite rapid for some interventions although there may be important differences between the bisphosphonates in this respect. Finally, treatment of individuals with a high fracture probability is most cost-effective and correspondingly there has been a move away from long-term preventive strategies towards shorter-term intervention in high-risk individuals.

Bisphosphonates are regarded as the front line option because of their proven efficacy against vertebral and non-vertebral fractures, including hip fractures. Teriparatide, the only anabolic agent currently available, also has proven efficacy against vertebral and non-vertebral fractures, although effects on hip fracture have not been demonstrated. Raloxifene is a useful option in women with vertebral osteoporosis but has not been shown to protect against non-vertebral fractures. Calcium and vitamin D have an important role as adjuncts to treatment and in the prevention of non-vertebral fractures in the frail elderly population.

The future promises new agents, for example strontium ranelate, new dosing regimens for bisphosphonates which aim further to improve tolerability and better tolerated modes of administration for parathyroid hormone peptides. Combinations of selective oestrogen receptor modulators and oestrogen are being developed with the aim of producing an optimal pharmacological profile. In addition, a number of molecules implicated in bone resorption and formation are being investigated for their potential to prevent osteoporotic fractures.

ABSTRACTS OP001 TO OP052

ORAL PRESENTATIONS

OP001

MICE LACKING FRA-2 ARE SEVERELY OSTEOPENIC DUE TO OSTEOCLAST AND OSTEOBLAST DEFECTS

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The three Fos proteins c-Fos, FosB, Fra-1 have been shown to play crucial roles in bone biology, but little is known about Fra-2, the fourth Fos protein. To study its role in bone development and remodeling, we generated Fra-2 knock-out (−/−) and Fra-2 overexpressing mice.

Fra-2 overexpressing mice show increased bone mass at 1 and 3 months of age, increased bone formation rate by osteoblasts *in vivo* and *in vitro*, but interestingly also increased bone resorption *in vivo* and *in vitro*, accompanied by higher expression levels of Carbonic Anhydrase II and MMP-9 in transgenic osteoclasts.

Fra-2−/− mice die postnatally between day 1–5, are growth retarded and display severe osteopenia, thus supporting our gain-of-function approach. Analysis by bone histomorphometry showed that bone volume is reduced by 50% and that numbers of osteoblasts were unchanged. However, both number and size of osteoclasts were dramatically increased, resulting in a "giant" osteoclast phenotype and most likely increased bone resorption.

During embryonic development, from E 14. 5 onwards, Fra-2 deficient mouse embryos display a reduced zone of hypertrophic, Collagen type X-positive cells in long bones. Moreover, chondrocyte differentiation and ossification of vertebral bodies is delayed throughout development, indicating a general chondrocyte maturation and mineralization defect.

Although the mature osteoblast marker osteocalcin shows a normal expression pattern during embryonic development up to E19.5, the expression of both osteocalcin and bone sialoprotein is almost completely abolished postnatally in Fra-2−/− mice. Since primary Fra-2−/− osteoblasts additionally showed a severe differentiation defect *in vitro*, this suggests that decreased bone formation by osteoblasts is also contributing to the dramatic bone loss *in vivo*.

To study the mechanism leading to the *in vivo* "giant" osteoclast appearance, we performed *in vitro* cultures of fetal liver-derived osteoclasts and M-CSF dependent newborn bone marrow precursors. Unexpectedly, primary osteoclast cultures showed a severe defect in differentiation and fusion; the numbers, but also the size, of TRAP-positive osteoclasts were smaller compared to controls. This defect could be partly rescued by addition of TGFbeta and TNFalpha to the culture medium. Although mutant osteoclasts show impaired differentiation and reduced expression of osteoclast marker genes such as Carbonic Anhydrase II, they are still capable of resorbing bone matrix at comparable levels to control osteoclasts. *In vivo*, yet unknown systemic or paracrine signals must be responsible for the "giant" osteoclast phenotype. In conclusion, we provide evidence that Fra-2 plays important roles in chondrocyte, osteoblast and osteoclast differentiation, and that loss or overexpression of Fra-2 result in imbalanced bone development and remodeling leading to severe bone diseases.

OP002

ENHANCED TRABECULAR BONE MASS AND ARCHITECTURE IN MICE OVER-EXPRESSING ATG1-TO-TTG1 MUTANT HISTONE H4 TRANSGENE

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The evolutionary conserved histone H4 genes encode at least two peptides: the 103 amino acid H4 protein and a circulating mitogen, Osteogenic Growth Peptide (OGP), which is identical to the 14 carboxy terminal residues of histone H4. OGP is synthesized de novo from H4 mRNA following leaky ribosomal scanning through the imperfect H4 AUG initiator. Consequently, alternative translation initiates at codon 85, a perfect AUG initiator, ultimately yielding OGP. Here we engineered transgenic mice, ubiquitously and constitutively expressing a mutant H4 mRNA, H4tTG1, which encodes OGP but not H4.

Quantitative micro-computed tomographic analysis of femora from 8, 17 and 34 week-old mice revealed a marked increase in trabecular, but not cortical, bone volume density at all ages. This effect was particularly strong in females, which exhibited a significant 2-fold increase in trabecular bone density compared to wild-type controls. The enhancement of trabecular bone density was accompanied by increased trabecular number and connectivity, parameters that contribute to bone strength. Dynamic histomorphometric analysis demonstrated a significant 35% increase in the percentage of trabecular surface engaged in bone formation and a significant 23% increase in the mineral appositional rate in females. Osteclast number was not significantly altered. No adverse effect of OGP over-expression was noticeable in transgenic mice up to 18 months of age. Thus, continuous OGP over-expression throughout life results in a specific augmentation of trabecular bone without noticeable effects on cortical bone or extra-skeletal tissues. In summary, expression of OGP-coding, H4-like mRNA in post-mitotic cells results in increased trabecular bone accrual.
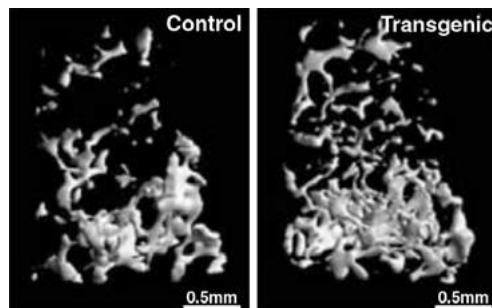
OP003

IDENTIFICATION OF NOVEL RUNX2 TARGET GENES

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The transcription factor Runx2 is considered a master gene for osteoblast differentiation. Although some transcriptional targets for Runx2 are known, it is believed that the osteogenic action of Runx2 requires additional target genes. However there is no established method for unbiased discovery of genomic sites directly occupied by transcription factors of interest. Therefore, we developed a novel approach, Chromatin Immunoprecipitation (ChIP) Display (CD), which allowed us to identify several novel Runx2 target genes.

The CD technique was successfully employed with a robustly mineralizing MC3T3-E1 osteoblastic subclone. Cells were subjected to ChIP with Runx2 antibodies, resulting in a precipitate containing Runx2 targets, but also the inevitable vast excess of non-specifically co-precipitated DNA. This background makes problematic identification of Runx2 targets by direct cloning of immunoprecipitated fragments. To overcome this obstacle, true Runx2 targets were separated from the background using restriction digestion with *Ava*II, which brought all the fragments representing any one target to a unique size. Furthermore, following linker ligation, the immunoprecipitated DNA fragments were PCR amplified in distinct families, segregated based on the identity of two nucleotides in the vicinity of the *Ava*II sites at the ends of each fragment. This results in improved signal-to-background ratio because each single target is wholly amplified within its family, while only a fraction of the background fragments is amplified in the same reaction. Indeed, upon gel electrophoresis, fragments representing true Runx2 target genes formed distinct bands. In contrast, the non-specifically precipitated DNA resulted in a background smear formed by fragments of diverse sizes. Bands of interest were excised, and the DNA fragments were identified by sequencing and comparison to public genomic databases. Of a total of 36 families of genomic fragments that would cover the entire genome, CD of the first two has already revealed several novel Runx2 targets. These include genes encoding the transcription factors Fli1 and Runx3 and the less well-known genes ORP8 and a novel EST with homology to the DYRK1 kinase. These genes were confirmed as Runx2 targets using conventional ChIP assay. Furthermore, ORP8 mRNA was shown to increase during BMP-2-induced osteoblast differentiation. In principle, CD can facilitate the discovery of novel target genes for any DNA-binding protein.

OP004

WNT-SIGNALING REGULATES HUMAN OSTEOBLAST DIFFERENTIATION AND MINERALIZATION IN A STEROID-DEPENDENT MANNER

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The wnt-signaling pathway regulates transcription of several genes linked to proliferation and differentiation. Wnt proteins prevent β-catenin degradation which results in β-catenin accumulation, its nuclear transport and regulation of several target genes. Mutations in the wnt co-receptor LRP5 that inactivate wnt-signaling result in decreased bone mass, whereas mutations that prevent inhibition by Dickkopf proteins (Dkk) result in sustained wnt-signaling and increased bone mass. These findings suggest a crucial role for wnt-signaling in bone metabolism. To study wnt-signaling in osteoblast we used the human osteoblast cell-line SV-HFO in which differentiation and mineralization is initiated by the presence of glucocorticoids. Cells were cultured for 23 days and throughout culture-time we quantified the expression of various wnt-signaling genes, in relation to differentiation and mineralization. Furthermore we stimulated wnt-signaling using lithium and monitored osteoblast differentiation by measuring alkaline phosphatase activity (ALP-activity) and mineralization.

Real-time PCR revealed that SV-HFO cells express various wnt-antagonists, including three members of the Dickkopf family: Dkk1, −2, −3, and two secreted frizzled-related proteins: sFRP-2 and FrzB. Glucocorticoid-induced differentiation resulted in increased expression of these wnt-antagonist, decreased β-catenin protein levels and reduced β-catenin transcriptional activity. Induction of wnt-signaling resulted in decreased ALP-activity and strongly reduced mineralization. In contrast, wnt-signaling enhanced ALP-activity in the absence of glucocorticoids. We have shown that 1,25(OH)2D3 vitamin D enhances osteoblast differentiation and mineralization. Interestingly, wnt-signaling could not inhibit ALP-activity and mineralization in the presence of vitamin D.

In conclusion, wnt-signaling acts in a vitamin D- and glucocorticoid-dependent manner. Furthermore these data suggest an inverse relationship between wnt-signaling and glucocorticoid-induced osteoblast differentiation and mineralization.

OP005

ZOLEDRONATE UP-REGULATES BONE SIALOPROTEIN EXPRESSION IN SAOS-2 CELLS THROUGH RHOA INHIBITION

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Zoledronate (ZOL), a new generation of nitrogen-containing bisphosphonate, is widely used for the therapeutic management of osteoporosis and skeletal complications of malignancy. Recent studies demonstrated that ZOL enhances bone formation, however a direct effect of this compound on the expression of proteins playing a pivotal role in the mineralization process has not been investigated yet. Several *in vivo* and *in vitro* studies indicate that bone sialoprotein (BSP) is a bone matrix protein that plays a major role during de novo bone formation. Because of its bone-inducing activity, the osteosarcoma Saos-2 cell line is considered as a model of osteoblastic function and as a source of bone-related molecules including BSP. The purpose of this study was to search for potential effects of ZOL on the expression of BSP in Saos-2 cells. ZOL at a concentration of 20 microM stimulated the synthesis of BSP mRNA in Saos-2 cells treated for 48 hours. Previous studies have shown that ZOL is able to interfere with the mevalonate pathway and inhibits the synthesis of mevalonate metabolites including farnesyldiphosphate (FPP) and geranylgeranyldiphosphate (GGPP) and thereby impairs the prenylation of small GTPases such as Ras and Rho. Therefore, we treated Saos-2 cells with ZOL in the presence of geranylgeraniol (20 microM) or farnesylfarnesol (20 microM) to test if these substances could overcome the inducing effect of ZOL on BSP expression. The effect of ZOL was reversed by geranylgeraniol but not by farnesylfarnesol. Because Rho GTPases undergo geranylgeranyl modification, we investigated the contribution of Rho to BSP mRNA up-regulation. Treatment with cytotoxic necrotizing factor-1 (200 ng/ml), an activator of Rho GTPases, decreased BSP mRNA expression. Furthermore, inhibition of RhoA synthesis by short interfering (si)RNA induced an increase of BSP mRNA expression. These results are the first demonstration that ZOL up-regulates BSP expression in Saos-2 cells suggesting a potential new mechanism for its bone protective effects. This study also emphasizes the role of the Rho GTPases in ZOL-mediated BSP up-regulation

OP006

DEFECTIVE BONE MINERALIZATION AND OSTEOPENIA IN YOUNG ADULT FGFR3-/- MICE

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Mutations that cause constitutive activation of fibroblast growth factor receptor 3 (FGFR3) result in skeletal disorders characterized by short limbed dwarfism and premature closure of cranial sutures. In previous work it was shown that congenital deficiency of FGFR3 led to skeletal overgrowth during development, as a result of defective endochondral ossification in the epiphyseal growth plates. Its role in post natal bone growth and metabolism have not, however, been characterized. Using a combination of imaging, classic histology and molecular cell biology we now show that young adult FGFR3−/− mice are osteopenic due to reduced cortical bone thickness and defective trabecular bone mineralization. The reduction in mineralized bone and lack of trabecular connectivity observed by micro computed tomography were confirmed in histological and histomorphometric analyses, which revealed a significant decrease in calcein labeling of mineralizing surfaces and a significant increase in osteoid in the long bones of 2 and 4 month old FGFR3−/− mice. These alterations were associated with increased staining for recognized markers of differentiated osteoblasts and increased numbers of tartrate resistant acid phsophatase postitive osteoclasts. Despite evidence of expression of mature osteoblast markers, primary cultures of marrow-derived stromal cells from FGFR3−/− mice failed to develop a significant accumulations of mineralized bone nodules compared with wild type cultures of the same age. This data reveals a novel role for FGFR3 in post-natal bone growth and remodeling, which identify it as a potential therapeutic target for osteopenic disorders and those associated with defective bone mineralization.

OP007

ACCELERATED BONE AGING IN DNA REPAIR DEFICIENT TRICHOTHIODYSTROPHY MICE

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Our genome is continuously damaged by environmental (e.g. UV light) and endogenous agents (e.g. reactive oxygen species). Lesions in DNA cause immediate effects on cell function (cell cycle arrest, temporary block of transcription, apoptosis) and have long-term consequences (mutagenesis, carcinogenesis and aging). In mammals, UV lesions and other helix-distorting damages are removed by the Nucleotide Excision Repair (NER) mechanism. In man, the importance of NER as a cancer/ageing preventing genome care taking system is illustrated by the occurrence of hereditary photosensitive DNA repair disorders.

Trichothiodystrophy (TTD) is a rare, autosomal recessive NER disorder in which patients present with an array of symptoms, including skeletal abnormalities. TTD is caused by a mutation in one of the genes of the TTD complementation group (XPD, XPB, TTDA). XPB and XPD are helicase subunits of the dual functional DNA repair/basal transcription factor TFIIH. We have mimicked a causative point mutation identified in the XPD gene of a photosensitive TTD patient. Previous work has shown that the phenotype of TTD mice is very similar to the symptoms of patients including the presence of premature aging features like osteoporosis, kyphosis, osteosclerosis, early greying, cachexia, ovarian dysfunction and reduced life span. These features of accelerated ageing may relate to a combination of enhanced accumulation of endogenously produced DNA damage and subtle transcription defects.

In the present cohort study, we have analysed the bone phenotype and hormone profile in aging wild type and TTD mice. Up to 39 weeks microCT analysis of tibiae showed no differences in bone fragment thickness, total bone area or moment of inertia (MOI) between wt and TTD mice, but from then on a more rapid cortical thinning was observed in TTD mice resulting in a similar bone fragment thickness at 52 weeks of age as in 78 weeks old wt mice. Bone area and MOI decreased up to 52 weeks in both wt and TTD mice after which they remained at the same level in TTD mice whereas an increase was observed in wt mice. This coincided with an increase in body weight in wt mice, which is lacking in TTD mice. Also for 1,25-(OH)2D3, PTH, osteocalcin and estradiol age-related differences were detected. In conclusion, TTD mice show an accelerated age-related bone phenotype and have an altered age-related pattern in calcium and bone regulating hormones.

OP008

GENETIC DEFICIENCY IN PERK CAUSE SEVERE OSTEOPENIA AND NEONATAL GROWTH RETARDATION

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We have generated a knockout mutation of the PERK eIF2 alpha kinase in mice. Perk−/− KO mice display extreme osteopenia and severe neonatal growth retardation virtually identical to the skeletal defects seen in the human Wolcott-Rallison syndrome recently shown to also be associated with Perk loss of function mutations. Micro CT analysis revealed extreme porosity and low bone density through the entire skeletal system with the most severe defects seen in the axial skeleton. Bone tissue in Perk KO mice exhibit an abnormally low level of mature type I collagen, whereas procollagen-1 was highly elevated. In addition the collagen-specific ER chaperone protein Hsp47 was elevated, reminiscent of specific cases of osteogenesis imperfecta in which procollagen-1 is abnormally retained in the endoplasmic reticulum. The examination of the subcellular distribution of procollagen-1 in primary cultures of calvarial osteoblasts showed that a large fraction of immunoreactive procollagen-1 was localized in the ER surround the nucleus in Perk−/− cells. We propose that PERK is required for the normal assembly and/or postranslational modification of procollagen-1 in the ER, and we are currently investigating the molecular mechanism of this function. In addition to these defects in cortical bone, Perk KO mice display a structural weak fraction in the hypertrophic zone of the growth plate and apparent diminution of aggrecan suggesting potential secretory defects in growth plate chondrocytes. Perk−/− KO mice display severe neonatal growth retardation. Analysis of circulatory growth factors revealed a deficiency in IGF-1, which is strongly correlated with a reduction in liver IGF-1 mRNA expression. These studies have shown that PERK regulates early neonatal expression of IGF-1 in the liver during the developmental period when IGF-1 is independent of GH. Moreover, we show that normal variation in neonatal IGF-1 serum level is strongly correlated with growth whereas variation in IGF-1 serum levels during the juvenile is not correlated. Thus PERK-dependent IGF-1 during neonatal growth is limiting for growth whereas GH-dependent IGF-1 expression during later stages of adult development is permissive for growth. In summary, the PERK eIF2 alpha kinase has major regulatory functions that impact bone development and growth particularly during the late embryonic-neonatal periods.

OP009

PROSTAGLANDINS-DEPENDENT ACTIVATION OF ERK MEDIATES CELL PROLIFERATION INDUCED BY TGFβ IN MC3T3-E1 OSTEOBLASTIC CELLS

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TGFβ is a major coupling factor for bone turnover and is known to stimulate osteoblastic proliferation and matrix deposition. The cellular and molecular mechanisms involved in these effects are uncompletely understood. Enhanced synthesis and autocrine action of prostaglandins (PGs) is considered as a potential mechanism for some of the TGFβ effects on osteoblasts. Activation of Smad and of MAP kinase pathways have been shown to be involved in mediating TGFβ effects but the precise function of each pathway remains unclear.

In the present study, we investigated the role of MAP kinases in mediating cell proliferation induced by TGFβ in MC3T3-E1 cells.

TGFβ (2. 5 ng/ml) induced activation of the three MAP kinases ERK, p38 and JNK in MC3T3-E1 cells. Surprisingly, whereas activation of Smad2 was rapid and maximal after 15 min incubation, activation of MAP kinases was delayed. p38 stimulation was detected after 1 h exposure whereas activation of ERK and JNK was only detected after 3 h suggesting indirect activation of MAP kinases by TGFβ. Associated with this effect, TGFβ enhanced cell proliferation by about two folds. Since, as mentioned above, PGs have been reported to mediate some of the TGFβ effects on osteoblastic cells, we investigated the influence of indomethacin (indo), a specific PGs synthesis inhibitor, on signaling and cell proliferation induced by TGFβ. Indo (10 μM) markedly reduced activation of MAP kinases without influencing Smad2 phosphorylation and completely blunted cell proliferation induced by TGFβ. These data strongly suggest that PGs play an important role in activation of MAP kinases and cell proliferation induced by TGFβ. Interestingly, exposure of PGE2 (1 μM) mimicked the effects of TGFβ on cell proliferation and MAP kinases without any delay in activation. Finally, the role of each MAP kinases in mediating TGFβ- and PGE2-induced cell proliferation was investigated using specific inhibitors. U0126 (10 μM), a specific inhibitor of the ERK pathway, completely blocked TGFβ- and PGE2-induced cell proliferation whereas SB203580 (10 μM) and SP600125 (20 μM), which are selective inhibitors of respectively p38 and JNK pathways had no effect.

In conclusion, data presented in this study strongly suggest that activation of ERK by prostaglandins that are synthesized in response to TGFβ mediates osteoblastic cell proliferation induced by this major coupling factor of bone turnover.

OP010

11β-HYDROXYSTEROID DEHYDROGENASE EXPRESSION AND GLUCOCORTICOID SYNTHESIS IS DIRECTED BY A MOLECULAR SWITCH DURING OSTEOBLAST DIFFERENTIATION

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Glucocorticoids (GCs) have profound effects on bone and when administered in pharmacological doses cause osteoporosis, mainly by suppressing bone formation. Paradoxically, glucocorticoids are crucial for human osteoblast differentiation. It can therefore be envisaged that glucocorticoid metabolism is an important target to control glucocorticoid action in bone and osteoblast development. The aim of the current study was to investigate in detail the glucocorticoid endocrine system during human osteoblast differentiation. In particular expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) an enzyme that locally converts inactive cortisone into active cortisol. To study this we used the human osteoblast cell-line SV-HFO. In the presence but not in the absence of glucocorticoids these cells produce an extracellular matrix and develop a peak alkaline phosphatase activity (ALP-activity) in the first 2 weeks of culture after which mineralization starts.

We show that SV-HFO osteoblasts express 11β-HSD1 and have the capacity to convert cortisone into active cortisol. GRalpha was expressed throughout culture-time however expression was reduced in the presence of glucocorticoids. Expression and activity of 11β-HSD1 was regulated in a differentiation dependent manner. During GC-induced differentiation 11β-HSD1 mRNA expression and activity were low and remained constant. However cultures that were not treated with glucocorticoids (no osteoblast differentiation) showed a strong induction of 11β-HSD1 expression and activity. Importantly, this switch in 11β-HSD1 expression was initiated after day 12 of culture, which coincides with the start of mineralization in GC-treated cultures. These results indicate that absence of glucocorticoids results in an autocrine feed-back mechanism which compensates for the absence of active glucocorticoids and delayed osteoblast differentiation by increasing local cortisol production. Indeed we proved that osteoblastic expression of 11β-HSD1 in the presence of cortisone, resulted in induction of ALP-activity and osteoblast differentiation.

In conclusion, the current study shows that human osteoblasts activate several means to secure glucocorticoid pathway-mediated stimulation of differentiation/mineralization, by increasing local cortisol production. This feedback mechanism is strongly directed by osteoblast differentiation. Thereby these data provide new insights into the versatile and paradoxical actions of glucocorticoids in bone.

OP011

CUMULATIVE RISKS OF FRACTURE IN PATIENTS USING ORAL GLUCOCORTICOIDS

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The aim of this study was to estimate 5-year risks of fractures in patients using oral glucocorticoids (GC).

The study population consisted of 191,752 patients aged > 40 years prescribed an oral GC (in the UK General Practice Research Database). The period of follow-up was divided into time-periods of current (i.e. duration of individual prescription plus 3 months) and no exposure, with patients moving between these exposures. Using Cox proportional hazards models, a risk score, indicating the association to risk, was initially estimated from daily and cumulative GC dose of each exposure period, body mass index (BMI), and disease and drug history. Then, the 5-year risk of fracture (Cox survival function) was estimated for each sum of scores.

7412 patients experienced an osteoporotic fracture. For each 10-year increment of age, the risk score for osteoporotic fractures increased by 4 points; for men −6, low BMI +3, fall history +8, fracture history +6, and rheumatoid arthritis (RA) +1. GC therapy increased the risk score. In patients aged 65 years, use of 7.5 mg prednisone per day increased the risk score by 8. The 5-year risk of osteoporotic fracture for patients with scores of 30, 40, 50, and 60 was 7.2, 18.5, 42.7, and 78.1%, respectively. The 5-year risk of osteoporotic

fracture for a 65-year female with RA, low BMI, and fracture and fall history using between 7.5 and 15 mg GC (total score of 52) was 49.4% (male with similar history 31.2%). Intermittent high-dose GC therapy (defined as short-term use with daily dose > 30 mg) was associated with only a small increased risk of osteoporotic fracture in first-time users (RR 1.20; 95% CI 0.98–1.46) and in patients with pulse dosing at least 3 months after end of prior GC use (RR 1.21; 95% CI 1.04–1.42).

In conclusion, this risk score allows for assessing an individual's risk of fracture.

OP012

CONTRASTING EFFECTS OF TERIPARATIDE AND ALENDRONATE ON BONE TURNOVER ASSESSED BY BONE HISTOMORPHOMETRIC PARAMETERS IN WOMEN WITH OSTEOPOROSIS

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We conducted a randomized double-blind study in postmenopausal women with osteoporosis to contrast the effects of teriparatide 20 mug/d (TPTD) and alendronate 10 mg/d (ALN) on bone remodeling as assessed by bone histomorphometry. Patients were randomly assigned to receive either TPTD (n = 102) or ALN (n = 101) for 18 months. Bone biopsies were obtained in separate subsets of patients at 6 (TPTD, n = 8; ALN, n = 9) and 18 months (TPTD, n = 8; ALN, n = 7). Histomorphometric indices of bone remodeling were significantly greater with TPTD than with ALN (mean ± SD). In trabecular bone, indices reflecting bone formation and activation frequency were generally greater at 6 vs. 18 months with TPTD, while the same indices remained suppressed at both time points with ALN. In TPTD, the peak in bone formation indices coincided with peak levels for biochemical markers of bone formation. Bone resorption, as reflected by erosion surface, although generally greater in TPTD compared with ALN, did not reach the magnitude of treatment differences attained in formation indices. Resorption remained relatively constant over time in both groups. Bone formation was greater at 6 vs. 18 months with TPTD. The values at both time points were significantly greater than that observed with ALN, confirming the opposite mechanism of action of the two treatments. Furthermore, these results reveal the sustained, positive formation-resorption balance achieved by teriparatide compared with alendronate.
Funded by Eli Lilly and Company

Table:

Bone envelope	6 months TPTD	6 months ALN	18 months TPTD	18 months ALN
Trabecular				
OS/BS	17.26** ± 7.94	6.83 ± 5.17	12.63* ± 6.64	5.29 ± 3.04
ES/BS	3.08 ± 2.03	2.17 ± 1.35	3.89 ± 2.30	2.59 ± 1.28
BFR	0.062** ± 0.036	0.002 ± 0.002	0.030*	0.003
Ac.f	0.99**	0.02	0.46**	0.04
Endocortical				
ES/BS	5.60*	2.79	5.62	4.06
MS/BS	18.73**	0.44	9.69**	1.02
BFR	0.098*	0.007	0.064*	0.009

OP013

BIOCHEMICAL MARKERS OF BONE FORMATION ARE POSITIVELY CORRELATED WITH VOLUMETRIC BMD FOLLOWING TERIPARATIDE THERAPY

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The effects of teriparatide 20 mcg/day [rhPTH (1–34),TPTD20] and alendronate 10 mg/day (ALN10) on bone were compared in a randomized controlled trial of postmenopausal women with osteoporosis. Previous results have shown significant increases of 100 to 300% in bone turnover markers (BTM) during the first 6 months of TPTD20 treatment compared with decreases of 60 to 70% during ALN10 treatment (McClung ASBMR 2003). Both lumbar spine areal BMD (aBMD) by DXA and trabecular volumetric BMD (vBMD) by QCT increased in each treatment group. While increases in aBMD were nearly 2-fold, vBMD increases were 4 to 5-fold greater in TPTD20 versus ALN10 treated patients. The aim of this study was to assess the correlation of early changes in BTM and improvements in bone mass as reflected by lumbar spine aBMD and vBMD changes after 18 months of ALN10 or TPTD20 therapy. Early changes in the collagen bone formation markers (PINP, PICP) were assessed using the area under the curve (AUC) from the first 6 months of treatment. Spearman's correlation coefficient was calculated between 6-month AUC values and last observed aBMD and vBMD. In TPTD20-treated patients, early changes in PINP and PICP had similar significant positive correlations with endpoint lumbar spine aBMD and vBMD (Table). In contrast, early changes in PINP and PICP had significant negative correlations with lumbar spine aBMD and lesser negative and nonsignificant correlations with lumbar spine vBMD following ALN10 therapy. These results highlight the difference in mechanism of action between the two therapies, and indicate that PINP and PICP are good markers for the skeletal response to teriparatide.

Table: PINP and PICP correlation with LS aBMD and vBMD

BMD Parameter Biochemical Marker	Spearman Correlation ALN10	Spearman Correlation TPTD20
Change in Lumbar spine aBMD	(N = 89)	(N = 90)
PICP first 6-months AUC	−0.51*	0.53*
PICP first 6-months AUC	−0.27*	0.42*
Change in Lumbar spine vBMD	(N = 23)	(N = 26)
PINP first 6-months AUC	−0.20	0.51*
PICP first 6-months AUC	−0.19	0.55*

*$P < 0.01$

OP014

URINE OSTEOCALCIN – A NOVEL MARKER OF BONE METABOLISM AND PREDICTION OF FRACTURES

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Osteocalcin (OC) is a 6 kDa protein produced by osteoblasts. Although most of synthesized OC is adsorbed to bone hydroxyapatite, some OC leaks into blood stream where it can be detected. Part of OC found in blood is also thought to originate from bone resorption. Levels of circulating OC have been widely used in clinical investigations as a marker of bone turnover. In addition to circulation, OC can be found in urine. OC is excreted into urine by glomerular filtration as midmolecule fragments and urine OC (U-OC) fragments can be classified into two main categories according to their size.

We developed three immunoassays for the detection of various molecular forms of U-OC. Assays were either two-site (MidOC and LongOC) or competitive assays (TotalOC) and the specificity towards different OC fragments was determined with OC purified from pubertal urine. TotalOC assay recognized OC fragments from both U-OC categories whereas MidOC and LongOC were unable to detected the more truncated fragments in the second category. U-OC was studied in healthy premenopausal (n = 58) and postmenopausal women with or without hormone replacement therapy (HRT) (n = 13 and n = 20, respectively) and in postmenopausal women receiving alendronate (n = 76) or placebo (n = 76). Furthermore, the ability of U-OC assays to predict fractures was evaluated in 1040 randomly recruited 75-year-old women. All U-OC values were corrected for urine creatinine.

Total concentration of U-OC (TotalOC) was significantly higher in postmenopausal women compared to premenopausals (P < 0.01) and all three U-OC assays were able to distinguish between postmenopausal women with and without HRT (P < 0.05). All U-OCs were significantly reduced in response to treatment with alendronate. Interestingly, U-OC was also able to predict fractures, in particular fractures engaging trabecular bone. When women in the highest U-OC quartile were compared to all other women, odds ratios for prospective clinical vertebral fracture were significant for U-OCs (OR = 2.71

for MidOC and 2.75 for LongOC) while non-significant for four different serum OC assays (ORs 1.33–1.51). Odds ratios for U-OCs remained significant after correction for spine BMD. In conclusion, U-OC offers an alternative method for monitoring bone metabolism and may have potential applications in diagnostics related to disorders of bone metabolism. Furthermore, U-OC is a promising new marker for prediction of fractures.

OP015

FRACTURE PREDICTION USING MARKERS OF BONE TURNOVER IN THE GENERAL POPULATION: THE OPUS STUDY

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Markers of bone turnover may be predictive of fracture in selected study populations. The relative value of bone turnover assessment versus densitometry to predict fracture within the general population is less certain. We recruited a population-based sample of 2374 postmenopausal women (ages 55 to 79 years) from 5 European cities as part of the Osteoporosis & Ultrasound (OPUS) study. Baseline assessment included DXA at the lumbar spine and total hip, serum PTH, PINP, OC, betaCTX (Roche Elecsys), and urine NTX/Cr (Ortho Diagnostics). Blood samples were collected within a narrow time-interval at mid day. Urine samples were second morning void. Biochemical measurements were performed concurrently in a single laboratory. Reported incident fractures were assessed 2 to 3 years after baseline evaluation, and were verified by a physician. There were 112 incident fractures, including 53 low trauma fractures in 2099 women available to followup. Association of baseline measurements with incident fracture were expressed as standardised risk ratios (sRR) per one SD decrease (densitometry) or increase (biochemical measurements) with 95% confidence limits. All biochemical measurements were significantly less predictive of fracture than DXA (ROC analysis, $P < 0.05$). sRR for prediction of all fractures by DXA spine was 1. 3/SD (1.06–1.59) and 1. 49/SD (1.10–2.02) for low trauma fractures. sRR for PTH, PINP, OC, serum betaCTX and uNTX/Cr were 0. 93 (0.75–1.15), 1.04 (0.84–1.29), 0.93 (0.75–1.16), 1.12 (0.78–1.17) and 0.95 (0.91–1.38) respectively for all fractures and similar for low trauma fractures. Study power for all fractures was 80% for detection of a RR of 1.3/SD ($P < 0.05$) and 98% for detection of a RR of 1.5/SD or greater. These data do not provide evidence favouring a role for these biochemical measurements in the primary assessment of all-fracture risk in unselected individuals from the general population. The performance of serum ° etaCTX is affected by feeding, and the conclusions for this analyte are applicable only to these sampling conditions. Further information about incident vertebral and clinical fractures will be assessed at a follow-up interval of 5 years in this cohort.

OP016

CANNABINOID RECEPTOR ANTAGONISTS INHIBIT OSTEOCLASTIC BONE RESORPTION IN VITRO AND PREVENT OVARIECTOMY INDUCED BONE LOSS IN VIVO

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Previous studies have shown that the hormone Leptin influences appetite and body weight by modulating levels of endogenous cannabinoids (endocannabinoids) in the hypothalamus. Since Leptin is also known to regulate bone turnover and bone mass, we used a pharmacological approach to study the effects of the endocannabinoid pathway on bone cell function *in vitro* and bone metabolism *in vivo*.

The cannabinoid receptor type 1 (CB1) selective antagonists AM251 and SR141417A and the cannabinoid receptor type 2 (CB2) antagonist SR144528 were found to inhibit osteoclast formation and bone resorption in a concentration-dependent manner in murine osteoblast bone marrow co-cultures with half-maximal effects (IC50) at 4–8 μM ($P < 0.0001$). All three compounds inhibited bone resorption by 80–90% in rabbit osteoclasts at concentrations of 10 μM and above ($P < 0.0001$), by promoting osteoclast apoptosis as defined by morphological criteria, TUNEL staining and cleavage of caspase 3. By way of comparison, we found that 100 M of the bisphosphonate alendronate was required to exert similar effects on osteoclast inhibition in this assay. Studies on osteoblasts showed that AM251 and SR141417A inhibited growth with an IC50 of 10–25 μM whereas the CB2 selective antagonist SR144528 had no inhibitory effects at up to 50 μM. In keeping with this, evidence of CB1 and CB2 receptor expression was detected on both osteoclasts and osteoblasts by im-

munohistochemistry, western blotting and RT/PCR. Further studies showed that both AM251 and SR144528 were highly effective at preventing ovariectomy induced bone loss in mice *in vivo* when injected intraperitoneally at 6mg/kg/day.

Our studies demonstrated that cannabinoid CB1 and CB2 receptors are expressed in bone cells and that cannabinoid receptor antagonists inhibit bone resorption *in vitro* and ovariectomy induced bone loss *in vivo*. We conclude that cannabinoid receptor antagonists represent a new class of antiresorptive drug and our studies identify the endocannabinoid pathway as a therapeutic target for the prevention and treatment of bone diseases.

OP017

ROLE OF INOSITOL POLYPHOSPHATE 4-PHOSPHATASE TYPE II IN OSTEOCLAST CYTOSKELETAL ORGANIZATION AND ACTIVITY

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Osteoclasts are unique hematopoietic cells that derive from the monocyte/macrophage lineage and are responsible for bone tissue resorption. Loss of osteoclast activity leads to malignant osteopetrosis, a severe genetic disease characterized by an abnormal increase in bone mass and a severe reduction in bone marrow space formation. We are studying the grey-lethal (gl) gene that is responsible for the development of malignant recessive osteopetrosis in mouse and human. In the mouse osteopetrosis is associated with a defect in cytoskeleton reorganization and gl osteoclasts display underdevelopment of the ruffled border. To identify both putative target genes of gl and osteopetrosis-associated genes, we have undertaken a differential display study. By comparing gene expression in wild type and gl mice, we have identified several differentially expressed mRNA in homozygous gl/gl mice. One of them corresponds to the mouse homologue of the human and rat inositol polyphosphate 4-phosphatase B (Inpp4b) and is specifically down regulated in gl osteoclasts as demonstrated by expression analysis. We have cloned the mouse Inpp4b cDNA that encodes a 105 kD protein that dephosphorylates the phosphatidylinositol(3,4)biphosphate (PI(3,4)P2) and generates phosphatidylinositol(3)monophosphate (PI3P). To investigate whether Inpp4b activity is required during osteoclast differenciation and activation, an Inpp4b-GFP fusion protein was produced. Ectopic overexpression of Inpp4b-GFP fusion protein in RAW 264.7 monocyte/macrophage cell line reduced the differentiation potential of these cells in osteoclast in response to RANKL. In contrast overexpression of a phosphatase dead mutant of Inpp4b potentiated the response to RANKL. Interestingly, multinucleated osteoclast overexpressing the phosphatase dead mutant, displayed multiple smaller actin rings and were unable to resorb dentine. These results suggest that normal Inpp4b enzymatic activity is required in osteoclast both for normal response to RANKL and for cytoskeleton reorganization and resorption.

OP018

OSTEOCLAST ALPHAVBETA3 INTEGRIN-INDUCED ERK1/2 ACTIVATION IS INTRACELLULAR CA2+/C-SRC-DEPENDENT, SHC/RAS/RAF-1-INDEPENDENT, AND IS TRIGGERED BY ENGAGEMENT OF PKCALPHA

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The alphaVbeta3 integrin is central to osteoclast function, is engaged by adhesion to bone matrix proteins and activates cytoskeletal reorganization and intracellular signalling. MAPK phosphorylation is observed on osteoclast adhesion, but the pathway leading to their activation is still unknown. To clarify this aspect, mouse primary osteoclast-like cells were allowed to adhere to immobilized LM609 alphaVbeta3 monoclonal antibody, to select the molecular events exclusively triggered by this integrin. We observed translocation (i.e. activation) of PKCalpha, but not of other isoforms, to the cytoskeletal compartments. Activation was independent of PLCgamma and PI3-K. Immunoprecipitation assays revealed that PKCalpha was engaged in a complex with alphaVbeta3, which also recruited PYK2 and Grb2. AlphaVbeta3 ligation induced ERK1/2 activation as suggested by its phosphorylation in the cytosol, membrane and cytoskeletal compartments. In contrast, JNK and p38 MAPKs were unaffected. Association between ERK1/2 and alphaVbeta3 or PKCalpha was undetectable, suggesting indirect interaction among these pathways. However, canonical Shc/Ras/Raf-1 signal was not involved as none of these molecules were tyrosine/serine-phosphorylated. AlphaVbeta3-dependent ERK1/2 phosphorylation was blunted by osteoclast pre-treatment with the PKCalpha

inhibitor Go6976 or by long-term treatment with TPA. Likewise, ERK1/2 phosphorylation was not observed in osteoclasts pre-treated with the c-Src inhibitor PP2, suggesting a dual, possibly independent, pattern of ERK1/2 regulation, as indicated by undetectable molecular interaction between c-Src and PKCalpha. Pre-treatment of osteoclasts with the intracellular calcium chelator BAPTA inhibited PKCalpha activation, its recruitment by alphaVbeta3 and ERK1/2 activation, demonstrating dependence of these events on intracellular calcium. PKCalpha inhibition did not impair osteoclast adhesion nor survival, but significantly reduced migration and bone resorption. This was at variance with what observed upon c-Src inhibition, which induced osteoclast apoptosis in a manner dependent on an early transient ERK1/2 de-phosphorylation followed by a late sustained re-phosphorylation overwhelming basal levels. In conclusion, we showed that ERK1/2 activation is central to alphaVbeta3 signalling and that intracellular calcium, c-Src and PKCalpha are key regulators affecting downstream events associated to osteoclast survival, motility and bone resorption.

OP019

TRANSDIFFERENTIATION OF HUMAN DENDRITIC CELLS TOWARDS OSTEOCLASTS

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Dendritic cells (DC) and osteoclasts (OC) are highly specialized cells that share a common myeloid precursor. We demonstrate here that human monocyte-derived DC exhibit potential towards OC differentiation in response to macrophage colony stimulating factor (M-CSF) and receptor activator of NF-kB ligand (RANKL). Indeed, DC-derived multinucleated osteoclastic cells display characteristics of functionally differentiated osteoclasts such as a strong avb3 and cathepsin K expression, a classical belt of acting podosomes, but especially the capacity to resorb dentine. Surprisingly, comparative studies show that DC-derived osteoclast formation is more efficient than the well known monocyte-derived osteoclast formation. Moreover, this transdifferentiation process is highly enhanced by recombinant pro-inflammatory cytokines such as interleukine-1a (IL-1a) or tumour necrosis-a (TNF-a) or by pro-inflammatory over-expressed in synovial fluids from rheumatoïd arthritis patients. This study emphasizes a remarkable differentiation plasticity within myeloid cell system and suggests that DC may be directly involved in the formation of osteolytic lesions developed in human inflammatory bone diseases such as Rheumatoid Arthritis. Of particular interest is the Langerhans Cell Histiocytosis (LCH), an immunological disorder characterized by abnormal proliferation of cells with a dendritic Langerhans cell phenotype and chronic lytic bone lesions in 50% to 80% of all patients.

OP020

ABD56 A POTENT INHIBITOR OF BONE RESORPTION, INDUCES OSTEOCLAST APOPTOSIS VIA NFKB AND ERK INHIBITION IN VITRO

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We have previously shown that the butanediol ester of biphenylcarboxylic acid (ABD56) inhibits osteoclast (OC) formation and activity in vitro and in vivo. However, the mechanism of action of this compound is presently unknown. As the NFkappaB and ERK signal transduction pathways have been shown to be essential pathways for osteoclast differentiation and survival, we studied whether inhibition of these pathways mediates the anti-resorptive effects of ABD56.

Rabbit osteoclasts were isolated from the long bones of newborn rabbits. Osteoblasts (OB) were isolated from the calvaria of newborn mice by collagenase digestion. Apoptosis was identified on the basis of characteristics in nuclear morphology using DAPI and by staining for fragmented DNA using the TUNEL assay. Activation of caspases was measured using the APO-ONE caspase activity kit (Promega) in cultures of J774 cells. Expression of intact (p-30) and cleaved (p-19) caspase-3, phosphorylated ERK, phosphorylated IKKα/β and phosphorylated IKappaB were evaluated in cell lysates by western blotting.

Exposing rabbit OC cultures to 100 μM of ABD56 1 hour prior to stimulation with TNFα, completely abolished TNFα-induced IKKα and IKappaB phosphorylation. In OB cultures, however, ABD56 caused only partial inhibition of IKappaB phosphorylation. Similarly, ABD56 completely suppressed TNFα-induced ERK activation in rabbit OC-like cells, whereas only partial inhibition of ERK phosphorylation was detected in mouse OB. ABD56 induced apoptosis in OC but not OB cultures as evidenced by caspase-3 activation, nuclear morphology and TUNEL-labelling. ABD56 did not affect IL-1-induced RANKL release and failed to stimulate OPG expression in OB cultures.

In conclusion, ABD56 inhibits activation of the NFkappaB and ERK pathways. Furthermore, ABD56 causes osteoclast apoptosis, possibly induced by the observed NFkB and ERK inhibition, as these pathways are crucial for osteoclasts survival. Since ABD56 only inhibits cells from the macrophage/osteoclast lineage, but not the osteoblasts lineage, ABD56 is a promising novel anti-resorptive drug with possible use in disorders characterised by increased osteoclast activity such as osteoporis and Paget's disease of bone.

OP021

RAS SIGNALING REGULATES OSTEOBLAST DIFFERENTIATION AND EXTRACELLULAR MATRIX MINERALIZATION. IMPLICATIONS FOR THE BONE MANIFESTATIONS OF NEUROFIBROMATOSIS TYPE I

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Neurofibromatosis type I (NF1) is caused by a mutation in the *Nf1* gene. The *Nf1* gene product, neurofibromin, is a GTPase-activating protein stimulating the intrinsic GTPase activity of ras. A significant proportion of patients affected by NF1 display bone abnormalities such as short stature, scoliosis, bowing of the tibia, sphenoid dysplasia and pseudoarthrosis, but the pathophysiology of these bone manifestations is unknown. Northern blot and immunocytochemistry analyses revealed that neurofibromin is expressed in osteoblasts and prehypertrophic chondrocytes. In order to understand the role of *Nf1* in bone homeostasis, we generated an osteoblast-specific deletion of *Nf1*. Bone specific *Nf1*-deficient mice (Nf1CKO) display an increase in osteoid surface and volume compared to control littermates. This osteoidosis is not accompanied by low serum phosphate level, confirming the osteoblast autonomous origin of this phenotype. As opposed to what is observed in other cell types affected by *Nf1* loss of heterozygosity, osteoblast-specific *Nf1* deficiency promotes cell differentiation, as demonstrated by the increased expression of *Osteocalcin* and *type I collagen* genes in Nf1CKO mice. To further characterize the phenotypic characteristics of Nf1CKO osteoblasts, we used bone marrow and calvariae cell culture from wildtype and Nf1CKO mice and found that osteoblast progenitor proliferation was increased in Nf1CKO cultures, while alkaline phosphatase activity and the formation of mineralized nodules were reduced. The ras signaling pathway is activated by *Nf1* deficiency in osteoblasts, as indicated by the increased phosphorylation of ERK in Nf1CKO osteoblasts and bone lysates. To test the hypothesis that the bone phenotypic manifestations of NF1 are due to the hyperactivation of ras signaling, we treated Nf1CKO osteoblasts and mice with nitrogen-containing biphosphonates in order to reduce the prenylation of ras, its membrane localization and therefore its activity. Nf1CKO mice are treated as well with alendronate to assess the possible rescue of their osteoidosis. Based on these preliminary results, we hypothesize that the ras signaling pathway may play an important role in the control of bone mineralization by osteoblasts.

OP022

FGFR2 DOWNREGULATION RESULTS FROM C-CBL-DEPENDENT FGFR2 PROTEASOME DEGRADATION AND CONTRIBUTES TO INCREASED OSTEOBLAST DIFFERENTIATION IN APERT SYNDROME

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Mutations in FGFR2 in Apert syndrome result in premature cranial ossification. We previously demonstrated that constitutive activation of FGFR2 induced by the activating Apert S252W FGFR2 mutation results in increased expression of alkaline phosphatase (ALP) and type I collagen (COLIA1) in human osteoblasts. Because activation of tyrosine kinase receptors is often associated with their ubiquitination, we examined FGFR-2 degradation in FGFR2 mutant osteoblasts. Western blot analysis in immortalised human Apert (Ap) calvaria osteoblastic cells bearing the activating S252 W FGFR2 mutation showed decreased expression of FGFR2 at the protein but not at the mRNA level compared to normal age-matched control (Co) calvaria cells. Treatment of Ap cells with lactacystin (10 μM, 48 hrs), a specific proteasome inhibitor, restored FGFR2 expression and ALP activity in Ap cells, indicating that the increased ALP activity induced by the mutation results in part from increased FGFR2 ubiquitination and proteasome degradation. Because c-Cbl acts as an ubiquitin ligase and is involved in receptor tyrosine kinase downregulation, we examined the role of c-Cbl in FGFR2 ubiquitination in Ap osteoblasts. Immunoprecipitation studies showed that FGFR2 is associated with c-Cbl in Ap cells. Moreover, FGFR2 immunoprecipitated with ubiquitin, indicating that Cbl interacts with FGFR2 to promote its ubiquitination. To determine the role of the RING and phosphotyrosine binding (PTB) domains in FGFR2 ubiquitination by c-Cbl, Ap cells were transfected with c-Cbl in which the RING finger is disrupted (70Z-Cbl), or with c-Cbl with a point mutation (G306E) that inactivates the PTB domain (vectors kindly provided by Dr. R. Baron). Transfection of Ap cells with 70Z-Cbl,

and to a lesser extent G306E inhibited FGFR2 degradation, as shown by increased FGFR2 levels. Moreover, the two vectors reduced ALP overexpression induced by the FGFR2 mutation in Ap cells. These data show that 1) constitutive activation of FGFR2 leads to downregulation of FGFR2 protein levels due to c-Cbl-mediated ubiquitination of FGFR2, 2) the ubiquitination of FGFR2 in Ap cells requires the RING finger domain and possibly the PTB domain of c-Cbl and 3) c-Cbl-dependent ubiquitination of FGFR2 mediates in part the increased osteoblast differentiation induced by FGFR2 activation in osteoblasts in Apert syndrome.

OP023

SEQUESTOSOME 1 GENE MUTATIONS LEAD TO DISREGULATED NF-KAPPAB SIGNALING IN PAGET'S DISEASE OF BONE

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Paget's disease of bone (PDB) is a common disorder characterized by focally increased and disorganized bone turnover. Mutations of the Sequestosome 1 gene (SQSTM1), which codes for the scaffolding protein SQSTM1/p62 involved in NFkappaB signaling, have been found to account for up to 50% of familial PDB cases. To date, 8 mutations (3 truncating: 390X, 394X, E396X and 5 missense: P387L, P392L, M404 V, G411S, G425R; data on the latter three submitted), all affecting the SQSTM1 ubiquitin-binding domain, have been identified in familial Paget's disease of bone.

We sought to determine whether the SQSTM1 mutations increase NFkappaB signaling *in vitro* in Paget's disease of bone.

Cultures of EBV-immortalised B-cells from patients carrying the P392L and 390X mutations, and normal controls were stimulated with human recombinant TNF-alpha for 0, 10, 15, 30, 60 and 90 minutes, and phosphorylation and degradation of the NFkappaB inhibitor IkappaB, was assessed by Western Blotting using P-IkappaB and IkappaB antibodies. NFkappaB activation was studied in HEK293 cells transfected with an NFkappaB-Luciferase reporter construct in combination with SQSTM1 mutation encoding p62 expression constructs.

At baseline P-IkappaB levels were upregulated by 50% in cells carrying SQSTM1 mutations, whereas IkappaB levels were not significantly different from those in wild type. Stimulation of cells with TNF-alfa resulted in a transient up-regulation of P-IkappaB levels in wild type returning to baseline within 15 min, whereas in the mutant cells P-IkappaB levels remained elevated for at least 90 min. Likewise, the luciferase activity was 50% higher at baseline in mutant HEK293 cells, however this was restricted to the truncating mutation-carrying cells only. TNF stimulation resulted in a tenfold increase in luciferase activity in both mutant and wild type p62 expressing cells.

In conclusion, SQSTM1 mutations lead to accumulation of P-IkappaB, suggesting impaired ubiquitination-dependent degradation of this protein. In addition, truncating mutations of SQSTM1 lead to constitutive activation of NFkappaB. This could account for the increased numbers of osteoclasts observed in PDB, as NFkappaB plays an essential role in osteoclastogenesis.

OP024

TWIST HAPLOINSUFFICIENCY IN THE SAETHRE-CHOTZEN SYNDROME REGULATES OSTEOBLAST GENE EXPRESSION INDEPENDENTLY OF FGFR2 SIGNALING

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Mutations of the Twist gene, a bHLH transcription factor, induce premature fusion of cranial sutures (craniosynostosis) in the Saethre-Chotzen syndrome (SCS). We previously showed that Twist haploinsufficiency in SCS increases alkaline phosphatase (ALP) and type I collagen (COLIA1) expression in human calvaria osteoblasts. In Drosophila, Twist was suggested to act upstream of Fibroblast Growth Factor receptor (FGFR), which play essential roles in osteoblast differentiation. We therefore tested the hypothesis that FGFR2 signaling is involved in the premature osteoblast differentiation induced by Twist haploinsufficiency. To test this hypothesis, coronal sutures were obtained from 3 SCS patients with Twist mutations (Y103X, Q109X, R118C), all inducing Twist haploinsufficiency. Immunohistochemical analysis of coronal sutures in these patients showed increased expression of FGFR2 in osteoblasts and pre-osteoblasts compared to age-matched normal control sutures. This effect was specific for FGFR2 as FGFR1 and FGFR3 expression was not reduced. Western blot analysis showed increased FGFR2 levels and receptor tyrosine kinase activity in Twist mutant (M-Tw) calvarial osteoblasts bearing the Y103X mutation compared to normal (Nl) age-matched calvarial cells cultured in serum-free medium. We therefore determined whether FGFR2 up-regulation may play a role in osteoblast differentiation in Twist mutant osteoblasts. Transient transfection with a dominant negative (DN)-FGFR2 vector increased ALP and COLIA1

expression in NI cells. However, DN-FGFR-2 did not correct the abnormal expression of ALP and COLIA1 in M-Tw cells, indicating that the increased FGFR2 expression and FGFR2 signaling in M-Tw cells is not involved in the premature osteoblast differentiation induced by Twist haploinsufficiency. We conclude that 1) Twist haploinsufficiency in osteoblasts increases FGFR2 expression and signaling and 2) the altered osteoblast differentiation induced by Twist haploinsufficiency does not result from alterations in FGFR2 signaling. This points to a direct rather than indirect role of Twist in the premature osteoblast differentiation in the Saethre-Chotzen syndrome.

OP025

ALTERATIONS IN TRANSCRIPTIONAL ACTIVITY OF THE 24-HYDROXYLASE GENE DUE TO A NOVEL PROMOTER POLYMORPHISM

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24-hydroxylase (24OHase) is involved in controlling local and circulating levels of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) by inactivating the active hormone to 1,24,25(OH)3D3. Additionally, this enzyme also generates 24,25(OH)2D3 from 25(OH)D3. This metabolite locally plays an important role and signals via a specific membrane receptor.

A region within the promoter of the gene 24OHase containing two A-stretches was analyzed by fragment analysis and sequencing using an ABI 310 sequencer. PCR products from genomic DNA were subcloned into the pGL3 basic plasmid. Plasmids were transiently transfected into osteoblasts and chondrocytes by electroporation and promoter activity was measured in triplicate and expressed as luciferase units per μg protein.

24OHase promoter sequences were amplified from 72 human DNA samples. Performing fragment analysis 27 PCR products displayed variations in peak patterns indicative for polymorphisms of the respective genomic DNA. Variations in the length of the oligo-A stretches in the 24OHase promoter were found in 1/3 of the samples and a 5 bp insertion was detected in 4 cases. Promoter constructs containing up to 580 nt's downstream of the transcriptional start site representing the published promoter of the 24OHase gene as well as constructs with the detected polymorphisms were transfected into human osteoblasts (hFOB-cells) and chondrocytes (T/C 28-1cells). Marked differences in promoter activity were observed reproducibly in both cell lines (n = 7 independent experiments). Addition of 1 or 2 A-residues within the A-stretches enhanced promoter activity 2.5 fold and 3 fold, respectively, compared to the wild type promoter. The plasmid containing an insertion within the A-stretch displayed a 1.5 fold reduced promoter activity. These altered transcriptional activities were obtained at basal conditions (no treatment of cells) and were maintained under conditions of 4–6 fold promoter activation due to treatment with 10 nM 1,25(OH)2D3.

In summary, several new polymorphisms were detected in the promoter of the human 24OHase gene. These sequences markedly influenced promoter activty *in vitro*. Therefore, the detected novel polymorphisms within the promoter might correspond to altered enzyme activity of 24OHase *in vivo* within the human population. We conclude that altered transcriptional activities of the alleles of the 24OHase gene *in vivo* might influence vitamin D metabolism as well as local signaling of 24,25(OH)2D3.

OP026

HEAD-TO-HEAD COMPARISON OF RISEDRONATE AND ALENDRONATE

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Previous clinical studies suggest there are differences in bisphosphonate pharmacokinetics. However, these were separate studies with different designs and dose levels. The purpose of the current study was to compare the pharmacokinetics of risedronate and alendronate following single intravenous doses (at clinical dose levels).

This is a 52-week, open label, randomized study in postmenopausal women with osteopenia or osteoporosis (ages 43–73 yr). Subjects were administered single intravenous doses of ^{14}C-risedronate sodium (230 ug; 2.9 uCi) or ^{14}C-alendronic acid (450 ug; 3.2 uCi), each approximately equivalent to an oral weekly dose, followed by weekly oral doses of unlabeled risedronate (35 mg) or alendronate (70 mg), respectively. Concentrations of ^{14}C-risedronate and ^{14}C-alendronate in serum and urine are measured (over the 52-week study period) by liquid scintillation counting as long as the samples contain adequate radioactivity, and thereafter by accelerator mass spectrometry (AMS). Use of AMS allows measurement of these compounds at concentrations of 10 pg/mL and

lower. The study in-life is on-going; presented here are urinary excretion results from the first 27-days (the primary end-point).

The cumulative urinary excretion of risedronate (66%) was significantly higher than that of alendronate (55%) over the first 27 days. This resulted in one-third more of drug retained on bone for alendronate compared with risedronate (34% risedronate; 45% alendronate).

The absolute amount of drug retained from the initial dose at 27 days was 78 ug risedronate sodium and 202 ug alendronic acid. This difference (2.5× at 27 days) is estimated to increase disproportionately with repeated dosing (to > 2.8× at 6-mo). This suggests a smaller skeletal burden is associated with long-term risedronate use, and indicates the potential for improved control of therapy for risedronate compared to alendronate.

Table: Percent of Dose Excreted in Urine (Median)

Interval	Risedronate (n = 15)	Alendronate (n = 17)	P-value*
0–24 hours	49.0	44.5	0.0966
0–72 hours	54.5	48.2	0.0235
0–336 hours	63.5	52.6	0.0025
0–648 hours	66.2	55.1	0.0020

*Wilcoxon Rank Sum Test

OP027

SUSTAINED EFFECT OF RISEDRONATE ON TRABECULAR ARCHITECTURE AND MINERALIZATION OVER 5 YEARS OF TREATMENT: TRIPLE BIOPSY STUDIES BY MICRO-CT

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We reported in previous studies that 3 years of risedronate treatment reduced bone turnover, leading to the preservation of trabecular architecture (B. Borah et al., JBMR, 2001) and increased mineralization (E. Ritman et al., CTI, 2003) in comparison to baseline and placebo. These changes may have contributed to the decrease in fracture risk observed at 3 years. This study compares the long term effect of risedronate on trabecular architecture and mineralization in a limited group of osteoporotic patients (n = 7), using iliac crest bone biopsy specimens taken sequentially at 0, 3 and 5 years from the same patient. The trabecular bone volume (BV/TV), and 3-D architectural parameters (connectivity density, trabecular number, thickness, separation, structure model index and marrow star volume) were measured by conventional bench-top 3-D Micro-CT at 30 micron resolution. These measurements at 5 years were not significantly different from those at 0 and 3 years, indicating a sustained preservation of trabecular architecture up to 5 years. High-resolution (4 micron) Synchrotron data provided quantitative delineation of the low-mineralized trabecular bone (mean density 0.865 g/cc) from the high-mineralized trabecular bone (mean density 1.041 g/cc). The ratio of low mineralized bone to high mineralized bone (LMB/HMB) decreased significantly after 3 years of risedronate treatment ($P < 0.05$) and is consistent with a reduction of bone turnover. After 5 years of continuous treatment, LMB/HMB was significantly lower in comparison to baseline ($P < 0.05$), but maintained at the 3-year level, indicating that there was no progressive increase in secondary mineralization of trabecular bone in the last 2 years of treatment. The persistence of low mineralized bone even after 5 years of treatment implies that bone remodeling continues under risedronate treatment albeit at a reduced rate. In conclusion, these unique data from sequential biopsy samples show that risedronate provides long-term beneficial effect on bone quality factors such as trabecular architecture and mineralization.

OP028

TERIPARATIDE (RHPTH [1–34]) TREATMENT IMPROVES THE STRUCTURE OF THE PROXIMAL FEMUR IN WOMEN WITH OSTEOPOROSIS: RESULTS FROM THE FRACTURE PREVENTION TRIAL

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Postmenopausal women with osteoporosis enrolled in the Fracture Prevention Trial who received daily injections of teriparatide (rhPTH [1–34]) showed significantly lower rates of vertebral and non-vertebral fragility fractures compared to placebo controls. These observations suggest that teriparatide improves bone mechanical strength. A subset of postmenopausal women enrolled in the Fracture Prevention Trial (n = 558; mean age 70 ± 7 years) were randomized to a once-daily self-

administered subcutaneous injection of placebo (n = 189), teriparatide 20 µg (TPTD20; n = 86) or teriparatide 40 µg (TPTD40; n = 183) for a median of 20 months. Dual energy x-ray absorptiometry (DXA) hip scans were analyzed with the Hip Structure Analysis (HSA) program to derive structural geometry. This HSA program measures BMD and the geometric properties of cortical bone within narrow regions across the femoral neck, intertrochanter and femoral shaft from images acquired by DXA. Linear models were used to assess the effects of teriparatide treatment on bone structure using baseline values as covariates. At the femoral neck, teriparatide increased bone mass and improved bone strength in a dose-related manner, compared to placebo. The mean increase (95% CI) in bone mineral density was 4.2% (2.4% to 6.0%) in the TPTD20 group and 7.2% (5.4% to 9.1%) in the TPTD40 group. The mean differences in axial strength (bone cross-sectional area) were 3.5% (1.8% to 5.3%) and 6.3% (4.5% to 8.2%), and in bending strength (section modulus) 3.6% (1.4% to 5.8%) and 6.8% (4.6% to 9.1%) in the TPTD20 and TPTD40 groups respectively. The mean increase in cortical thickness was 4.5% (2.6% to 6.4%) and 7.7% (5.7% to 9.7%), while buckling ratio (local cortical instability) decreased by 5.5% (3.5% to 7.5%) and 8.6% (6.6% to 10.5%) in the TPTD20 and TPTD40 groups respectively. Changes at the intertrochanteric region were comparable to those at the narrow neck; however, teriparatide treatment effects at the purely cortical femoral shaft were not significantly different from placebo. In conclusion, compared to placebo, teriparatide treatment increased bone mass, improved axial bending strength, increased cortical thickness and stability, and reduced the buckling ratio at the intertrochanteric region and the femoral neck.

OP029

STRONTIUM RANELATE REDUCES THE RISK OF VERTEBRAL AND NON-VERTEBRAL FRACTURES IN CAUCASIAN WOMEN WITH POST-MENOPAUSAL OSTEOPOROSIS

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Strontium ranelate, a new orally active anti-osteoporotic agent, has been reported to have a dual action on bone metabolism, simultaneously increasing bone formation and decreasing bone resorption. A large phase 3 program including 2 multinational, double blind, placebo controlled studies has been performed. In both studies, patients were randomly assigned to receive strontium ranelate 2 g/day or placebo for 3 years associated to calcium and vitaminD supplementation according to the patient's status.

The SOTI study (Spinal Osteoporosis Therapeutic Intervention) focused on vertebral anti-fracture efficacy of strontium ranelate, in 1649 post menopausal women with osteoporosis [age: 69.7(7.3) years; Lumbar Bone Mineral Density (BMD Tscore: −3.6(1.3); mean(SD)], 87.5% having at least one prevalent vertebral fracture (2.2 per patient), has demonstrated a rapid and sustained vertebral anti-fracture efficacy of strontium ranelate in the intent-to-treat population, with a relative risk reduction of vertebral fracture of 49% ($P < 0.001$) the first year of treatment and 41% ($P < 0.001$) over 3 years. At 3 years lumbar BMD in strontium ranelate group increased by 14.4% as compared to placebo ($P < 0.001$) and femoral neck BMD by 8.3% ($P < 0.001$).

The TROPOS study (Treatment Of Peripheral OSteoporosis) investigated the efficacy of strontium ranelate on non-vertebral fractures in 5091 women with post-menopausal osteoporosis [age: 76.8(5) years, Femoral Neck BMD T-score −3.1(0.6), 36.8% had at least one prevalent non-vertebral fracture]. In the intent-to-treat population, the risk for experiencing a new non-vertebral fracture during

the 3 years was reduced by 16% (RR = 0.84 95% CI [0.702; 0.995], $P = 0.04$, adjusted cox model on pre-defined influential covariates age, BMI, FN BMD and country). 41% reduction of the hip fracture risk (RR = 0.59; 95% CI [0.37; 0.95], $P = 0.03$) was also achieved in patients having taken strontium ranelate for at least 18 months.

At 3 years femoral neck BMD in strontium ranelate group increased by 8.2% as compared to placebo ($P < 0.001$) and lumbar BMD by 14.7% ($P < 0.001$).

In both studies strontium ranelate had a good bone and general safety profile.

The present data support the efficacy of strontium ranelate in reducing the risk of vertebral and non vertebral fracture in post-menopausal women with osteoporosis, representing a new candidate in the treatment of postmenopausal osteoporosis.

OP030

EFFECTS OF 10 YEARS OF ESTRADIOL ON FRACTURE RISK AND OTHER OUTCOMES IN EARLY POSTMENOPAUSAL WOMEN - THE DANISH OSTEOPOROSIS PREVENTION STUDY

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Aim: To study the fracture reducing potential of estradiol used alone or combined as primary prevention in early postmenopausal women.

Design: Comprehensive cohort study with one study arm randomised to hormonal replacement therapy (HRT, n = 502) or no HRT (n = 504) and one study arm where the participants were allowed to choose HRT (n = 221) or not (n = 789) by their own free will.

Material and methods: A total of 2,016 randomly selected early postmenopausal (from 3 to 24 month past last menstrual bleeding) women with a median age of 50 years (range 45–58) were included and followed for 10 years. The first line HRT contained estradiol with or without norethisterone. Fractures were the primary end-point, and BMD of the spine, hip and forearm the secondary end-point. BMD was measured using Hologic 2000 DXA scanners at baseline, after 6 months, 1, 2, 3, 5, and 10 years.

Results: The women were followed for 18,653 person years. The risk of any fracture was borderline significantly reduced with HRT (OR = 0.78, 95% CI: 0.60–1.03) mainly due to a decline in forearm fractures (OR = 0.60, 95% CI: 0.39–0.93). The number of spine fractures was not reduced (OR = 0.95, 95% CI: 0.66–1.39). The BMD decreased in untreated women at all measurement sites and was significantly below that of HRT treated. Among the treated, there was an increase in the spine, a relatively stable level in the hip and a small decrease in the forearm. The number of breast cancers (OR = 1.64, 95% CI: 1.03–2.60) and events of cholecystolithiasis (OR = 1.70, 95% CI: 1.01–2.85) were increased among HRT treated. There was a trend towards more cardiovascular events (OR = 1.35 vs. 0.66) and breast cancer events (OR = 2.95 vs. 1.07) in those on HRT by own choice compared to those on no HRT by own choice. This trend was not found in the randomised group.

Conclusion: HRT containing estradiol has only a modest fracture reducing potential in a low risk group of early postmenopausal women. There was a significant increase in adverse events.

OP031

GENE THERAPY WITH GROWTH HORMONE ALONE NORMALIZES BONE MASS AND BONE STRUCTURE IN HYPOPHYSECTOMIZED MICE

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Patients with growth hormone (GH) deficiency exhibit decreased bone mass and increased risk of fractures and these effects can be prevented by growth hormone treatment. However, these patients are usually substituted by multiple hormones and therefore the role of GH in increasing bone mass is not clear. Thus, we investigated the effects of GH treatment on bone mass and structure in

a hypophysectomized mouse model. Male mice (Bom:NMRI mice, M & B, Ry, Denmark) were hypophysectomized at 5 weeks of age and underwent non-viral gene therapy with a human GH expressing plasmid (via hydrodynamic gene transfer) at 8 weeks of age. Previous study demonstrated a maintained high serum levels of human GH during the 68 days duration of the study (Søndergaard et al., 2003, Am J Physiol Endocrinol Metab 285: E427–E432). After sacrifice, the mice were subjected to DEXA and pQCT measurements (Lunar PIXImus2 and XCT 2000, Stratec, respectively). In addition, changes in serum levels of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP-3) showed a positive correlation with bone mass and bone structure measurements. Our results demonstrate that GH exerts anabolic effects on bone that are sufficient to restore bone mass and bone structure of hypophysectomized mice in presence of thyroid hormone, glucocorticoids and sex-hormone deficiencies. These data may encourage re-evaluation of the role of GH in the treatment of age-related and osteoporosis-related bone loss. Results are represented as mean ± SEM, *) Sig. diff. from "Intact, untreated", $P < 0.05$, #) Sig. diff. from "Hypophysectomized untreated", $P < 0.05$

Table:

Treatment Parameter	Intact untreated control (n = 7)	Hypophy sectomized untreated (n = 7)	Hypophysect. + hGH-plasmid (n = 13)
Total BMC (mg/mm) (pQCT)	2.174 ± 0.088	1.363 ± 0.064*	1.847 ± 0.073*#
Cortical BMC (mg/mm) (pQCT)	1.94 ± 0.09	1.270 ± 0.066*	1.626 ± 0.055*#
Cortical BM area (mm2) (pQCT)	2.50 ± 0.10	1.547 ± 0.096*	2.22 ± 0.13#
Cancellous BMC (mg/mm) (pQCT)	0.2329 ± 0.0092	0.0929 ± 0.0064*	0.224 ± 0.030#
Cancellous BM area (mm2) (pQCT)	1.214 ± 0.059	0.600 ± 0.037*	0.925 ± 0.082*#
Cortical thickness (mm) (pQCT)	0.467 ± 0.016	0.389 ± 0.022*	0.459 ± 0.019#
Periosteal circumf (mm) (pQCT)	6.83 ± 0.11	5.196 ± 0.097*	6.26 ± 0.18*#
Spine BMD (mg/cm2) (mg/cm2) (DEXA)	0.0705 ± 0.0024	0.0629 ± 0.0018*	0.0787 ± 0.0022*#

OP032

DLK1/PREF-1 (EGF–LIKE HOMEOTIC PROTEIN) INHIBITS GENE EXPRESSION OF INSULIN–LIKE GROWTH FACTOR (IGF)-BINDING PROTEINS DURING OSTEOBLAST DIFFERENTIATION OF HUMAN BONE MARROW STROMAL CELLS

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Insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) are essential regulators for osteoblast proliferation and differentiation. We have recently demonstrated that dlk1/Pref-1, (a member of Delta-Serrate-Notch family) inhibits human mesenchymal stem cell (hMSC) differentiation into mature osteoblasts (Abdallah B., et al., JBMR (2003), in press). However, the molecular mechanisms of these inhibitory effects are not known. Thus, we studied the effects of Dlk1/Pref-1 on IGF system during osteoblast differentiation. We employed a telomerase-immortalized hMSC line (hMSC-TERT) (Simonsen JL., et al., Nat Biotechnol. (2002), 20:592-6.) and a cell line derived from it overexpressing Dlk1/Pref-1 (hMSC-Dlk1). Real time RT-PCR analysis was used to compare the expression pattern of IGFBPs and Western ligand blotting to determine the levels of IGFBPs in the conditioned media. hMSC-TERT expressed high levels of IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5 and IGFBP-6. Dlk1/Pref-1 overexpression exhibited a lowered steady state levels of these

binding proteins: 5.1, 6.7, 2.3, 22.0 and 1.1 folds respectively compared to hMSC-TERT. Similarly, hMSC-Dlk1 cells secreted lower level of IGFBP-2, −3, and −4 (= 3 folds less) than the control hMSC-TERT cells. Treatment the cells with calcitriol (10 nM) upregulated the osteoblastic differentiation markers of hMSC-TERT as well as increased the production of IGFBP-3 (3.6 folds), IG-FBP-4 (2.5 folds), IGFBP-5 (3.0 folds) and IGFBP-6 (4.5 folds) at mRNA level in time and dose dependent manner. In contrast, Dlk1/Pref-1 overexpressing cells showed an impairment of osteoblast differentiation in response to calcitriol with inhibition of gene expression of Alkaline phosphatase (98%), collagen type I (87%) and Osteocalcien (84%). These effects were associated with downregulation of IGFBP-3, IGFBP-4, IGFBP-5 and IGFBP-6 gene expression by 88%, 84%, 92%, and 65.5% respectively. Our results suggest that the inhibitory effect of Dlk1/Pref-1 on osteoblast differentiation of hMSC is partly mediated by the regulation of IGFBPs production. These changes may alter the availability and the biological functions of the IGFs.

OP033

GHRELIN IS EXPRESSED IN HUMAN BONE CELLS AND ENHANCES THE PROLIFERATION OF OSTEOBLASTS

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Ghrelin is the natural ligand for the growth hormone secretagogue receptor (GHS-R1a). However, recent studies have uncovered the pleiotropic nature of this peptide hormone, whose functions include regulation of energy metabolism through a number of mechanisms and the inhibition of apoptosis. Furthermore, ghrelin, but not GHS-R1a, appears to be expressed widely among tissues but its presence in bone cells has not been reported.

Therefore, we have measured mRNA levels of ghrelin and the two GHS-R isoforms (1a and 1b) in biopsy samples from whole bone, using real-time PCR. Both ghrelin and the GHS-R1b were expressed in bone biopsies but not the cognate receptor for ghrelin, GHS-R1a. This prompted us to study gene expression in cultures of human fetal osteoblasts (SV-HFO). They were cultured in 6-well plates and from day 2 their medium was supplemented with 10mM β-glycerophosphate with or without 1 μM dexamethasone (dex). The cells were cultured for a further 21 days during which total RNA was isolated at various timepoints. Ghrelin and GHS-R1b, but not GHS-R1a, mRNAs were detectable in SV-HFO cells throughout the differentiation period. Ghrelin was not significantly regulated during the culture period. On the other hand, GHS-R1b mRNA was expressed at consistently low levels during the first 19 days of culture, but rose during the last four days. This late increase in GHS-R1b expression was most marked in dex treated cells, reaching 670% ($P = 0.006$) and 800% ($P < 0.002$) of day 7 levels at days 21 and 23, respectively. To establish whether ghrelin can modulate osteoblast cell growth, we treated SV-HFO cells at culture day 5 (±dex) with ghrelin concentrations of 1–100 nM for 24 hours and then measured thymidine uptake. The effect of ghrelin on cell proliferation was biphasic, inducing thymidine up to 25 nM (250% and 190% of controls; + dex and −dex, respectively) but not at 100nM. Interestingly, unacylated ghrelin (UAG) had the greatest effect, causing an ~280% increase in non-dex (but not in dex) treated cells. The combination of UAG and ghrelin had greatest effect causing ~310% and 200% increases in thymidine uptake in dex as well as non-dex treated cells, respectively.

Overall, our data suggest the presence of ghrelin and a possible role for ghrelin in modulating bone cell growth and differentiation. However, the mechanism by which this is manifested remains unclear, since GHS-R1a, ghrelin's cognate receptor, does not appear to be expressed by these cells.

OP034

NOVEL INSIGHTS INTO THE MECHANISM OF ACTION OF VITAMIN D METABOLITES IN BONE: EVIDENCE FOR 1,25-DIHYDROXYVITAMIN D3 SYNTHESIS BY OSTEOBLASTS AND DIRECT STIMULATION OF MINERALIZATION

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Vitamin D plays a major role in the regulation of mineral homeostasis and affects bone metabolism. However, so far it is unclear whether vitamin D directly stimulates bone mineralization and detailed knowledge on the vitamin D endocrine system in human osteoblasts is actually lacking. In this study we investigated the direct effects of $1\alpha,25-(OH)_2D_3$ on osteoblast differentiation and mineralization. Also, we studied the impact of 24-hydroxylation, generally considered as first step in the degradation pathway of vitamin D, as well as the role of the nuclear and presumed membrane vitamin D receptor (VDR). Finally, we investigated whether human osteoblasts are able to synthesise $1\alpha,25$-

$(OH)_2D_3$. For this we used a human osteoblast cell line (SV-HFO), that has the potency to differentiate during culture, forming a mineralized extracellular matrix in a 3-week period.

Treatment with both $1\alpha,25-(OH)_2D_3$ and the 24-hydroxylated metabolites $24,25-(OH)_2D_3$ and $1\alpha,24,25-(OH)_3D_3$ dose-dependently increased osteocalcin (OC) production (protein and RNA), and alkaline phosphatase (ALP) activity. All three compounds directly enhanced mineralization. $1\alpha,24,25-(OH)_3D_3$ stimulated ALP activity and OC production most potently, while for mineralization it was equipotent to $1\alpha,25-(OH)_2D_3$. The nuclear VDR antagonist ZK159222 almost completely blocked the effects of all metabolites. To test the involvement of a membrane VDR, we used $1\beta,25-(OH)_2D_3$, an inhibitor of membrane effects of $1\alpha,25-(OH)_2D_3$ in the intestine. $1\beta,25-(OH)_2D_3$ did not block the effects of the vitamin D metabolites but, remarkably, it increased ALP activity and mineralization by itself. Finally, we demonstrated that human osteoblasts have 1α-hydroxylase activity as they synthesised in 48 hours about 600 nM $1\alpha,25-(OH)_2D_3$ out of 1 μM of 25-(OH)D$_3$ which was accompanied by increased mineralization.

In conclusion, not only $1\alpha,25-(OH)_2D_3$, but also the presumed 24-hydroxylated 'degradation' products stimulated differentiation of human osteoblasts and we show that they directly enhance mineralization in which the nuclear VDR plays a central role. The intestinal antagonist $1\beta,25-(OH)_2D_3$ acts in bone as an agonist and directly stimulates mineralization. Finally, bone cells contain 1α-hydroxylase activity and produce active $1\alpha,25-(OH)_2D_3$, which points towards an active vitamin D metabolism in bone cells that may play a role in the observed strong effects on differentiation and mineralization of different vitamin D metabolites.

OP035

AN UPSTREAM REGION OF MOUSE OSTEOPROTEGERIN BINDS ESTROGEN RECEPTOR AND CONTRIBUTES TO ESTROGEN RESPONSIVENESS

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The mechanisms of estrogen regulation of adult skeletal metabolism are thought to involve the modulation of paracrine factors produced by osteoblastic cells that act on osteoclastic lineage cells, especially, the RANK/RANKL/OPG system. Pertubations of this pathway result in pathophysiological processes, such as osteoporosis, osteolytic metastasis and tumor associated hypercalcaemia. Estrogen modulates gene transcription via binding of the estrogen receptor (ER) to an estrogen responsive element (ERE) within the promoter of a target gene. This classical pathway of estrogen action via direct binding to an ERE has not been reported for OPG up to now.

2031 bp of the OPG promoter were isolated by genomic walking. This fragment as well as the fragments, which were 5′deleted to sequentially reduce the number of EREs, were cloned upstream to a luciferase reporter gene and transfected into osteoblastic and non-osteoblastic cells. The putative binding sites for the ERalpha to the OPG promoter were tested with DNA immuno-precipitation and electrophoretic mobility shift assay (EMSA).

Computer analysis of the isolated 5′flanking region, which overlapped with the exon 1 mRNA, revealed four putative estrogen EREs. Transient transfections of the promoter constructs into ST2 and ROS17/2.8 cells and treatment with estrogen upregulated the transcription of all OPG promoter constructs, whereby in U2OS and MCF-7 cells estrogen resulted in a downregulation of the reporter activity. Coactivators and corepressors are described to regulate the actions of steroid hormone receptors and may cause the different regulation pattern, although the level of two coactivators investigated, GRIP1 and AIB1, were found to be the same in all cell lines. DNA immunoprecipitation revealed a binding for the ERalpha to the OPG promoter within the proximal 385 bp but not for the 1406 bp further upstream, although each fragment contained two putative EREs. By means of EMSA the position from −158 to −133 was verified to be a direct binding site for the ERalpha. Analysis of this ERE site showed that the two half sites for the ER binding are homologue to that described for a functional ERE in the rabbit uteroglobin promoter. In summary, we identified an ERE binding site for the ERalpha in the OPG promoter which contributes to the regulation of OPG through estrogen.

OP036

LOCI FOR REGULATION OF BMD IN MEN AND WOMEN THE FAMOS STUDY

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Osteoporosis is a major public health problem characterised by reduced bone mass and an increased risk of fragility fractures. Bone mineral density (BMD) is under strong genetic control and is the most important determinant of osteoporotic fracture risk, but the genes responsible for BMD regulation are incompletely defined. In this study we sought to identify quantitative trait loci for regulation of BMD in men and women by conducting a genome-wide linkage scan in 3658 individuals from 730 families who were selected on the basis that probands had low bone mass, defined by a BMD Z-score at the lumbar spine (LS-BMD) or Femoral Neck (FN-BMD) of −2.0 or below. Multipoint linkage analysis was conducted separately for men and women on the autosomes using variance components methodology (SOLAR software) and on the X-chromosome using regression based methodology (MERLIN software) with correction for ascertainment, age, height and weight. Analysis of the whole study population identified three quantitative trait loci (QTL) showing evidence of suggestive linkage to LS-BMD. Two were in men, on chromosomes 1q (LOD 2.38; 111 cM), and 3q (LOD 3.19; 177 cM) and one was in women on chromosome 7q (LOD 2.20; 141 cM). On further analyses of the subjects who were age 50 years and under, we identified additional QTL for LS-BMD on chromosome 16p in men (LOD 2.52; 31 cM); on chromosome 16q in women (LOD 2.28; 116 cM) and on chromosome 20q13 in women (LOD 3. 19; 91cM). Two QTL for regulation of FN-BMD were identified in men; one on chromosome 4q (LOD 2.22; 117cM) and another on chromosome 10q21 (LOD 4.40; 80cM) where the lodscore exceeded the threshold for genome wide significance This study provides evidence for sex-specific, site specific and age-specific quantitative trait loci for regulation of BMD and has identified a major locus for regulation of peak bone mass in men on chromosome 10q21. This study, which represents the largest genome wide scan for osteoporosis phenotypes performed to date, illustrates the importance of stratifying for sex and age in the analysis of genome wide search results for complex diseases. Further research is warranted to identify the gene variants responsible for the effects observed.

OP037

ETHNIC VARIATION IN HAPLOTYPE STRUCTURE OF THE COMPLETE VDR GENE AND ASSOCIATION WITH FRACTURE RISK

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Background: Polymorphisms of the vitamin D receptor (VDR) gene have been found to be associated with complex diseases, including osteoporosis. Most studies have used polymorphisms at the 3′end or in exon 2, but information on other polymorphism across the VDR gene is scarce.

Materials and Methods: In 15 Caucasians, we sequenced 22 kb in the regions displaying high homology between human and mouse genomic VDR sequence, including the 3.2 kb 3′-untranslated region (3′-UTR), 4.1 kb of all coding exons and flanking introns and 14.7 kb of the 6 promoter exons 1a −1f. We determined linkage disequilibrium (LD) between single nucleotide polymorphisms (SNPs) across the gene in 234 Caucasians, 107 Chinese and 58 African Americans. We performed association studies with "haplotype tagging" SNPs (htSNPs) and fracture risk in 6535 elderly Caucasians from the Rotterdam Study.

Results: We determined the structural organisation of the VDR promoter region by aligning our sequencing results and the Celera database, resolved a 500 bp gap in front of exon 1b. We identified 62 polymorphisms, including 55 SNPs and 7 tandem repeats. 22 SNPs (40%) were new and not contained in the NCBI and Celera databases. In the VDR promoter region, 14 polymorphisms change the putative recognition sequences of transcription factors, while 4 SNPs are located in destabilizing elements (DE) in 3′UTR. LD analysis of common SNPs (frequency 35 %) revealed 4 −8 high linkage haplotype blocks (D' > 0.8) which are conserved among Caucasians and Asians, but more fragmented in Africans. The haplotype allele frequencies differ extensively among ethnic groups. 15 htSNPs are diagnostic for the VDR haplotype blocks for large-scale association studies in Caucasians. The alleles of haplotype-block 2 in the promoter region, containing the Cdx-2 and GATA SNPs, were found to be associated with 20% increased risk for the vertebral fracture. Alleles of haplotype-block 5 in the 3′-UTR were previously observed to be associated with 30% increased risk of fracture.

Conclusion: We found 62 polymorphisms in the VDR gene and identified ethnic-specific haplotype maps and htSNPs in three ethnic groups. Significant associations were observed between the haplotypes defined by htSNPs in the promoter region and 3′-UTR with fracture risk. Our "whole gene" analysis demonstrates intragenic interaction of polymorphisms in the VDR gene to contribute to fracture risk.

OP038

POLYMORPHISMS OF OESTROGEN RECEPTOR ALPHA PREDICT BONE LOSS IN PERIMENOPAUSAL WOMEN

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The XbaI, PvuII and TA repeat polymorphisms of the oestrogen receptor (ER) alpha gene have been proposed as candidate genetic markers of bone mass, osteoporotic fracture HRT response and bone loss in numerous studies. Most of these studies have been small however and the results inconclusive. In this study we conducted a large scale analysis of the XbaI, PvuII and TA repeat polymorphisms of the ER gene on bone mass and bone loss in a population based study of 2876 perimenopausal women from the UK. All genotypes were in Hardy Weinberg equilibrium and genotype frequencies were similar to those which have previously been reported in Caucasian subjects. We found no significant association between any of the individual polymorphisms and bone density at the lumbar spine (LS-BMD) or femoral neck (FN-BMD). However, in non-HRT treated women there was a significant association between the PvuII polymorphism and bone loss, after correction for age, years since menopause, and body mass index, with greater rates of loss in the Pp and pp genotype groups ($P = 0.017$). When data were combined from both groups (i. e. PP vs Pp/pp) the result was highly significant ($P = 0.004$) and equivalent to a 1% difference in rate of loss between the groups. We found no association between the XbaI or TA repeat polymorphisms and bone loss in non-HRT treated women, although individuals who carried 1 or 2 copies of the px haplotype did have increased rates of FN-BMD bone loss ($P = 0.01$), consistent with the results seen with the PvuII polymorphism alone. There was no significant association between any ER polymorphism or haplotype and response to HRT. We conclude that the ER PvuII polymorphism acts as a predictor of perimenopausal bone loss, but we failed to confirm the results of previous studies which have shown an association between ER alleles and bone mass and response to HRT.

OP039

GENE INTERACTION BETWEEN ESTROGEN RECEPTOR ALPHA AND IGF-I DETERMINES HIP BONE STRENGTH IN ELDERLY MEN

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Estrogen receptor alpha (ERalpha) male mice KO's have decreased periosteal apposition. Likewise, IGF-I has been shown to mediate bone apposition. In humans, polymorphisms in the ERalpha gene are thought to influence bone phenotypes in women through differential estrogen receptor expression and circulating E2 levels. No associations have been demonstrated consistently in men. Previously, we found the 192-bp (or wild type) allele of an IGF-I promoter polymorphism associated with higher IGF-I serum levels. In concordance, the allele was associated in males with bone strength (section modulus) but not with BMD. In this study, we examined in elderly men from the Rotterdam Study (a population-based, prospective study of disease in the elderly) the relation of the ERalpha gene to height, FN BMD and hip bone geometry, also in interaction with the IGF-I gene. ERalpha PvuII-Xbal haplotypes, IGF-I gene promoter polymorphism genotypes and DEXA scans were present in 2259 men. In addition to BMD, hip structural geometry of the femoral neck (FN) was approximated from DEXA using a computational algorithm to obtain the section modulus (Z), an index of bone strength, and the cortical buckling ratio (BR), an index of bone stability. ANCOVA was used to compare mean estimates adjusted for age, height and weight across ERalpha PvuII-Xbal haplotype 1 genotypes and multiple linear regression to test for interaction with IGF-I promoter genotypes (defined on the number of 192-bp alleles). The presence of ERalpha PvuII-Xbal haplotype 1 was significantly associated with an allele dose dependent decrease in BMD (0.88, 0.88, 0.87 g/cm^3 p-trend = 0.03) and Z (1.36, 1.34, 1.33 cm^3 p-trend = 0.02) in non-carriers, heterozygotes and homozygotes, respectively. A borderline significant trend was observed with height. No genotype differences were observed with bone instability (BR). These findings are in line with the assumption that haplotype 1 causes lower ERalpha expression.

Interestingly, a significant gene interaction (p = 0.04) with the IGF-I gene promoter polymorphism was observed with bone strength, where differences in Z are significantly enhanced (192-bp allele homozygotes 1.40, heterozygotes 1.35 and non-carriers 1.28 cm^3 p-trend = 0.006) in the group of non-carriers of the ERalpha haplotype 1. These results suggest a biologic interaction in men, where ERalpha genotype differences in BMD and section modulus (bone strength) probably reflect differential IGF-Iaction on periosteal apposition by ERalpha haplotype 1 genotype.

OP040

LDL-RECEPTOR RELATED PROTEIN 5 GENE POLYMORPHISMS INFLUENCE THE NORMAL VARIATION OF BONE MINERAL DENSITY

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Osteoporosis is a highly heritable condition with genetic and environmental etiologies. Genetic factors account for 50–80% of the inter-individual variation in BMD. Studies of cohorts characterised by extremely low or high BMD show the LRP5 gene is an important genetic modulator of bone mineral density (BMD). However, little is known about the frequency of LRP5 polymorphisms within the normal population and the contribution of this gene to the development of osteoporosis and determination of BMD in a normal population.

This study examined the role of the LRP5 gene in determining normal population variation of BMD using family-based and case-control approaches. To examine the entire BMD spectrum, lumbar spine, femoral neck and hip BMD were measured in 152 osteoporotic probands, their families (597 individuals) and 160 women with elevated BMD. Polymorphisms detected by denaturing high performance liquid chromatography and polyacrylamide gel electrophoresis were genotyped using mass spectrometry and restriction fragment length polymorphisms. Within-family association studies were performed by QTDT and linkage analysis by SOLAR. Case-control comparisons of genotype and haplotype frequencies between groups with divergent BMD were performed by contingency-table analysis.

8 single nucleotide polymorphisms (SNPs) with allele frequencies of >5% were found in exons 8, 9, 10, 15, 18 and in introns 6, 7 and 21. Family-based association studies revealed the C171346A SNP in intron 21 was associated with hip BMD ($P < 1 \times 10$-5 in men only, $P = 0.0019$ in both men and women). This association was confirmed in comparisons of osteoporotic probands and woemn with elevated BMD ($P = 0.03$). Polymorphisms in exons 8 (C135242T $-P = 0.007$) and 9 (C141759T $-P = 0.02$) were also associated with BMD. Haplotypes composed of the SNPs G121513A, C135242T, G138351A and C141759T were associated with BMD when comparing osteoporotic probands and high BMD cases ($P < 0.003$). The C165215T SNP in exon 18 was linked to lumbar spine, femoral neck and total hip BMD (parametric LOD scores = 2.8, 2.5 and 2.2 and non-parametric LOD scores = 0.3, 1.1 and 2.2 respectively). Lastly, linkage disequilibrium was present between the polymorphisms in exons 10, 15 and 18.

These results show that LRP5 polymorphisms are common and contribute to the determination of BMD in the general adult population. In particular, male BMD is strongly influenced by LRP5 gene polymorphisms.

OP041

RESISTANCE TO THE BONE ANABOLIC EFFECT OF PTH(1-34) ON AN ISOCALORIC LOW PROTEIN DIET

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PTH stimulates bone formation and increases bone strength. Severe osteoporosis is frequently associated with malnutrition in elderly. We hypothesized that protein intake could influence the response to PTH, as it does for IGF or GH. To test this hypothesis, six-month old female rats were fed isocaloric diets containing 2.5% (low Protein) or 15% (normal Protein) casein for 2 weeks. PTH(1-34) (5 or 40 µg/kg BW) or its solvent was then given subcutaneously to rats on either diet daily for 4 weeks. Bone strength and its determinants like BMD, geometry and microarchitecture were measured at the level of the proximal and midshaft tibia. PTH(1–34) dose-dependently increased ultimate strength (+55.3% ± 14.3* and +96. 5% ± 16.1*) and BMD (10.0% ± 2.2* and +21.5% ± 2.2*), in rats treated with 5 or 40 µg/kg BW respectively, which were fed the normal protein diet. In rats fed a low protein diet, only the higher dose of PTH significantly increased ultimate strength (+4.2% ± 8.4 and +43.8% ± 13.0*) and BMD (+4.1% ± 2.0 and +11.0% ± 2.7*). The higher dose of PTH significantly increased ultimate strength and BMD in rat fed a normal casein diet but not in rat fed low protein diet at the level of the midshaft tibia. MicroCT analysis showed a dose-dependent increment of trabecular bone volume and thickness in rat fed the normal protein diet, an effect less pronounced in rat fed a low protein diet. PTH dose-dependently increased midshaft tibia external diameter, bone volume and cortical thickness in rats fed the normal protein diet but not with the low protein diet. Thus, under a low protein diet, bone formation and the anabolic effect of PTH were reduced. These results indicate that an isocaloric protein restriction attenuates the anabolic response to PTH by reducing its positive effect on bone formation, bone geometry and micro-architecture.

OP042

ARRESTINS REGULATE BONE REMODELING INDUCED BY OVARIECTOMY AND INTERMITTENT PTH TREATMENT

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Intermittent PTH increases bone turnover, with net anabolic effects on bone mass. Although PTH increases cancellous bone volume, its effects on cortical bone remain poorly understood. Estrogen-deficiency, on another side, triggers cancellous bone loss, but may allow periosteal expansion and cortical bone widening. PTH activity is regulated by cytoplasmic beta-arrestin2, and we observed that beta-arrestin2 KO, intact female mice have increased skeletal response to intermittent PTH compared to wild type (WT). In this study, we examined the interaction between estrogens and arrestins in regulating PTH activity on bone compartments.

The skeletal response to ovariectomy (OVX, 4 wks) followed by intermittent PTH (80 ug/kg/d) or vehicle (VEH) for 4 wks was evaluated in WT and beta-arrestin2 KO adult female mice (N = 8 − 11/group) using pDXA, uCT and biochemical markers.

After 4 weeks, OVX significantly decreased total body (TB) BMC compared to sham in both WT and KO mice (WT: −3.4 ± 2.7% vs +2.6 ± 1.9%; KO: −2.2 ± 0.9% vs + 5.8 ± 1.6% in OVX and sham respectively, $P = 0.0006$). After 8 weeks, midshaft femur thickness (MfemTh) was significantly lower in both WT and KO OVX vs sham (-7.7%, $P < 0.0001$), with a marginal increase in marrow volume (MV) and no changes in total volume (TV). In turn, compared to VEH, PTH increased MfemTh in both OVX WT and KO (+8.4%, $P < 0.0001$), with a concomitant decrease in MV ($P = 0.036$) but no changes in TV. These observations are in strong contrast with those obtained in intact beta-arrestin2 KO mice, in whom PTH significantly increased femur cortical TV, i.e. bone widening. In contrast, vertebral trabecular bone density (V BV/TV), trabecular thickness (TbTh) and number (TbNb) were all significantly lower in OVX vs sham ($P = 0.037 − 0.008$), but not in WT, and PTH significantly increased V BV/TV in KO (+27.8%, $P = 0.010$), but not in WT (+7.8%, ns). A significant interaction between treatment and genotype occurred on osteocalcin ($P < 0.0001$) and deoxy-piridinoline ($P < 0.05$), indicating that PTH-stimulated bone turnover was significantly higher in KO than WT mice.

These data indicate that endocortical bone response to PTH is primarily regulated by estrogens, whereas estrogens and arrestins cooperate in the regulation of periosteal and cancellous bone remodeling in response to PTH.

OP043

ROLE OF OSTEOBLASTIC MATRIX METALLOPROTEINASES (MMPS) ON THE ANABOLIC ACTION OF PARATHYROID HORMONE IN VIVO

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Our aim was to evaluate *in vivo* the role of MMPs in the anabolic action of PTH using transgenic mice over expressing the TIMP-1 (tissue inhibitor of

MMPs) specifically in osteoblastic cells. We have previously shown that these mice present an increase in bone mineral density and bone mass resulting from a decreased bone turnover associated with a decrease in bone mineralising surfaces and bone formation rate. In this study, 10-week-old female mice were treated with 40 or 150 µg/kg of weight/day for 1.5 months. DEXA analysis (PIXIMUS) was performed before and after treatment and histomorphometric analysis on femoral metaphysis at the end of the experiments. The densitometric analysis showed a increase in bone mineral density (BMD) and bone mineral content (BMC) in PTH-treated animals at all analysed sites (total body, femur, vertebrae and tibial metaphysis). Only measurements of BMC at the total body and the femur showed a significative positive interaction between the genotype and the treatment.

The histomorphometric analysis indicated that anabolic PTH treatment induced an increase of bone volume (BV/TV), and trabecular thickness (Tr. Th.) associated with a decrease in trabecular separation (Tr. Sp.). Mineralizing surfaces (MS), matrix apposition rate (MAR) and osteoclastic surfaces (Oc. S/BS) were increased with the treatment indicating an increased bone turn-over. Our data also indicate that there was an interaction between the PTH treatment and the genotype for BV/TV, Tr. Th., and MS that were significantly higher in the transgenic mice than wild type mice for the dose of 40 µg/kg PTH. Further more there was an interaction between treatment and cortical thickness that was increased in transgenic mice for the dose of 40 µg/kg PTH. We did not observe any interaction between the genotype and the treatment for resorption parameters (Oc. S/BS and Tr. Sp).

In conclusion, by measuring BMD, we could not capture the interaction between transgene and the PTH treatment in the cancellous bone. By contrast the interaction observed when measuring BMC at cortical sites is in accordance with the measurement of cortical thickness by histomorphometry. Furthermore, we showed that inhibition of osteoblastic MMPs induced further increase of active osteoblast and therefore of trabecular thickness in cancellous bone in mice treated with PTH. Our data suggest that inhibiting MMPs can potentiate the anabolic effect of low dose of PTH both at trabecular and cortical sites.

OP044

ANABOLIC ACTIONS OF PTH ARE ASSOCIATED WITH AN EARLY INHIBITION OF MINERALIZATION IN VIVO

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Skeletal responses to parathyroid hormone (PTH) are anabolic or catabolic, depending on the dosing regimen, but the mechanisms of the anabolic actions remain unclear. A novel tissue engineering model was used to gain a better understanding of PTH anabolic actions. Bone marrow stromal cells (BMSCs) were isolated from C57B/6 mice and utilized to form ectopic ossicles in athymic mice. We previously reported that ossicles contain hormonally responsive cortical, trabecular bone, and a hematopoietic marrow. PTH (40 ug/kg) or vehicle was administered subcutaneously beginning 1wk after cell implantation and continuing for 1wk (group 1), 3wks (group 2), or 7wks (group 3). A fourth group also had PTH or vehicle administration for 3wks but injections did not begin until 12wks after implantation to represent a more mature bone. Microradiography, histomorphometry, and BrDU labeling were used to analyze the ossicles and host vertebral bone. Raman spectroscopy was utilized to determine the phosphate mineral content and northern blot analysis to evaluate gene expression (OC) mRNA expressed in the ossicles and lower incidence of phosphate mineral. PTH-treatment resulted in more widespread BrDU labeling in the bone marrow of ossicles from PTH-treated mice versus a more focused BrDU positivity along the trabecular bone in the controls. Over the course of time, the effects of PTH seemed to plateau and decline (group 3). Reduced bone was observed in ossicles from both vehicle- and PTH-treated mice of group 4 compared to group 2 and 3. Interestingly, vertebral trabecular bone was not affected in any of the groups, which suggests that the anabolic effects of PTH are more pronounced in growing bone than more mature bone. These results indicate that; 1)tissue engineered bone is particularly responsive to PTH during the modeling phase, and 2)PTH inhibits mineralization early in ossicle development, with a later augmentation of bone formation. These data suggest that anabolic doses of PTH act early to promote pre/osteoblastic cell proliferation at the expense of mineralization but this translates into increased matrix for progression to mature bone later.

OP045

HIGH-IMPACT EXERCISE AND BONE MINERAL DENSITY IN RANDOMLY SELECTED POPULATION OF PREMENOPAUSAL WOMEN

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Introduction: The number and incidence of osteoporotic fractures have dramatically increased and become one of the major health problems in developed countries. It has been suggested that regular exercise, especially high-impact activities, contributes to development of high bone mass. The aim of this study was to evaluate the effects of high-impact exercise on bone mineral content (BMC) and density (BMD) in a population-based randomised cohort of premenopausal women.

Materials and Methods: The study population consists of a random sample of 400 Finnish women from a cohort of 5161 women, aged 35 to 40 years. One hundred and twenty (120) of them fulfilled the inclusion criteria and agreed to participate. They were randomly assigned to an exercise and a control group. The exercise regimen consisted of supervised, progressive high-impact exercises three times per week and an additional home program for 12 months. BMC and BMD were measured on the lumbar spine (L1-4), proximal femur and distal forearm by dual-energy x-ray absorpti-ometry (Hologic Delphi QDR) at baseline and after 12 months.

Results: Thirty-nine women (65%) in the exercise group and 41 women (68%) in the control group completed the study. Average compliance defined as exercise sessions attended was 0.9 times per week in supervised sessions and 2.2 in home sessions. The exercise group demonstrated significant gain compared to control group in femoral neck BMD(1.1% vs. −0.4; $P = 0.003$), intertrochanteric BMD (0.8% vs. −0.2%; $P = 0.029$) and BMC (0.7 vs. −0.8%; 0.026), and total femoral BMC (1.2% vs. −0.2%; $P = 0.005$) over 12 months study period. Significant positive changes from baseline were observed within the exercise group also in trochanter and Ward's triangle BMD, but the differences between the groups were not significant. Lumbar BMC (1.3% vs. 0.3%; $P = 0.042$) and L1 BMC (4.4% vs. 0.0%; $P < 0.001$) and BMD (2.2% vs. −0.4%; $P = 0.002$) increased significantly more in the exercise group than in the controls. There were no significant differences between or within the groups in distal forearm.

Conclusions: This study indicates that high-impact exercise is effective in improving BMC and BMD in weight-bearing bones in randomly selected population of premenopausal women. This type of training may be a feasible, efficient and inexpensive way to prevent osteoporosis and osteoporotic fractures later in life.

OP046

INHIBITION OF BONE RESORPTION AND INCREASE IN BONE FORMATION CONTRIBUTE TO THE HIGH BONE MASS OF MICE DEFICIENT IN BET A2-ADRENERGIC RECEPTOR, A RECEPTOR DOWNSTREAM OF LEPTIN FOR ITS ANTIOSTEOGENIC FUNCTION

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Leptin is a hormone secreted by adipocytes whose binding to neurons in the ventromedial hypothalamus (VMH) eventually controls bone formation. Results from parabiosis experiments and clues from the literature suggested that the efferent signal from hypothalamic neurons to bones was of neuronal rather than humoral nature. Indeed, catecholamine-deficient (DBH) mice displayed a mild increase in bone mass compared to wildtype littermates. In order to demonstrate in a more direct way that the sympathetic nervous system controls bone formation, we analyzed the bone phenotype of mice deficient for beta1 (beta1ARKO mice) or beta2-adrenergic receptors (beta2ARKO mice). beta2ARKO mice, but not beta1ARKO mice, display a high bone mass phenotype of similar severity to the one observed in leptin signaling deficient mice. In contrast to what is seen in leptin signaling deficient mice, the high bone mass of beta2ARKO mice is not accompanied by any hormonal abnormalities. Leptin hypothalamic infusion decreases bone mass in wildtype mice but not in beta2ARKO mice, indicating that beta2-adrenergic receptors are necessary molecular intermediaries downstream of leptin hypothalamic signaling for the control of bone mass. Preliminary results indicate that the sympathetic nervous system controls both bone formation and bone resorption: the increased bone resorption induced by ovariectomy in wildtype mice is blocked by beta-blocker treatment, and osteoclast number and surface are decreased in beta2ARKO mice. In vitro co-culture experiments using wildtype and beta2ARKO osteoblasts and osteoclasts are ongoing to delineate further the mode of action of catecholamines on bone resorption. Altogether these results indicate that the sympathetic adrenergic nervous system regulates bone mass downstream of leptin hypothalamic signaling, via its action on osteoblasts and osteoclasts. Therefore beta2-specific adrenergic antagonists are potential promising anabolic and anti-catabolic agents for the treatment of osteoporosis.

OP047

SEVERELY DIMINISHED BONE RESORPTION AS WELL AS REDUCED BONE THICKNESS IN MICE LACKING THE TRANSIENT RECEPTOR POTENTIAL CHANNEL TRPV5

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The maintenance of body calcium homeostasis by kidney, intestine and bone is of crucial importance for many vital functions including neuronal excitability, muscle contraction and bone formation. Two novel transient receptor potential channels, TRPV5 and 6, have been designated as the gatekeepers of calcium-selective renal reabsorption and intestinal absorption. In this study, we genetically ablated TRPV5 (TRPV5−/−) in mice to investigate its role in calcium homeostasis and bone. As expected, renal calcium reabsorption was reduced, resulting in hypercalciuria and polyuria. To compensate for this, intestinal hyperabsorption occurred through upregulation of TRPV6. Serum calcium and parathyroid hormone levels remained constant but the concentration of 1.25(OH)2D3 was greatly increased in the TRPV5−/− versus wildtype (TRPV5+/+) mice. In the femurs analysed by microcomputed tomography, trabecular thickness as well as cortical thickness and volume were reduced in the femoral head and diaphysis, respectively. In addition, the moment of inertia, an indicator of bone strength, was reduced. To gain more insight into the mechanisms underlying reduced bone thickness/quality, we studied osteoclasts in bone sections and performed a resorption pit assay on cultured bone marrow cells from TRPV5+/+ and TRPV5−/− mice. In the bone sections, osteoclast number and osteoclast surface/bone surface were increased in the TRPV5−/− mice, reaching significance in the females. Similarly, *in vitro*, the number of osteoclasts as well as the number of nuclei per osteoclasts increased. Surprisingly, no resorption pits were present in the osteoclast cultures. Perhaps the number of osteoclasts is increased to compensate for reduced resorptive capacity. In support of this, the urinary bone resorption marker, deoxypyrillinodine, was also reduced in the TRPV5−/− mice.

In conclusion, TRPV5 plays a crucial role in renal Ca2+ absorption and osteoclastic bone resorption. The apparent discrepancy with the observed reduced bone thickness and quality can possibly be explained by the extremely high 1.25(OH)2D3 levels *in vivo* (Smith et al. J. Endo, 165: 163; 2000). Future studies should point out whether bone formation is affected in these mice.

OP048

INHIBITION OF GSK3BETA, A KEY REGULATOR OF THE WNT SIGNALING PATHWAY, REVERSES THE OSTEOPOROTIC PHENOTYPE IN LRP5 KNOCK-OUT MICE

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Low-density lipoprotein receptor related protein-5 (LRP5), a Wnt co-receptor and a homologue of Drosophila Arrow, has been shown to regulate bone mass accrual. LRP5 knockout mice (LRP5−/−) have reduced bone mass and decreased osteoblast number. Primary calvarial osteoblasts derived from LRP5−/− mice display a delay in differentiation and exhibit a significant decrease in their responsiveness to BMP-2 compared to wild-type cells. Stimulation of LRP5−/− primary calvaria cells with Wnt3a fails to induce beta-catenin nuclear translocation or TCF1-mediated transcriptional activity. In contrast, the GSK3 beta inhibitor lithium chloride (LiCl) increases both beta-catenin nuclear translocation and TCF1 activity. Since the inhibition of GSK3 beta is a key event downstream of LRP5, we then examined whether LiCl treatment could improve bone mass in LRP5−/− mice. We found that LiCl markedly increased bone volume, trabecular number, osteoblast number and bone formation rate in LRP5−/− animals. These data strongly indicate that disruption of canonical Wnt

signaling is responsible for reduced bone mass in Lrp5−/− mice. Enhancing the canonical Wnt signaling pathway by inhibiting GSK3 beta could be useful for treating other disorders of low bone mass.

OP049

ASSOCIATION OF AROMATASE (CYP19) GENE POLYMORPHISMS WITH ESTRADIOL AND ESTRONE LEVELS IN POSTMENOPAUSAL WOMEN

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Individual variation in postmenopausal estradiol (E2) level is an important determinant of diseases such as breast cancer and osteoporosis. A large portion of the individual variation in E2 levels is determined by genetic variations (i.e. polymorphisms) in genes that control hormone biosynthesis. The aim of our study was to determine if a common, possibly functional polymorphism located in exon 10 (a T to C substitution in the 3′UTR) of the CYP19 gene is associated with variation in serum estradiol levels in the general population.

In 719 postmenopausal women and 589 men from the Rotterdam Study, a population-based cohort study of individuals 55 years and older, CYP19 genotypes and serum E2 levels were determined. The C-allele, which was previously related to decreased CYP19 mRNA by others, was significantly associated with decreased serum E2 in postmenopausal women in an allele-dose dependent manner. Serum E2 levels decreased by 2.23 pmol/L per C-allele copy (p-trend 0.005); extreme genotypes varied 4.53 pmol/L. In a subset of 836 participants DHEA-sulfate, androstenedione, testosterone, bioavailable testosterone, estrone (E1) and bioavailable E2 were determined. In women, the C-allele was associated with decreased serum E1 and bioavailable E2 levels (p-trend 0.0001 and 0.001, respectively). In men, no association between the CYP19 polymorphism and sex-hormones was observed.

We previously demonstrated the estrogen receptor (ER) apha gene PvuII-XbaI haplotype 1 to be associated with a 2 pmol/L decrease in E2 levels in postmenopausal women. An additive effect of the CYP19 exon polymorphism and ER alpha haplotype 1 on E2 levels was observed. There was a 40% reduction in E2 levels in homozygous carriers of both risk alleles as compared to non-carriers. Together polymorphisms in these two genes explained 1.6% of the variance of E2.

In conclusion, we have demonstrated a role for a polymorphism located in exon 10 of the CYP19 gene in determining postmenopausal E2 and E1 levels women.

OP050

NE-10790, A PHOSPHONOCARBOXYLATE ANALOGUE OF THE BISPHOSPHONATE RISEDRONATE, EXHIBITS DIRECT ANTITUMOR ACTIVITY *IN VIVO*.

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In addition to being powerful inhibitors of bone resorption *in vivo*, bisphosphonates (BPs) also exhibit potent antitumor activity. *In vitro*, BPs inhibit tumor cell adhesion, invasion and proliferation, and they induce apoptosis of tumor cells. *In vivo*, BPs reduce skeletal tumor growth. However, because of their high affinity for bone mineral and rapid uptake in bone, tumor cells in the bone marrow may be exposed to BPs for too short a period to observe cytotoxicity. It is most likely that the antitumor activity of BPs in bone is mediated through inhibition of bone resorption which, in turn, deprives tumor cells of bone-derived growth factors. Conversely, a BP having a low bone affinity could act directly on tumor cells in the bone marrow because of its rapid release from bone mineral. To address this question, we compared the antitumor potency of the nitrogen-containing BP risedronate with that of the phosphonocarboxylate analogue of risedronate (NE-10790) in which one of the phosphonate groups is substituted by a carboxyl group. NE-10790 had a 15-fold lower affinity for bone mineral compared to that observed with risedronate. *In vitro*, NE-10790 and risedronate inhibited proliferation of GFP-expressing B02 breast cancer cells (IC$_{50s}$: 3.4 ± 0.9 and 0.4 ± 0.2 mM, respectively). Continuous treatment of mice with risedronate (0.15 mg/kg/day) almost completely inhibited bone destruction caused by GFP-expressing B02 breast cancer cells (as judged by radiography) and substantially reduced skeletal tumor burden (as judged by external fluorescence imaging and histomorphometry). NE-10790 (0.15 mg/kg/day), under similar experimental conditions, did not inhibit bone destruction whereas it did drastically inhibit skeletal tumor burden (70% reduction). This lack of

inhibitory effect of NE-10790 on bone destruction was consistent with the observation that NE-10790 was 8,000-fold less potent than risedronate (on a mg/kg basis) in inhibiting bone resorption in ovariectomized rats. Moreover, a continuous treatment of mice with NE-10790 (or risedronate), at a daily dose (0.15 mg/kg) that inhibited skeletal B02 tumor burden, did not inhibit the subcutaneous growth of GFP-expressing B02 cells. Overall, these findings strongly suggest that NE-10790 (because of its low bone affinity) transiently accumulates in bone and subsequently act on tumor cells to inhibit their growth.

OP051

SERUM CONCENTRATIONS OF AMG 162 WERE DETECTABLE AND BONE RESORPTION SUPPRESSED FOR 6 TO 9 MONTHS FOLLOWING A SINGLE DOSE IN POSTMENOPAUSAL WOMEN

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Objectives: To determine the pharmacokinetics and bone anti-resorptive effect (based on urinary NTx/creatinine (NTX)) of a single subcutaneous (SC) or intravenous (IV) dose of AMG 162, a fully human monoclonal antibody to Receptor Activator of Nuclear Factor-kB Ligand (RANKL), in postmenopausal women.

Methods: A randomized, double-blind, placebo-controlled, single-dose, dose escalation study in healthy postmenopausal women was conducted. Six cohorts of 8 or 9 each women received single SC or IV injections of either AMG 162 or placebo (3:1 ratio). Doses were 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg. Serum levels of AMG 162 were measured by a validated enzyme-linked immunosorbent assay. Urine was collected at specified time points up to 36-weeks post-dose and analyzed for creatinine and N-telopeptide (NTX) levels using Osteomark kit (Ostex, Bothell, WA).

Results: The pharmacokinetics of AMG 162 were non-linear with dose. SC and IV serum profiles were characterized by 3 distinct phases: 1) either an absorption phase with maximum concentrations that increased (2.6-fold) with dose and occurred 5–21 days after SC administration, or a rapid distribution phase with an initial volume of distribution similar to plasma volume after IV administration; 2) a prolonged beta-phase, characterized by half-lives that increased with dose to a maximum of 37 days; 3) a more rapid terminal elimination phase at concentrations below 1000 ng/mL with half-lives from 3 to 10 days. AMG 162 serum mean residence time increased with dose from 6 to 50 days, and relative exposure (SC:IV) ranged from 36% to 78%. AMG 162 produced dose-dependent, rapid (within 12 hours), profound (up to 93%), and sustained (up to 31 weeks) decreases in NTX. At month 6, there was a mean change from baseline of −81% and −77% in the 3.0 mg/kg SC and IV dose groups, respectively. There appeared to be a good correlation between serum AMG 162 levels and suppression of NTX, as suppression of NTX was maintained (< −70%) while AMG 162 serum levels were above approximately 200 ng/mL.

Conclusions: AMG 162 serum levels were detectable for 36 weeks following a single SC or IV dose of 3.0 mg/kg, and produced rapid and sustained decreases in the bone resorption marker NTX that were maintained for the duration of the study. The favorable pharmacokinetics and pharmacodynamics indicate that AMG 162 may allow for infrequent dosing in the treatment of bone disorders, such as osteoporosis and cancer related bone diseases.

OP052

KYPHOPLASTY - RESULTS OF THE FIRST PROSPECTIVE CONTROLLED TRIAL

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Evidence-based treatment with calcium, vitamin D3, bisphopshonates and raloxifene does not solve the clinical severe complication of osteoporosis, pain and decreased mobility of patients after vertebral fracture. This prospective controlled trial evaluates changes in pain, mobility, number of new fractures and economic aspects of kyphoplasty.

60 out of 211 patients with primary osteoporosis and painful vertebral fractures were included in this cohort study after an interdisciplinary team of endocrinologists/osteologists, trauma surgeons, and radiologists discussed every single patient in detail on the basis of the clinical complaints, x-rays, CT and MRI scans of the spine and finally agreed on the indication. 40 patients received kyphoplasty while 20 served as controls. Outcomes assessed were pain (VAS spine score), the European vertebral osteoporosis study score addressing mainly mobility in daily life (EVOS), radiomorphometric assessments and healthcare contacts. All patients received medical treatment with 1000 mg calcium, 1000 IE vitamin D3, the standard dose of an oral aminobisphosphonate, pain medication as required and standard physical therapy.

Both groups were comparable prior to treatment. Kyphoplasty increased midvertebral height by 15% while in the control group vertebral height decreased by 20% ($P < 0.001$). Internal stabilization and augmentation of vertebrae by kyphoplasty resulted in a 69% ($P < 0.001$) reduction of back pain while mobility increased by 23% ($P < 0.001$). There are no significant changes in control patients. The number of back pain related doctor visits within the 6 months follow up period decreased significantly after kyphoplasty with 8.6 visits/patients in the control group and with 3.3 visits/patient in the kyphoplasty group. There was a non significant trend to less new vertebral fractures in the kyphoplasty group compared to the control group.

Kyphoplasty performed in elderly patients with primary osteoporosis and painful vertebral fractures results in patial normalization of vertebral height, reduction of pain, increase in mobility with the consequence of reduced pain medication and doctors visits. Therefore, kyphoplasty performed in appropriately selected osteoporotic patients with chronic painful vertebral fractures is a promising addition to current medical treatment.

ABSTRACTS P001 TO P328

POSTER PRESENTATIONS

Poster viewing

Posters will be displayed in the Hall Agora 2 and will be attended as follows:

Odd numbered boards (P1, P3, P5 etc) are to be attended from 11.00 to 12.45 on Sunday 6 June 2004.

Even numbered boards (P2, P4, P6 etc) are to be attended from 11.00 to 12.45 on Monday 7 June 2004.

All posters are to be attended from 11.00 to 12.45 on Tuesday 8 June 2004 and from 10.00 to 11.00 on Wednesday 9 June 2004.

Early morning poster viewing

Posters are also available for early morning viewing from 08.00 to 09.00.

Abstracts

Cell Biology: Osteoblasts, Osteocytes and Bone Formation

P001

DIFFERENTIAL EXPRESSION OF NONCOLLAGENOUS BONE MATRIX PROTEINS IN CORTICAL AND TRABECULAR BONE

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The skeleton consists of 80% cortical and 20% trabecular bone whose differences lie in the organization of the collagen fibrils and the degree of porosity. The cortical bone, for its compact structure, fulfills mechanical and protective function while trabecular bone, having higher surface area, is a site of greater bone turnover and involving in metabolic function. Ninety percent of the protein in bone is type I collagen while smaller noncollagenous proteins (NCPs) comprise the rest. Several studies provided evidence on the diverse functions of NCPs such as regulation of mineralization and modulation of cell migration, differentiation and maturation. Since cortical and trabecular bone have structural and functional heterogeneity, it is conceivable that the composition of NCPs are different in these two types of bone. Transiliac crest bone biopsy obtained from 10 volunteers, 3 males and 7 females were subjected to study. Immunohistochemistry of the NCPs was performed on multiple undecalcified bone sections cut at steps of 50–100 mum apart in the same biopsy specimen. At least 30 microscopic fields of cortical and 40 of trabecular bone were subjected to quantitation using KS-300 digital image analysis and percentages of area of positive staining per total cortical or trabecular bone area were reported. Each protein has specific pattern of distribution but overlap in localization between the proteins were observed. Osteopontin and bone sialoprotein express strongly in the area of the bone matrix adjacent to the harversian canals, cement lines and variably in the osteocyte lacuna. Osteocalcin expresses in the cement lines while harversian canals and osteocyte lacuna are devoid of staining. Trabecular bone was 31% positive for osteopontin comparing to 22% in cortical bone ($P < 0.0005$). Similarly bone sialoprotein (17% trabecular vs. 9% cortex, $P < 0.02$) and osteocalcin (17% trabecular vs. 10% cortex, $P = 0.07$) also preferentially express in trabecular bone. Oteonectin staining falls in a different pattern. In addition to the intense staining observed in outer lamellae osteon, the protein also expresses more in the cortical than trabecular bone (13% trabecular vs. 21% cortex, $P < 0.01$). In conclusion, each NCP has specific pattern of distribution and differential expression of NCPs were observed in the cortical and trabecular bone suggesting distinct role of NCPs in bone formation and remodeling.

This work was supported by Thailand Resarch Fund

P002

COMPARATIVE STUDIES ON THE THERMAL ALTERATION OF THE CENTRAL DARK LINE IN BIOLOGICAL APATITE AND SYNTHETIC OCTACALCIUM PHOSPHATE

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The central dark line (CDL) is recognized as platy nuclei of the biologically induced apatite. Although it has been widely accepted that octacalcium phosphate (OCP) might be a strong candidate for the CDL, the temperature of thermal change of OCP seems to be lower than that of the disappearance of CDLs in apatitic crystals in calcifying hard tissues. Therefore, as part of our serial studies on biomineralization, the present study was conducted to demonstrate the difference between the constituent of CDL in the biologically induced apatite and synthetic OCP from the viewpoint of physical properties.

The samples of enamel, bone, and synthetic OCP were heated at 150 degrees to 600 degrees. Then they were processed for transmission electron microscopy and X-ray diffraction analysis.

X-ray diffraction analysis has revealed that the sharp reflection peak of 4.7 degrees 2Theta characteristic for OCP disappeared after being heated up to 150 degrees. However, electron microscopic observations have clearly shown the existence of CDLs in both enamel and bone crystals heated at 600 degrees.

Our results strongly indicate that OCP would not be a potential candidate for the constituent of CDLs in biological apatite crystals and CDL-bearing crystals might utilize this pathway for apatitic formation in the biological system.

P003

NUCLEAR FACTOR KAPPA B IN THE RESPONSE OF THE MURINE OSTEOCYTE-LIKE CELL LINE MLO-Y4 TO IONIZING RADIATION

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Important factors in manned space flight are the weightlessness leading to loss of bone mass and the exposure to cosmic ionizing radiation. Concerning the bone metabolism, effects of ionizing radiation and their interaction with microgravity have not been investigated, especially their influence on gene expression in osteocytes. An important transcription factor which is activated in response to different kinds of stressors is the Nuclear Factor kappa B (NF-kappa B). Activation of this pathway as a possible antiapoptotic route could explain high survival rates after exposure to toxic agents. Furthermore, activation of NF-kappa B is supposed to be involved in induction of cyclooxygenase 2 expression leading to increased production of the osteoblastic differentiation promoting prostaglandin E2. In osteoblasts and osteocytes, this pathway could explain differentiation events initiated by radiation. To elucidate these mechanisms, radiation effects on survival and NF-kappa B activation in osteocyte-like cells were examined. First, the murine osteocyte-like cell line MLO-Y4 and the osteoblast-like cell line OCT-1 (kindly provided by L. Bonewald, San Antonio, Texas, USA) were screened for different markers. MLO-Y4 displayed a stellate morphology and a low alkaline phosphatase expression. Both cell lines expressed osteocalcin and the transmembrane protein E11, as determined by RT-PCR. Survival after exposition to X-rays was determined in colony forming ability tests. The resulting dose-effect relationships revealed a higher resistance of MLO-Y4 compared to OCT-1 cells. To investigate the role of NF-kappa B in the radiation response of MLO-Y4 cells, the cell line was stably transfected with a vector carrying the genes for the reporter proteins Enhanced Green Fluorescent Protein (EGFP) or its destabilized variant d2EGFP under control of four copies of the NF-kappa B response element. Treatment with the NF-kappa B activating tumor necrosis factor alpha gave rise to EGFP/d2EGFP expression in several stably transfected clones, measured by Fluorescent Activated Cell Scanning (FACS). Two clones, expressing EGFP or d2EGFP and showing stellate morphology, were chosen for X-irradiation. Only in the high dose range (> 8 Gy), a substantial activation of NF-kappa B could be monitored by FACS analysis. So far, the higher resistance of MLO-Y4 cells to X-rays can not be explained by an activation of NF-kappa B, which may protect from apoptosis, in a larger part of the population.

P004

FMS*CALCIUMFLUOR INCREASES SPECIFICALLY MRNA LEVELS AND INDUCES SIGNALING VIA MAPK 42,44 AND NOT FAK IN DIFFERENTIATING RAT OSTEOBLASTS

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The homeopathic compound of resonance FMS*Calciumfluor was shown to induce increase in the level of Alkaline Phosphatase (AP) mRNA and of the expression of the enzyme, of the accumulation of Calcium in the extracellular matrix and of nodule formation in rat osteogenic cultures differentiating *in vitro*. The osteogenic process takes place more rapidly in presence of FMS*Calcium-fluor. We here report studies to determine the effect of continuos exposure to FMS*Calciumfluor during differentiation of rat osteogenic cells on the level of mRNA for markers known to be developmentally modulated during osteogenesis. Biglycan (BG), Collagen type I (Coll I), and integrin b1 mRNA levels are not changed in cultures treated with FMS*Calciumfluor. AP, Osteocalcin (OC), Metalloproteinases (MMP)-2 and −14 and the Procollagenase C BMP-1 are expressed at higher level in FMS*Calciumfluor-treated osteoblasts than in control cells. Early signaling events associated with the effects of the exposure to FMS*Calciumfluor do not involve activation by phosphorilation of p120 (FAK) while phosphorilation of MAPK 42,44 is induced in maturing osteoblasts (AP positive). Pre-osteoblasts do not respond to FMS*Calciumfluor treatment with phosphorilation of mitogen activated protein kinases (MAPK) 42,44, the effect being differentiation stage-dependent.

These data, confirm previous indications that FMS*Calciumfluor increases the efficiency of differentiation in early and maturing osteogenic cells and suggest a possible mediator of its effects is the activation of MAPK 42,44, which might participate of a cascade of transcription enhancement (or of stabilization of mRNA) affecting selectively some of the genes whose expression is modulated during the osteogenic progression. These data also confirm the efficacy in producing molecular changes consistent with the enhancement of the osteogenic phenotype of a fluoride compound which is effective at concentrations far below those of NaF and AlFx, and suggest that also FMS*Calciumfluor action is mediated through G-proteins.

P005

RADIATION RESISTANCE OF MURINE OSTEOBLAST CELL LINES OF DIVERSE DIFFERENTIATION LEVELS

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During longterm space missions, astronauts suffer from the loss of minerals especially from weightbearing bones due to prolonged sojourn under microgravity. In addition to weightlessness, exposure to cosmic ionization radiation is another space related factor endangering health and productivity of astronauts. In order to elucidate changes in bone cell metabolism by ionizing radiation, ground-based bone cell models have been developed. Osteoblast differentiation is a key aspect of bone formation and remodeling. This model comprises a collection of immortalized murine osteoblast and pre-osteoblast cell lines representing discrete stages of differentiation: the osteoblast cell line OCT-1 (kindly provided by D. Chen, San Antonio, Texas), and the subclones 4 and 24 of the osteoblast cell line MC3T3-E1 displaying varying potential to produce mineralized bone matrix.

Upon incubation of cell lines with ascorbic acid and beta-glycerophosphate, growth and differentiation characteristics were examined using a DNA binding fluorescent dye and the von Kossa stain. OCT-1 showed the highest proliferation rate which was not altered by culturing in osteogenic medium. Subclone 4 had the lowest proliferation rate, which was further reduced by addition of ascorbic acid and beta-glycerophosphate to the culture medium. Only this subclone showed a positive von Kossa reaction indicating mineralized bone matrix after culture in osteogenic medium. All cell lines expressed osteocalcin, as determined by Reverse Trancriptase PCR. The activity of alkaline phosphatase was highest in the cell line OCT-1.

After exposition to X-rays, survival was determined using the colony forming ability test. The resulting dose-effect relationships revealed a radiation resistance (compared to human fibroblasts). Cell clone specific variations (subclone 4 and 24) in the radiation resistance may be due to the differentiation level. The quantitative acquisition of DNA-strand breaks was performed by Fluorescent Analysis of DNA-Unwinding (FADU). Results can be correlated with the survival curves of the three cell lines. In conclusion, the cell line with the highest differentiation level displays a higher radiation resistance compared to the less differentiated osteoblast cell lines. The high survival rate could result in continued growth of cells carrying mutations in genes relevant for cell cycle and apoptosis control.

P006

A THROMBIN PEPTIDE (TP508) PROMOTES FRACTURE HEALING BY MODULATING IMMUNE RESPONSE AND ANGIOGENESIS

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TP508, a synthetic 23 amino acid peptide, represents a receptor-binding domain of human thrombin, an important growth factor and immunoregulator of tissue injury. When clots dissolve, thrombin fragments act on receptors to initiate healing. A single injection of TP508 accelerates fracture healing in a closed rat femoral fracture model. The aim of this study was to uncover the molecular mechanisms of TP508 actions using Affymetrix genome-scale profiling.

Methods: Closed fractures of the femoral midshaft were created in 10-month-old Sprague-Dawley rats (3 animals/group/time point). 1, 10, or 100 ug of TP508 in 100 ul saline was injected percutaneously into the fracture site one hour post-fracture. Saline alone was injected in the controls. Midshaft femurs were collected on days 1, 2, and 4 after fracture for RNA extraction. Affymetrix Rat U34A Gene Array was used. For histology, femurs were collected at 21 days, followed by routine sectioning and Trichrome staining.

Results: I. Some major genes related to immunological reactions, such as Interferon-γ and several MHC Class II genes, increased expression levels in the TP508 treated group compared with controls at day 1. Increasingly expressed Interferon-γ activates macrophages, which secrete several cytokines including IL-1b to promote bone healing. II. There were significant increases in some critical growth factors in the TP508 treated group compared to controls, including early growth response factor 1 (Egr-1), and C-fos. Egr-1 expression increased in all fractures (*$P < 0.01$) compared to non-fractured femurs, and 1ug TP508 treated group increased (#$P < 0.05$) compared to the saline controls. III. More blood vessels were observed in the fracture site of TP508 treated animals compared with controls in the late stage of fracture healing. Treatments with 1 and 10 ug are more effective than 100 ug.

Conclusion: TP508 promotes fracture healing through a mechanism that involves early induction of a number of growth factors, enhanced expression of inflammatory mediators, and angiogenesis-related genes.

P007

ARG-VASOPRESSIN STIMULATES PROLIFERATION AND DECREASES PRODUCTION OF MACROPHAGE-COLONY STIMULATING FACTOR AND INTERLEUKIN-6 IN HUMAN OSTEOBLAST-LIKE CELLS

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Arg-vasopressin (AVP) is a hypothalamic nonapeptide which is released from the neurohypophysis into the blood. Patients with central diabetes insipidus have a deficiency in AVP. Recently, it was reported that patients with central diabetes insipidus have a lower bone density compared to healthy subjects. The aim of the present study was to investigate whether AVP could stimulate proliferation of human osteoblast-like (hOB) cells and influence some osteoblast derived factors, macrophage-colony stimulating factor (M-CSF) and interleukin-6 (IL-6), involved in bone metabolism. Primary cultures of hOB-cells were prepared from bone fragments from patients during orthopedic surgery. The cells were incubated with different concentrations of AVP and cell proliferation was measured as DNA synthesis by [3H] thymidine incorporation. M-CSF and IL-6 were determined by ELISA.

Incubation of hOB-cells with AVP 10–100 pmol/l during 48 hours increased cell proliferation significantly (10 pmol/l: 164%, $P < 0.01$; 100 pmol/l: 126%, $P < 0.05$). In addition, AVP (10 pmol/l) significantly decreased the production of M-CSF from 126 ± 14.0 to 104 ± 11.6 pmol/l ($P < 0.01$) and IL-6 from 171 ± 7.35 to 149 ± 14.8 pmol/l ($P < 0.01$). Both IL-6 and M-CSF are synthesized by osteoblastic cells and acts on osteoclast progenitors to stimulate their replication, differentiation and proliferation. In conclusion, AVP increased cell proliferation in hOB-cells. Since 10 pmol/l is very close to physiological levels it is possible that AVP can affect bone metabolism *in vivo*. In addition, AVP might inhibit bone resorption since M-CSF and IL-6 stimulate osteoclast activity and they were both decreased in response to AVP. It is possible that AVP may affect bone formation also during physiological situations *in vivo*.

P008

INTERACTIONS BETWEEN ESTROGEN AND MECHANICAL STRAIN EFFECTS ON OSTEOBLASTS ARE NOT INFLUENCED BY ESTROGEN RECEPTOR TYPE

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Estrogens (E) and mechanical strain exert direct effects on osteoblast activity, with good evidence of interactions between their respective effects. Osteoblasts express both forms of estrogen receptors (ER) ER alpha and ER beta, and previous studies have suggested a specific role for each receptor. Therefore, our working hypothesis was that the interactions between E and mechanical strain on osteoblast activity vary depending on which ER is preferentially activated.

Using the human osteoblastic cells, U2Os, stably transfected either with ER alpha or ER beta, we evaluated the effects of cyclical cell loading on a F-3000 Flexercell Strain Unit (1.5% elongation, 10 min./day), in presence of estradiol (E2) 10-8 M or not. The U2Os cell lines, either ER alpha or ER beta transfected or the original U2Os cell line which does not express ER, were characterized by low alkaline phosphatase (AP) activity. They expressed all osteoblastic markers but osteocalcin because of a specific gene mutation. In both U2Os-ER alpha and U2Os-ER beta cell lines, mechanical strain induced similar increases in AP activity and gene expression as measured by quantitative RT-PCR (Light Cycler, Roche), and a decrease in type I collagen gene expression. No change in proliferation rate was observed. Strain and E2 had a synergistic effect on AP activity as compared to each stimulus alone. Neither proliferation nor differentiation of the original U2Os cell line, was altered by strain or E2.

In summary, the differences observed between the U2Os and the U2Os-ER alpha or -ER beta cell lines are consistent with previous studies suggesting that ER play a critical role in mechanotransduction. However, these data do not support the hypothesis of differing roles for ER alpha and ER beta in these defined experimental conditions. Understanding the mechanisms mediating interactions between estrogens and mechanical strain at the cellular level still requires further investigations.

P009

MECHANICAL STRAIN ON OSTEOBLASTS ACTIVATES AUTOPHOSPHORYLATION OF FAK AND PYK2 TYROSINE SITES INVOLVED IN ERK ACTIVATION

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The mechanisms involved in the mechanical loading-induced increase in bone formation remain unclear. In this study, we showed that cyclic strain (CS)(10 min, 1% stretch at 0.25Hz, performed on a Flexcell apparatus) stimulated proliferation of overnight serum-starved ROS 17/2.8 osteoblast-like cells, plated on Type I collagen coated silicone membranes. This increase was blocked by a MEK inhibitor PD98059. Signaling events were then assessed 0,30 min, and 4 h after one CS period with Western blotting and co-immunoprecipitation. CS rapidly and time-dependently promoted phosphorylation of both ERK2 at Tyr-187 and focal adhesion kinase (FAK) at Tyr-397 and at Tyr-925 leading to the activation of the Ras/Raf/MEK pathway. Cell transfection with FAK mutated at Tyr 397 completely blocked ERK2Y187 phosphorylation. Quantitative immunofluorescence analysis of phosphotyrosine residues showed a significant increase in focal adhesion plaque number and size in strained cells compared to control cells. CS also induced both SrcY418 phosphorylation and Src to FAK association. Treatment with the selective Src family kinase inhibitor pyrazolopyrimidine 2 did not prevent CS-induced FAKY397 phosphorylation suggesting a Src-independent activation of FAK. In order to identify other proteins involved in strain-induced signaling, we performed a WB with anti P-Tyr Ab (PY99). We found that, besides the 125 Kd band corresponding to FAK, another 110 Kd protein was highly phosphorylated in both ROS17/2.8 and rat primary cells. We identified this protein as PYK2, a protein kinase highly homologous to FAK, phosphorylated at Tyr 402.CS promoted PYK2 association to FAK in a time-dependent manner. Mutation of PYK2 at the Y402 site prevented the ERK2 phosphorylation only at 4 h. Intra and extracellular calcium chelators prevented PYK2 activation only at 4 h. In summary, our data showed that osteoblast response to mitogenic CS was mediated by MEK pathway activation. The latter was induced by ERK2 phosphorylation under the control of FAK and PYK2 phosphorylation orchestrated in a time dependent manner.

P010

THE EXPRESSION OF TSG101 DURING DIFFERENTIATION OF HUMAN OSTEOBLAST CELL LINE, hFOB

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The tumor susceptibility gene 101 (Tsg101) was first discovered in murine fibroblasts in a screen for potential tumor suppressors. Various biological functions of Tsg101 have been postulated. Several reports show that Tsg101 may influence cell cycle control and cell differentiation. This study is design to test the effect of Tsg101 in osteoblast differentiation. As a model system for such testing, the conditionally immortalized human osteoblast cell line, hFOB was used. This line was immortalized by transfection with a temperature-sensitive SV40 large T antigen. When cultured at the permissive temperature of 34 °C, these cells express large T antigen and undergo continuous proliferation. In contrast, when cultured at the nonpermissive temperature of 39 °C, large T antigen does not accumulate, and these cells stop proliferating, become quiescent, and express typical markers for differentiated osteoblasts.

Firstly, Tsg101 promoter was cloned and characterized. This 2.6-kb Tsg 101 promoter contains important regulatory elements require for transcription. A Tsg101 promoter-luciferase reporter plasmid was constructed and transfected into hFOB at 34°C. Then the cells were culture in different temperatures, 34°C and 39°C, for 2 days. Using luciferase assay, the promoter activity is more than 2-fold at 39°C. We also found the Tsg101 protein production was decreased about 50% during culture at 39°C in western blot analysis.

The expression of Tsg101 is down-regulated during osteoblast differentiation in hFOB cell line.

P011

BONE MORPHOGENETIC PROTEIN EXPRESSION IN DIFFERENTIATING OSTEOBLASTS IS REGULATED BY 17-β ESTRADIOL

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We have recently shown that MC3T3-E1 cells during differentiation process *in vitro* synthesize only BMP-4 which is necessary and sufficient for osteoblastic differentiation and extracellular matrix deposition. In the same system the function of BMP-4 could be replaced by BMP-7, another BMP family member. In this study we explored the effect of 17-β estradiol on regulation of BMP and osteoblastic markers gene expression during *in vitro* differentiation of MC3T3-E1 cells. At designated time points (days 0, 3, 7, 12 and 17) parallel cultures were treated with 17-β estradiol (10^{-7} M to 10^{-11} M) for 12 and 72 hours. Total RNA was isolated using TRIzol reagent and cDNA was synthesized from 4 μg of total RNA with Superscript II RNase H-Reverse Transcriptase (Gibco BRL). The expression of BMP-2 to BMP-7, collagen type I, osteocalcin and osteopontin was analyzed by RT-PCR.

Treatment of MC3T3-E1 cultures with 17-β estradiol, like BMP-7, reduced BMP-4 gene and protein expression following 48 h or 72 h of treatment. Evaluation of the expression of several other BMP family members indicates that only BMP-6 gene transcripts were induced upon 17-β estradiol treatment in the dose dependent manner. The expression of genes associated with osteoblastic differentiation, osteopontin, collagen type I and osteocalcin were markedly stimulated by 17-β estradiol, as well as bone nodule formation at day 21. The stimulation of osteocalcin gene expression was further confirmed by ELISA in the conditioned media of treated cultures. In conclusion, these results suggest that estradiol selectively induces expression of BMP-6, which can also replace for the role of BMP-4, an endocrine signal for osteoblasts, in initiation and maintenance of sequential differentiation of osteoblastic MC3T3-E1 cells and extracellular matrix deposition.

P012

ANTIOXIDATIVE ENZYME SYSTEMS IN HUMAN MESENCHYMAL STEM CELLS

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Reactive oxygen species (ROS) of cellular and environmental origin are involved in redox signalling, cumulative cell damage, senescence and tumour development. The selenoenzymes glutathione peroxidases (GPx) and thioredoxin reductases (TrxR) as well as the selenium-independent superoxide dismutases (SOD) and catalase (CAT) regulate ROS neutralisation, compartmentalisation and steady state levels. Standard cell culture systems (e.g. 5–10% FCS) are selenium deficient (5–10 nM) which influences the activity of selenium dependent enzymes as it depends on selenocysteine incorporation. Telomerase-immortalised (hTERT4) and primary mesenchymal stem cells express TrxR 1 and TrxR 2, GPx 1–3, SOD 1 (CuZnSOD) and SOD 2 (MnSOD) and CAT. After 5–7 days of sodium-selenite supplementation (100 nM) TrxR and GPx activity is enhanced

2-4-fold. SOD 1 but not SOD 2 expression and activity was enhanced 2-4-fold in selenium supplemented cell cultures as measured by dot blot hybridisation, quantitative PCR, Western blotting and SOD enzyme assay. In selenium supplemented cultures H2O2 (50µM) stimulated SOD1 mRNA steady state levels as determined by quantitative PCR which was markedly reduced in selenium deficient cultures.

Our results indicate that mesenchymal stem cells express antioxidative enzyme systems. In selenium deficiency, where the hydrogenperoxide neutralising GPx activities are reduced, the expression of the hydrogenperoxide producing SOD 1 is diminished to prevent the cells from hydrogenperoxide overload.

In the context of tissue engineering and transplantation procedures the scavenge of the stem cell genome and proteome during *in vitro* procedures by adding selenium appears to be of utmost importance.

P013

EFFECT OF BONE EXTRACTS ON THE RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KB LIGAND/OSTEOPROTEGERIN SYSTEM IN OSTEOBLAST-LIKE CELLS

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Background: Osteoclast-mediated bone degradation has the potential to release matrix proteins and growth factors. Previous studies have shown that bone extracts are capable of stimulating osteoblast proliferation therefore the resorptive activity of osteoclasts could potentially influence differentiation and maturation of the local osteoblast lineage cells. Osteoblasts regulate the recruitment and activity of the osteoclast through the RANKL/OPG system. Cells of the osteoblast lineage produce the membrane bound protein RANKL (receptor activator of nuclear factor-kB ligand) which is a positive regulator of osteoclast formation along with the decoy receptor osteoprotegerin (OPG). OPG is able to bind RANKL and prevent it from binding to it's receptor RANK (receptor activator of nuclear factor-kB) on the osteoclast surface. The activation of RANK by its ligand RANKL is necessary for osteoclastogenesis. The aim of this work was to investigate the effect of crude bone extracts on the RANKL/OPG system in osteoblast-like cells.

Methods & Results: Partially purified extracts were prepared from normal human cortical bone using 0.5 M EDTA followed by separation on a C18 SPE column (Jones Chromatography). The resulting eluant fractions were lyophilised and reconstituted in PBS. The effect of the extracts (approximately 5mg protein/ml) on osteoblast proliferation, differentiation and capability of supporting osteoclastogenesis was examined over a 2 week period. Synthesis of OPG was determined in culture supernatants by ELISA and levels of RANKL expression were examined using immunocytochemistry.

The bone extracts contained a range of molecular weight proteins ranging from 90 to 5 kDa including the bone protein osteocalcin. Preliminary results in the human osteosarcoma cell line MG-63 show that the bone extracts inhibit OPG secretion. The inhibition of OPG secretion by bone extracts appears to be dose dependent and is significantly different to the untreated controls on day 7, $P < 0.05$. Heat treatment of the extract, 95°C for 3 minutes, partially abrogates this inhibition. Conversely, RANKL immunopositivity appeared to be enhanced by treatment with bone extract.

Conclusion: This preliminary study demonstrates that bone extracts can modulate osteoblast-mediated OPG expression, and indicate that the RANKL/OPG pathway may have a role to play in coupling osteoclast and osteoblast activity.

P014

THE OSTEOGENIC EFFECTS OF GREEN TEA CATECHINS IN PLURIPOTENT STEM CELLS

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Green tea was reported to possess antioxidant activity, antitumorigenesis, antibacterial activity and regulation of endocrine system. Previous epidemiological studies found that the bone mineral density (BMD) of post-menopausal women with tea drinking habit was higher than that of without tea consumption. However, the effects of green tea catechins on osteogenic function have rarely been investigated. In our preliminary study, (-)-epigallocatechin-3-gallate EGCG had more potent effects than other catechins. In this study, we tested EGCG on the mRNA expression of type I collagen (COL I), alkaline phosphatase (ALP),

osteocalcin (OC), osteoprotegerin (OPG), macrophage-colony stimulating factor (M-CSF), core binding factors a1 (Cbfa1) and bone morphogenetic protein 2 (BMP2). Our results showed that the mRNA expressions of ALP, OC, OPG and Cbfa1 were ameliorated but that of M-CSF was deteriorated in murine pluripotent stem cells upon 48-hour treatment of EGCG. The upsurges of Cbfa1, OC and ALP expression in pluripotent stem cells provided the evidence of increasing osteogenesis after EGCG treatment. Osteoclastogenesis may also be thwarted by up-regulation of OPG and down-regulation of M-CSF in osteoblastogenic cells. Together with our result and the previous reports, we suggest that green tea may be a beverage for preventing osteoporosis. Further studies and effects upon post-translation changes of the osteogenic genes affected by catechins, longer treatment of catechins are necessary to investigate.

P015

CHARACTERIZATION OF THE EFFECT OF TITANIUM CARBIDE ON HUMAN FETAL OSTEOBLASTIC CELLS (HFOB1.19) ADHESION, PROLIFERATION AND DIFFERENTIATION

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Characterization of the effect of titanium carbide on human fetal osteoblastic cells (hFOB1.19) adhesion, proliferation and differentiation

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Modifications in the commercially pure titanium (Ti) surface may be undertaken to improve its biological properties. Enhancement of normal osteoblast function and appositional bone formation after implant placement represents a strategy which could be useful for the purpose of improving osseointegration. To further improve the osteointegration between the implant and bone, titanium substratum (Ti) was covered with its carbide (TiC), deposited by pulse laser deposition (PLD). Aim of this study was to characterize the effect of TiC on human osteoblastic cells (hFOB1.19) adhesion, proliferation and differentiation. To evaluate the effects of substrata on osteoblast morphology, cells were plated on TiC and Ti and analysed by electromicroscophy (SEM) after 6, 12 and 24 hrs. After 6 hrs TiC enhanced spreading and induced a more rapid formation of focal adhesions in comparison to Ti. Furthermore the effects of TiC on osteoblast gene expression was evaluated. We examined the regulation of important genes involved in osteoblast differentiation by semiquantitative PCR. Interestingly, alkaline phosphatase (ALP), osteocalcin (OC), α2 pro-collagen type I chain, BMP 2-5-7 and the osteopontin mRNAs expression were up-regulated. Additionally, since osteoblast contribute to osteoclastogenesis via cell-cell interaction and paracrine stimulation, we analyzed changes in the expression of osteoblast genes involved in modulation of osteoclastogenesis and osteoclast activity. No significative modulations were observed in IL-6, IL-1beta and M-CSF expression.

Finally, cell proliferation was evaluated by [3H]-thymidine incorporation. [3H]-thymidine uptake was significantly higher (1.8-fold) in bone cells cultured on TiC than on control. In conclusion, our data suggest that TiC might be used as a valid prostheses material, since improves osteoblast proliferation, adhesion and activity, without changing osteoclast functions.

P016

THE NONCOLLAGENOUS PROTEINS OF THE CHONDROID TISSUE EXTRACELLULAR MATRIX: IDENTIFICATION BY IMMUNOHISTOCHEMISTRY

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Chondroid tissue, which is a calcified tissue involved in the growth of particular skeletal sites like cranial sutures or mandibular symphysis, differs from bone and cartilage in that its extracellular matrix contains both types I and II collagen. This histological entity, which derives from the neural crests in the prechordal skeleton, constitutes the primordial vector of any membranous ossification. The present study was designed to further investigate the extracellular matrix of the human chondroid tissue by immunohistochemical screening of the noncollagenous proteins osteocalcin, osteonectin, osteopontin and bone sialoprotein. Osteocalcin seems to be almost absent from the early chondroid tissue, but was detected in more mature stages. Bone sialoprotein, osteopontin and osteonectin are much more concentrated in the matrix of chondroid tissue than in adult lamellar bone. These observations, which complete the morphological and biochemical description of chondroid tissue, strengthen its unique functional identity among the skeletal tissues.

P017

EFFECT OF HUMAN SERUM ON MESENCHYMAL STEM CELL (MSC) PROLIFERATION AND DIFFERENTIATION

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The cellular mechanisms mediating the age-related osteoblast defects are not known in details. In the present study we hypothesized that deficiency of signals in bone microenvironment is responsible for decreased osteoblast function observed during aging. As a model for bone microenvironment, we examined the effects of serum obtained from young (age 20–30 yo, n = 5) and old (65–80 yo, n = 5) female donors on cells proliferation and differentiation of human mesenchymal stem cell line transduced with human telomerase gene (hMSC-TERT) described previously by our group (Simonsen et al Nature Biotechnology 2002;20:592). We first assessed the ability of hMSC-TERT to grow and differentiate into osteoblasts and adipocytes in presence of human serum (HuS) as compared with fetal bovine serum (FBS). Growth and differentiation media supplemented with 5% HuS was as effective as media supplemented with 10% FBS in supporting hMSC-TERT cell proliferation (estimated by determining cell number in short-term and long-term cultures) and differentiation (assessed by gene expression markers and immunocytochemistry) in vitro. Sera from older donors stimulated cell proliferation as effective as sera obtained from young donors. Adipocyte differentiation media containing sera from young and old donors induced similar number of adipocytes and similar levels of adipocytic gene expression in hMSC-TERT. However, incubation of hMSC-TERT in sera of old donors resulted in impaired gene expression of osteoblastic gene markers: alkaline phosphates $56.1 \pm 10.6\%$, collagen type I $58.8 \pm 10.4\%$ and osteocalcin $67.2 \pm 14.1\%$ as compared to levels obtained in cells incubated in sera from young donors. In conclusion, human serum can support proliferation and differentiation of human mesenchymal stem cells in vitro. Age-related changes in composition of bone microenviroment may play an important role in the impaired osteoblast functions. The nature of these changes remain to be determined.

P018

EFFECT OF SIMVASTATIN AND ATORVASTATIN ON CELLULAR PROLIFERATION AND DIFFERENTIATION IN PRIMARY CULTURES OF HUMAN OSTEOBLASTS AND MG-63 CELLS

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Introduction: Statins are widely used for treatment of hypercholesterolemia. Recent studies showed an action on bone formation in mice model through an increase of BMP-2 gene expression related with osteoblast (OB) differentiation. We have performed an experimental study to analyze simvastatin and atorvastatin effects on cellular proliferation and gene expression of collagen type I (COL1AI) and osteocalcin (BGP) in primary cultures of human osteoblasts and MG-63 cells.

Material and methods: Explants of bone from patients without any metabolic diseases under orthopedic hip and knee procedures to make human osteoblast cultures have been used. Cell proliferation ELISA Colorimetric immunoassay based in BrdU incorporation at time 24,48,72 and 96 h. was used for quantification. Cell cultures were performed using DMEM medium with and without FBS at 10% and different concentrations of statins were added (10-9 M, 10-8 M, 10-7 M y 10-6 M) every 24 hours during the experiment. COL1AI and BGP expression levels were quantified by real time PCR after synchronization and 24 h. incubation with statins.

Results: Osteoblast proliferation was inhibited by statins at different concentrations. A correlation between inhibition and concentration of statins was found. When COL1AI and BGP expression were analyzed, a tendency of increase on OB function in presence of statins was detected. At greater statin concentrations higher levels of COL1AI and BGP seemed to be find, not reaching statistical significance. When BSA or FBS were added, cultures with BSA showed a significant increase of expression of COL1AI and BGP. Human OB and MG-63 cultures had similar results in this experimental model (numeric results showed in the poster).

Conclusion: Even though the inhibitory effect of statins in OB proliferation seems to exist an stimulatory effect of gene expression of COL1AI and BGP. A possible explanation for this effect may be a less proliferation of OB in favour of more OB function.

P019

HISTOMORPHOMETRIC ANALYSIS OF OSTEOID/BONE DEPOSITION AFTER THREE DIFFERENT ROOT CANAL THERAPY PROCEDURES

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Aim: The aim of this research was to analyse osteoid/bone deposition during initial phases of periapical lesion healing after three different protocols of root canal therapy.

Methods: Periapical lesions were induced in 9 mongrel dogs by exposing the pulps to the oral environment for 35 days. Seventeen root canals were instrumented and filled to the apical delta, which was confirmed radiographically (group 1). Animals in group 2 (21 root canals), after the same procedure, received 10 mg/kg doxycycline (Hiramicin, Pliva d.d., Zagreb, Croatia) per os daily for 12 days. In group 3 (20 root canals) we performed instrumentation reaching periapical area to the point determined by apex locator (EED 11, Struja, Zagreb, Croatia). Roots were filled to the length of physiological foramen which was confirmed radiographically. At the same day, one animal in groups 1 and 3 received intraperitoneal injection of vital dye Procion Red. Animals were sacrified 70 days after pulp exposure. Undemineralized unstained sections 5–7 mm thick were analysed for traces of doxycycline and vital dye with fluorescent microscope. Osteoid surface, osteoid thickness and osteoclast index were measured by light microscope on Toluidine Blue stained sections. Statistical analyses were performed by ANOVA test.

Results: Qualitative analysis of new bone formation with vital dye and doxycycline fluorescence showed lines close to the bone surface in group 1, but further away from bone surface in groups 2 and 3. There was statistically significant difference in osteoid thickness between groups 1 (15.62 mum $\pm$ 7.41) and 2 (14.32 mum $\pm$ 5.06) ($P < 0.05$), and groups 2 and 3 (16.26 mum $\pm$ 6.46) ($P < 0.001$). There was statistically significant difference in osteoid surface between groups 1 (10.34% $\pm$ 11.60) and 3 (33.21% $\pm$ 21.43) ($P < 0.001$), and groups 1 and 2 (30.36% $\pm$ 15.51) ($P < 0.001$). There was statistically significant difference in osteoclast index between groups 1 (111.34 mm^{-1} $\pm$ 115.46) and 2 (43.13 mm^{-1} $\pm$ 41.25) ($P < 0.001$), and groups 1 and 3 (27.01 mm^{-1} $\pm$ 39.03) ($P < 0.001$).

Conclusion: Administration of doxycycline in group 2 and more invasive therapeutic approach in group 3, enhanced healing potential demonstrated with lower osteoclast index and greater osteoid surface in comparison to group 1. It is debatable how doxycycline affects osteoid deposition because lowest osteoid thickness was measured in group 2.

P020

STUDY OF THE EFFECTS PRODUCED BY 17-BETA-ESTRADIOL AND RALOXIFENE ON FAS-MEDIATED APOPTOSIS IN MG-63 CELLS

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It is known that estrogen deficiency produces an increase in the production of the proinflammatory cytokine TNF-alfa in bone. TNF-alfa stimulates bone resorption and is implicated in bone loss after menopause. TNF-alfa can induce apoptosis in cells of the osteoblast lineage and this mechanism could regulate osteoblast cell number and bone loss. We investigated the effect of Fas activation in MG-63 osteoblast-like cells survival and the potential modulatory effect of estrogen (E) and raloxifene (R). Fas-mediated apoptosis was determined by flow cytometry of permeabilized, propidium iodide stained cells. MG-63 cell membrane Fas receptor expression was analyzed by flow cytometry and Fas mRNA levels were measured by semiquantitative rt-PCR using Fas specific primers. FasL mRNA, by semiquantitative rt-PCR, and protein level, by Western blot, were also measured. MG-63 cells were cultured in DMEM without FBS and treated with TNF-alfa (200U/ml) or TNF-alfa and 10-8 M E or R for 48 h. TNF-alfa did not modify spontaneous MG-63 cells apoptosis, but addition of agonistic anti-Fas antibody CH-11 (1microg/ml) to cultures during 24 h induced a significant increase in apoptosis: $419 \pm 66\%$ with respect to untreated cells (100%). In these conditions, pretreatment of cells with TNF-alfa further increased the number of apoptotic cells: $2466 \pm 363\%$ with respect to untreated cells (100%). The addition of 10-8 M E or R did not modify the level of apoptosis induced by CH-11 and TNF-alfa. Fas mRNA expression significantly increased in MG-63 cells pretreated with TNF-alfa for 24 h: $200 \pm 70\%$ versus untreated cells (100%). Expression of Fas receptor increased significantly ($158 \pm 58\%$) in cells pretreated with TNF-alfa for 48 h respect to untreated cells (100%). Neither E nor R modulated the increase in Fas at the mRNA or protein level induced by TNF-alfa. MG-63 cells constitutively express FasL mRNA and protein. We did not observe any change in FasL expression under our experimental conditions. In conclusion, while MG-63 cells are sensitive to Fas-mediated apoptosis, specially in the presence of certain cytokines, such as TNF-alfa, hormonal therapies for postmenopausal osteoporosis do not modulate this form of osteoblast cell death.

P021

CONGENITAL PSEUDARTHROSIS IN TWO CASES OF NEUROFIBROMATOSIS TYPE 1: IMPAIRED DIFFERENTIATION AND ACTIVATION OF BONE STEM CELLS

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Neurofibromatosis type 1 (NF1) is one of the most common heritable diseases, affecting one in 3,500 newborns worldwide. Skeletal manifestations of NF1 include congenital bowing and pseudarthrosis (PA) of tibia. The gene for NF1 encodes neurofibromin, a protein that negatively regulates signals transduced by Ras proteins. The roles played by neurofibromin in bone development and pathogenesis of bone abnormalities in cases of NF1 patients have not studied using bone cell experimentation.

In this study, mesenchymal stem cells (MSC) were cultured from two children with NF1 from site of the PA of the tibia. MSCs were also cultured from three healthy controls and from the iliac spine of the NF1 patient. The ability of MSCs to differentiate into bone forming cells *in vitro* was examined by specific alkaline phosphatase (ALP) activity, secretion of amino-terminal propeptide of type I procollagen (PINP), calcium quantification and von Kossa staining. MSCs were also analysed using western blot analysis (neurofibromin and phosphorylated p44/42) and indirect immunofluorescence labeling (neurofibromin and vimentin). Pseudarthrotic tissues were analysed using immunohistochemistry (HE-staining, neurofibromin and p-p44/42) and *in situ* hybridisation for NF1 mRNA.

It was found that the ALP, PINP, proportional area of positive von Kossa staining, calcium deposition and NF1 protein expression levels were significantly lower in the MSCs from the site of the PA compared to controls and even to NF1 patient cells from iliac spine. Interestingly, p-p44/42 MAPK levels were elevated in the NF1 cells only at the site of PA. MSCs from the site of PA showed no colocalization to vimentin which is in contrast to NF1 cells from iliac spine and controls. Pseudarthrotic tissue displayed prominent bone remodeling and p-p44/42 MAPK labeling was markedly elevated in endosteal osteoblasts adjacent to the PA.

These observations demonstrate for the first time a disturbed bone development at the cellular level of the pseudarthrosis in NF1 patients. We hypothesize that loss of heterozygosity at the site of the PA and impaired NF1 protein function lead to disturbed ras-GAP (GTPase activating protein) signalling. This causes impaired osteoblast differentiation and activation of MSCs. In conclusion, our findings suggest that local loss of heterozygosity affects the MSCs at the site of tibial bowing and pseudarthrosis in NF1 patients.

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P022

ONCOSTATIN M INDUCES DIFFERENTIATION OF OSTEOSARCOMA AND BONE MARROW MESENCHYMAL STEM CELLS INTO GLIAL-LIKE CELLS

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Oncostatin M (OSM) is a multifunctional cytokine of the Interleukin-6 (IL-6) family which is implicated in embryonic development, organogenesis, differentiation, inflammation and regeneration of various tissues, mainly the liver, bone, the central nervous and hematopoietic systems. One particularity of OSM rely on its growth inhibitory and pro-differentiating effects on a variety of carcinoma cell lines such as melanoma, breast cancer and osteosarcoma, providing arguments for a therapeutic application of OSM.

In this study, we show that OSM inhibits the growth of rat osteosarcoma cell lines as well as non transformed mature osteoblasts, in correlation with the induction of the cyclin dependent kinases inhibitor p21WAF1. However, OSM does not induce osteoblastic differentiation in these cells but rather reduces osteoblast markers such as alkaline phosphatase (AP), type I collagen (Coll I), osteocalcin (OC) and bone sialoprotein (BSP), leading to strong inhibition of mineralized nodules formation. This shutt-off of osteoblastogenesis is restricted to differentiated cells since OSM effectively stimulates AP, Coll I, BSP and OC and bone nodules formation in early, but not late, bone marrow mesenchymal stem cell (BMSC) cultures.

These results raise the possibility that OSM induces trans-differentiation of osteoblastic cells into another lineage. We observe that OSM induces mRNA and protein levels of the Glial Fibrillary Acidic Protein (GFAP) and Nestin, in osteosarcoma cells or BMSC, suggesting a differentiation into pre-glial cells. Ultrastructural examination of OSM-treated osteosarcoma cells confirms their glial-like phenotype with an elongated shape, the formation of a network of long cell processes with numerous desmosome-like and gap junctions as well as bundles of microfilaments. Cytoskeleton reorganization could be induced by GFAP, a major protein implicated in intermediate filaments assembly.

These results highlight the particular plasticity of OSM-treated osteosarcoma cells and BMSC that can efficiently trans-differentiate into glial-like cells, offering new lines of therapeutic investigations for osteosarcoma and degenerative diseases of the central nervous system.

P023

IDENTIFICATION OF GENES IMPLICATED IN MINERALIZATION OF FISH BONE-DERIVED CELL LINES

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Mechanisms of tissue mineralization in higher vertebrate models are complex and still insufficiently understood, and study of simpler models derived from lower vertebrates has shown in multiple occasions to be valuable in identifying key mechanisms. In this context, recently developed VSa13 and VSa16 fish bone-derived cell lines (Pombinho et al., 2004), appear as promising *in vitro* model systems. We describe here the construction of a bone-specific cDNA macroarray and its use to assess gene expression and identify proteins involved in the mineralization of VSa13 and VSa16 cell extracellular matrix. Different strategies were used to obtain the cDNAs to construct the array: (1) PCR amplification using specific primers designed against sequences that have been reconstructed from ESTs and genomic DNA fragments present in public databases, (2) PCR amplification of fish homologues using degenerate primers designed against conserved regions that have been identified from alignment of already known sequences (mainly mammalian proteins), and (3) the cloning of differentially expressed cDNAs using subtractive libraries prepared from VSa13 and VSa16 cell lines grown under normal or mineralizing conditions. All these approaches identified cDNAs coding for fish extracellular matrix proteins, transcription factors, nuclear and xenobiotic receptors, hormones, and growth factors. A standard macroarray containing selected cDNAs was constructed and probed with mRNA prepared from control or mineralized cells. Results are being analyzed and should provide important data on genes involved in tissue mineralization and on the similarities/differences with mammalian systems.

Pombinho et al. (2004) Development of two bone-derived cell lines from the marine teleost Sparus aurata; evidence for extracellular matrix mineralization and cell-type-specific expression of matrix Gla protein and osteocalcin. Cell & Tissue Research, in press

P024

EXTRACORPOREAL SHOCK WAVES INDUCE PRODUCTION OF BONE GROWTH FACTORS IN OSTEOBLASTS

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The molecular events following shock wave treatment of bone are too a large extent unknown. Nevertheless patients with osteonecrosis and non unions are already treated partly successfully with extracorporeal shock waves. In our study we isolated osteoblasts from bone pieces of patients undergoing knee or hip-replacement surgery, subjected the cultured cells to shock waves and investigated the supernatants for bFGF, TGFbeta1 and VEGF.

After collagenase treatment cells were cultivated and characterised using FACS analysis. 95% of the cells were CD 44+ and CD 34−, CD 14−, CD 3− and CD 4−. After conditioning with an osteogenic medium containing Dexamethasone, Ascorbate and Beta-Glycerolphosphate cells showed a homogenous mineralisation-pattern in the v. Kossa staining.

These cells were subjected to 250 or 500 shock waves at 25 kv using an experimental electrohydraulic lithotripter (Dornier XL 1). After shock wave treatment cell viability was determined and cells were seeded at 1×10^5 cells in 12 well plates. After 24, 48 and 72 h the cell number was determined and the supernatant was frozen. The levels of the bone and vascular growth factors bFGF, TGFbeta1 and VEGF were examined using ELISA. A control group was treated in the same way without receiving shock waves.

After 24 h there was a significant increase in bFGF levels ($P < 0.05$) with significant correlation ($P < 0.05$) to the number of impulses. TGFbeta1 showed an time dependent increase with a peak at 48 h which was not significantly different from the control group. VEGF showed also a tendency to be shock wave induced but with no significance.

For the first time it was shown that bFGF as an important growth factor in new bone formation is produced by human osteoblasts treated with shock waves. This may be one piece in the cascade of new bone formation following shock

wave treatment and may lead to a more specific application of shock waves in orthopaedic surgery.

P025

NON-GENOMIC ANDROGEN ACTIVATION OF PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALING PATHWAY IN MC3T3-E1 OSTEOBLASTS

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Androgens have important effects on the human skeleton in both males and females. Hypogonadism in men is associated with increased bone turnover and bone loss, which is reversed after treatment with androgen. However, the mechanism of androgen action on bone metabolism remains unknown. The aims of this current study were to determine the effect and mechanism of androgen action on the osteoblast cells. Here we demonstrated that 5?-dihydrotestosterone (DHT) accelerates cell proliferation of MC3T3-E1 cell line in time and dose dependent manner. The specific phosphatidylinositol 3-kinase (PI 3-kinase) inhibitor LY294002 and kinase-deficient Akt mutant can repress the androgen mediated cell proliferation. Western blot analysis showed that DHT, 17?-estradiol and testosterone(T) induce a rapid and transient phosphorylation of Akt in MC3T3-E1 cells. This activation reached to the climax after 15 min and gradually diminished after 60 min of DHT treatment. Nifedipine and Verapmil, the voltage-gated calcium channels blockers enhanced the phosphorylation of Akt induced by DHT and blockade of the intracellular calcium by BAPTA/AM prevented the DHT-induced Akt phosphorylation. Fluorescence Microscopy showed an evidence increase in immunostaining intensity in the nuclear interior after androgen treatment but no changed in the subcellular distribution of Akt when hydroxyflutamide (HF) or LY294002 pretreatment was administrated to the cells. In addition, RNA-interference against androgen receptor (RNAi-AR) each prevented DHT-induced Akt phosphorylation and cell proliferation. These findings strongly suggest that this non-genomic action is mediated by androgen receptor and the androgen-induced activation of Akt is an important step in androgen/AR signal pathway that mediates osteoblast proliferation.

P026

EFFECT OF NICOTINE ON HUMAN OSTEOBLASTS CULTURED ON COMMERCIALLY PURE TITANIUM DISKS: A PRELIMINARY STUDY

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Smoking is a major risk of endoosseous dental implants failures. The aim of this preliminary study is to examine the effect of different nicotine concentrations and time exposures on human osteoblasts cultured on commercially pure titanium disks.

Confluent cultures of human osteoblasts on commercially pure titanium disks were incubated with varying concentrations of nicotine (10-2 to 10-8 mol/L) added to the culture medium for 2 to 72 hours. Alkaline phosphatase activity was measured by spectrofluorometry and cell morphology was observed by scanning electron microscopy (magnification : X200 to X5000).

Nicotine doesn't seem to modify the alkaline phosphatase activity of human osteoblasts cultures for any concentration and time exposure. Morphologic changes occurred in nicotine-treated cells including rounding up and detachment.

Our preliminary results suggest that nicotine has critical effects on morphology of human osteoblasts cultured on commercially pure titanium disks. Further investigation will be necessary to evaluate nicotine impact and tobacco use on the osseointegration of dental implants.

P027

TISSUE DISTRIBUTION OF TELOMERIZED HUMAN MESENCHYMAL STEM CELLS AFTER SYSTEMIC INFUSION

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Systemic transplantation of mesenchymal stem cells (MSCs) has been suggested as a potential treatment option for generalized bone diseases and bone loss. Previous studies have demonstrated that the ability of MSC to home and to survive after systemic transplantation in vivo was variable, which may be explained by the short life span of MSC after transplantation in vivo. In order to test this hypothesis, we examined the in vivo distribution of a human MSC cell line transduced with human telomerase gene (hMSC-TERT) that exhibits extended life span in vitro (Simonsen et al, Nature Biotechnology 2002;20:592) and may thus have a prolonged life span in vivo. hMSC-TERT were labelled with enhanced green fluorescent protein (eGFP) by retroviral transduction. eGFP-hTERT-hMSCs retained the ability to differentiate into osteogenic and adipogenic lineages in vitro and to form bone after subcutaneous implantation with hydroxyapatite/tricalcium phosphate powder in NOD/SCID mice.10⁶freshly trypsinized, viable eGFP-hTERT-hMSCs were transfused into NOD/SCID mice by intravenous (IV) or left ventricle intracardial (IC) routes. Mice were killed after one hour (IV, n = 4 and IC, n = 4), one week (IV, n = 4 and IC, n = 5), or four weeks (IV, n = 4 and IC, n = 2), and formalin fixed, paraffin embedded sections were prepared for the detection of eGFP fluorescent cells. Polymerase chain reaction (PCR) was performed on frozen tissue samples to detect the eGFP transgene. One hour after IV injection, cells were found almost exclusively in lungs (4 out of 4 animals), whereas after IC injection, distribution was more widespread with fluorescent cells detected in heart (4/4), lung (4/4), kidneys (4/4), liver (2/4), spleen (1/4), brain (1/4), intestine (1/4), skin (1/4), skeletal muscle (1/4), and bone marrow (1/4). This pattern of distribution was confirmed by PCR. After four weeks, only few cells remained and these were restricted to lungs after IV injection (3/4), and to heart (1/2) and kidneys (1/2) after IC injection. None were found in bone at any time point.

In conclusion, eGFP-TERT-hMSCs are trapped in lungs after intravenous injection, whereas after intracardiac injection, cells can be found in most organs examined although to varying extent. eGFP-hTERT-hMSCs did not home specifically to bone marrow or bones. Future interventions are needed to increase the homing and integration of hMSC into the skeleton.

P028

ORGANIC BONE MATRIX IS DEGRADED BY OSTEOBLAST AND BREAST CANCER CELLS LEADING TO RELEASE OF TYPE I COLLAGEN FRAGMENTS *IN VITRO*

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Osteoblasts are usually referred as bone forming cells and osteoclasts as bone degrading cells. Some recent studies have, however, suggested that other cells in bone might also participate in degradation of organic bone matrix. In this study, we examined collagen degradation activity and bone synthesis by osteoblast precursor cells and MDA-MB-231 breast cancer cells.

Mesenchymal stem cells, derived from human bone marrow, were cultured in vitro and induced to differentiate into osteoblast-like cells. After a two-week culture period, most of the cells were alkaline phosphatase (ALP) positive, and there were no TRACP (tartrate-resistant acid phosphatase) positive cells in cultures as confirmed by staining. These pre-osteoblast cells, as well as breast cancer cells, were cultured on bovine bone slices, which were resorbed by rat osteoclasts and thoroughly washed after the first culture.

We found that both breast cancer and osteoblast cells degraded bone collagen, and released a significant amount of type I collagen degradation product (ICTP) into the culture medium. Degradation of organic bone matrix was also demonstrated by field emission scanning electron microscopy (FESEM). Matrix metalloproteinase (MMP) inhibitor Galardin dramatically inhibited the release of ICTP into culture medium, while cysteine proteinase inhibitor or estrogen had no effect. Western blot analysis revealed that MMP-8, −13 and −14 were present in pre-osteoblast cells, and gelatin zymography showed activity of MMP-2 in cultured cells. Osteoblast precursor cells also synthesised high amounts of PINP (aminoterminal propeptide of type-I procollagen), which was stimulated by estrogen. The culture substrate influenced the phenotype of cultured mesenchymal cells, and the increase in PINP synthesis was highest in cells cultured on bone slices. Breast cancer cells did not synthesise type I collagen.

In conclusion, we demonstrate here that human osteoblast precursor cells as well as metastatic MDA-MB-231 breast cancer cells are capable of degrading bone collagen remnants, which are left to the bottom of resorption lacunae by osteoclasts. Organic matrix degradation was facilitated by MMPs in both of these cells. It was further demonstrated that pre-osteoblasts also synthesise new collagen, which was stimulated by estrogen. We hypothesise that bone degradation by the very same cells that are responsible for bone formation may be an important step coupling bone formation into bone resorption.

P029

THE INFLUENCE OF LOW-INTENSITY, HIGH-FREQUENCY VIBRATION ON BONE FORMATION IN AGED RATS

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In a previous study, it was found that low-intensity, high-frequency vibration prevented the decrease in strength of long bones associated with ovariectomy of adult rats, and increased the periosteal bone formation. In the present study, the effect of low intensity, high frequency vibration on bone formation and bone strength was studied in an aged rat model. Twenty-three-month-old male rats were allocated randomly to the following groups: 1) start control, 2) mock vibrated control and 3) vibration at 45 Hz ($3.0 \times g$). Vibration was given 30 min/day for 90 days. During vibration the rats were placed in a box on top of the vibration motor. The amplitude of the vibration motor was 1.0 mm. The animals were labelled with calcein at day 79 and with tetracycline at day 86. The tibia mid-diaphysis was studied by mechanical testing and dynamic histomorphometry. The vibration increased (2p = 0.03) the periosteal bone formation rate ($103 \times 1.66 \pm 0.24$ cubic micrometer/day, mean ± SEM) by 2 fold compared with the mock vibrated group ($103 \times 0.82 \pm 0.27$ cubic micrometer/day) at the tibia mid-diaphysis. Furthermore, the percentage labelled circumference of the vibrated group ($35.5 \pm 4.3\%$) was increased (2p < 0.03) by 86% compared with the mock vibrated group ($19.1 \pm 5.4\%$). These alterations did not result in significant increases in the breaking strength of the tibia diaphysis of these old rats. In conclusion, the results support the hypothesis of a possible beneficial effect of passive physical loading on the preservation of bone in aged animals.

P030

MIGRATION ARREST STIMULATES NODULE FORMATION IN OSTEOBLAST CULTURES

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We have used bone nodule formation assay to follow the effects of proteinase inhibitors on bone formation *in vitro*. Rat primary osteoblasts were induced from bone marrow stromal cells in the presence of beta-glyserophosphate, ascorbic acid and dexamethasone for 2–4 weeks. ALP-positive, early osteoblasts (2 weeks in culture) were plated inside type I collagen matrix (350 000 cells/well) and cultured in the presence of MMP-inhibitors (1,10-fenantrolin, marimastat) or specific MMP9-antisense sequence. After 2 weeks, the samples were fixed and von Kossa-stained to visualize bone nodules. The presence of gelatin- and type I collagen degrading enzymes was studied with help of zymograms and immunoassays. Late osteoblasts (3–4 weeks in culture) were isolated with type I collagenase-treatment afer microscopically evident collagen formation. Isolated cells were seeded on 24 well plates (20 000 cells/well) and cultured in the presence of MMP-inhibitors (1,10-fenantrolin, marimastat, galardin) or E-64 (cystein proteinase inhibitor). After 7 days, the cells were fixed and stained either for ALP or von Kossa.

In the early osteoblast cultures, a dramatic degradation of the type I collagen network was seen in the control cultures, and, to a lesser extent, in the 1,10-fenantrolin -treated cultures. The matrix remained intact in the marimastat- and antisense-treated samples due to decreased MMP-activity, as shown by the zymograms. When the von Kossa- stainings were studied, we found a significant increase in the number of bone nodules in the marimastat- or antisense-treated samples, as compared to the control group. This suggested that early osteoblasts were capable of degrading the type I collagen matrix that they were plated in, and that matrix degradation could be blocked with MMP-inhibitors. Similarly, late osteoblasts formed more bone nodules in the presence of MMP-inhibitors, as compared to the control group. Cystein proteinase inhibitors, however, had no effect on nodule formation. There best inhibition was seen with the specific antisense molecules and with galardin, while 1,10-fenantrolin showed non-significant inhibition of MMP-activity.

In conclusion we find that osteoblasts secrete a vast population of MMPs that are involved in cell migration and invasion. Blocking the cell movement with MMP-inhibitors stimulated bone formation *in vitro*. The role of specific MMPs in nodule formation are currently under investigation.

P031

CYCLOOXYGENASE INHIBITORS INTERFERE HUMAN MESEMCHYMAL STEM CELL DIFFERENTATION INTO OSTEOBLASTS BY CONVERTING THE MESENCHYMAL CELLS INTO ADIPOCYTES

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Non-steroidal anti-inflammatory drugs (NSAID's) inhibit cyclo-oxygenase activity and are widely used as musculoskeletal painkillers. *In vitro* studies have suggested that prostaglandins may play a role in osteoblast recruitment and differentiation. NSAID's have been reported to influence bone by causing delayed unions and non-unions of fractures in animal models. The clinical data, however, is controversial.

In this study we examined the effects of three different cox-2 inhibitors, firstly indomethacin as the least specific, secondly clinically used parecoxib and thirdly NS398, which is a selective inhibitor but used only *in vitro*. Human bone marrow derived mesenchymal stem cell (hMSC) differentiation into osteoblasts or adipocytes and mouse osteoclast differentiation and activity were assayed. The osteoblast differentiation was characterised by measuring the specific alkaline phosphatase activity (ALP), PINP and PIIINP and calcium deposition. Osteoclasts were studied by counting the numbers of differentiated osteoclasts and resorption activity of mature osteoclasts.

After a two-week culture period the PINP production was significantly and dose-dependently decreased by NS398 and parecoxib. Simultaneously, the amount of adipocytes increased significantly as measured by morphology and also by red oil-o stained areas. In the osteoclast assay the number of rat osteoclasts decreased dose-dependently by indomethacin, whilst there were no significant changes by parecoxib or NS398. In the osteoclast differentation assay NS398 decreased the number of osteoclasts dramatically and dose-dependently.

In conclusion our data strongly suggests that the presence of specific cox-2 inhibitors interferes the differentiation of mesenchymal stem cells converting them to adipocytic lineage in conditions that usually promote osteoblast differentiation. Indomethacin affected only osteoclasts. These data suggests that routine use of cox-2 inhibitors in the treatment of post-fracture pain might influence the hMSCs and fracture healing.

P032

FRACTURE HEALING IN ALPHA10 INTEGRIN-DEFICIENT MICE

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Introduction. The integrin subunit alpha10 forms a heterodimer with the beta1 subunit and is closely related to the other collagen-binding integrins alpha1beta1, alpha2beta1 and alpha11beta1. alpha10beta1 is the predominant collagen-binding integrin during embryonic cartilage development and appears simultaneously with the onset of chondrogenesis (1). Fracture repair of the long bones provides an interesting model for endochondral ossification in adult mice as it recapitulates the same steps encountered during embryonic bone formation.

Methods. In this pilot study, bone from the alpha10 integrin knockout (KO) mouse was challenged by creating stabilized tibial fractures. Extracellular matrix (ECM) development was then followed 7–28 days post-fracture by X-ray, histological analysis and Northern/real time QPCR techniques.

Results. The organisation of the ECM structural components was more irregular in KO calluses including the periosteum than in the wild-type (WT) controls. High levels of type II collagen and aggrecan mRNA were observed in 7-day old KO calluses, two days prior to that in the WT controls. At day 14, the mRNA for these two cartilage-specific proteins had decreased in KO specimens below that of the WT controls. At day 7, type X collagen, which is considered a marker for chondrocyte hypertrophy, was expressed only in KO calluses. By days 9 and 14 similar levels of collagen X were observed in the controls (WT). During the second week of healing, the expression of type I collagen increased somewhat faster in the KO samples. However, by day 28, the type I collagen signal was not as high as in the WT controls. The callus size of KO mice was not altered at any of the time points studied when compared to WT mice.

Conclusion. These preliminary results indicate that the endochondral bone formation proceeds faster during fracture healing in mice deficient in the integrin alpha10-subunit than in WT mice.

Reference

1. Camper et al. Cell Tissue Res 306:107–116, 2001

P033

COURSE OF HEALING IS INFLUENCED BY EARLY FIXATION STABILITY

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Mechanical conditions are known to influence healing outcomes. The aim of this study was to investigate the healing path of fixation systems of differing mechanical stability and to identify the upper limit of fixation stability required for healing.

Two groups of sheep underwent a mid-shaft tibial osteotomy. The osteotomy was stabilized with either a hard (H) or soft (S) mono-lateral external fixator. The course of healing was investigated at 2, 3, 6, and 9 weeks post-op. Computer tomography (CT) scans were performed at sacrifice. The fractured tibiae were explanted and tested in torsion until failure. Sagittal histology sections (4 mm) were stained with Safranin-Orange/von Kossa and Safranin-Orange/Lightgreen. Tissue differentiation was quantified for bone, cartilage and fibrous tissue according to location. The callus densiometric moment of inertia was determined from CT scans.

Histomorphometric analyses revealed that the maximum callus area was reached at 3 weeks in group H (275 ± 35 mm2 (Mean $\pm$ SD)) and at 6 weeks in group S (346 ± 67 mm2). At 9 weeks group S had both a larger callus diameter and callus area ($P < 0.05$) but no differences in mechanical strength were determined. Fibrous tissue content at 3 weeks was similar, but in group H the fibrous tissue content dropped dramatically over the next 3 weeks, while in the group S it remained high until the 6th week. Mineralised bone area in group H increased up until the 6th week but no difference was determined between the 6th and 9th week. In contrast, the mineralised bone area in group S increased till the final investigated point at 9 weeks. The moment of inertia in both groups increased over the healing period. At 3 weeks the MOI of both callus groups reached approximately 100% of the corresponding intact tibia. At 6 weeks, the MOI in the soft fixator group tended to be greater (H: 114%, S: 125%). The trend increased at the final investigated time point of 9 weeks (H: 164%, S: 187%).

While decreased fixation stability seems to have had a negative effect on the course of healing, an initial delay and an over-compensatory final stage, the same mechanical callus strength was reached. The less stable fixator did not result in delayed healing, but a less optimal and less efficient healing path was taken. An upper limit of stability required for successful healing remains unknown, however an upper limit in which healing is less optimal has been determined.

P034

CBFA1 REGULATES THE EXPRESSION OF CD99, A EWING TUMORS MARKER

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Among genes known to be specific of different maturation stages of bone differentiation, Cbfa1 (core binding factor alpha 1) is currently defined as an essential transcription factor for osteogenesis and osteoblastic differentiation. The purpose of this study was to identify Cbfa1-responsive genes. In this end, Saos-2 an osteosarcoma cell line was stably transfected with a dominant-negative mutant of Cbfa1 (DeltaCbfa1). Comparison of gene expression patterns of selected clones by differential display allowed identification of Cbfa1 suppressed or induced genes such as CD99/MIC2. Level of this gene expression was around 10 fold higher in cells overexpressing the dominant-negative form (DeltaCbfa1) than in control cells (transfected with the vector alone). CD99 is an integral transmembrane protein that is described in Ewing tumor family for its functional role in inducing apoptosis and for its use as a diagnostic tool for the differential evaluation of small round cell tumor of childhood. CD99 is a sensitive but non-specific marker for Ewing tumor cells because its expression has been demonstrated in a large series of normal tissues and tumor types. Engagement of CD99 in hematopoietic system has been described for its implication in cell-to-cell adhesion, apoptosis of immature thymocytes and up-regulation of several transmembrane proteins. Even if CD99 function is not specifically defined, it's the first time that its positive regulation by a transcriptional regulator like Cbfa1 is observed.

P035

STRETCH-INDUCED MODULATION OF MATRIX METALLOPROTEINASE EXPRESSION IN DIFFERENTIATING OSTEOBLASTS VIA EXTRACELLULAR SIGNAL-REGULATED KINASE-1/2

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Mechanical loading is known to influence bone modeling and remodeling processes. Matrix metalloproteinases (MMPs) play an important role in the remodeling of collagenous extracellular matrices, including skeletal tissue. To understand the relevance of mechanical loading in relation to MMP levels in osteoblasts, we examined the effect of stretch and differentiation stage on MMP−3, −9, and −13 mRNA expression levels. Because the extracellular signal-regulated kinase-1 and −2 (ERK1/2) pathway plays an important role in the transduction of mechanical signals, we tested whether blocking this pathway can modify the effect of mechanical stretch on MMP expression.

A human osteoblastic cell line (SV-HFO) was cultured for 21 days in the presence of dexamethasone and β-glycerophosphate to enter a defined pathway of osteogenic differentiation, resulting in mineralization of the extracellular matrix (ECM) after 21 days. After 7, 14, or 21 days of culture, stretching experiments were performed for 15 minutes at a frequency of 0.5 Hz with an adapted loading unit (Flexercell, McKeesport, PA) producing strains of 0.4%. Experiments were performed in the presence or absence of a selective mitogen-activated protein kinase kinase (MEK)1/2 inhibitor U0126 (Promega). Cells were collected 2, 6 or 22 hours after stretching for mRNA extraction. Relative gene expression was measured using Q-RT-PCR (Taqman® assay). MMP-3 (stromelysin), −9 and −13 levels remained similar during osteoblast differentiation, with average CT values between 31 and 33. At day 7, stretching for 15 minutes resulted in an increase of MMP-3, up to 25-fold after 24 hours. The same effect, but less pronounced, was seen at day 14. At day 21, no effect of stretch was seen. Stretching experiments performed in the presence of the MEK1/2 inhibitor U0126 showed a strong suppression of the stretch-induced increase in MMP-3 expression at all tested days. MMP-9 levels increased upon stretching at all tested days, but the increase was less abundant than for MMP-3. Also, the expression was not affected by the presence of U0126. No clear effect of stretch was observed for MMP-13.

In conclusion, we show an interaction between stretch and MMPs in human osteoblasts. Short term (15 minutes) cyclic stretch induces MMP-3 and −9 expression. For MMP-3, this induction is dependent on osteoblast differentiation and is mediated via the ERK1/2 pathway. So far this seems to be specific for MMP-3.

P036

THE OSTEOGENIC POTENTIAL OF THE INITIAL FRACTURE HEMATOMA

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Fracture repair is a complex biological event. Initial fracture hematoma contains cytokines and growth factors who are mandatory for recruitment of mesenchymal progenitor cells and their differentiation to osteoblasts. Even though this is generally accepted, it remains so far unclear to what extend and when cytokines and growth factors emerge in the fracture hematoma. The goal of this study was to charakterize the initial fracture hematoma. Six female Merinomix sheep received bilateral, transverse, mid-tibial open osteotomies with a 3 mm gap using a standard osteotomy model (Klein et al.2003). The osteotomy site was stabilized with an external fixator. The fracture hematoma at the osteotomy underwent irrigation at 1 hour and 4 hours after osteotomy and was compared to peripheral blood. Total RNA isolation was performed with the Rneasy Maxi Kit (Qiagen, Germany). Transcription of RNA to cDNA followed by RT-PCR. Following ovine primers were used for amplification: ovine IGF 1, IGF 2, VEGF, PDGF, Il1β, Il 6, TGFβ, osteopontin and procollagen1, BMP2 and BMP4. Semiquantitative PCR reactions were set up using platinum Taq-DNA Polymerase and run on a thermocycler (Eppendorff, Germany). PCR analysis showed amplification of the following osteogenic markers: Il 1, procollagen 1a and osteopontin were amplified only in fracture hematoma. Osteopontin expression was stronger at 4 h after osteotomy. Il 6 was expressed at both time points in hematoma but to lesser extent in peripheral blood. VEGF was strongly amplified similar to PDGF at 4 h, but not in the peripheral blood. BMP2 amplification was found in blood and in osteotomy. BMP4 expression was weaker in hematoma and also in the blood. The temporal analysis of the expression of osteogenic markers showed amplification of all proteins at very early time points compared to peripheral blood most cytokines and growth factors were already expressed 1 to 4 h post osteotomy. Interestingly there was an expression of procollagen 1 as early as 1 h after osteotomy. These results go ahead with the study of Cho et al. 2002, who showed a peak expression of collagen I in the first 24 hours after fracture. There was a strong expression of VEGF, PDGF and osteopontin 4 h after osteotomy only in the fracture hematoma and not in the blood. This could be an evidence for the correlation between hypoxia, angiogenesis and migration of primitive mesenchymal cells stimulated by VEGF and PDGF, BMPs and osteopontin (Rasubala et al. 2003).

P037

MECHANICAL LOADING OF THE MURINE TIBIAE PROVIDES UNIQUE EVIDENCE SUPPORTING ADAPTATIVE REMODELLING OF TRABECULAR BONE AS WELL AS INCREASED CORTICAL BONE FORMATION IN NOVEL NON-INVASIVE AXIAL LOADING OF MOUSE TIBIAE

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Although bone's response to mechanical loads can be studied in several *in vivo* models, axially loading the murine tibia, non-invasively, through its articulations would have advantages. These include: i) larger bone size; ii) scope to apply disuse; iii) larger region of cancellous bone volume, and iv) scope to apply strain distributions similar to those engendered during locomotion. Herein, we explore such a model in which cortical and cancellous bone adaptation to mechanical loading is examined.

We used 8 groups of female, 14 week old C57Bl/J6 mice: 5 groups were loaded so that 1200–2000mE were produced on the lateral midshaft cortex. 2 groups were submitted to sciatic neurectomy (SN, 114 days) with and without loading. 1 group was sham-operated. Animals were loaded on alternate days for 3 weeks. They received fluorochrome label on third and last days and were killed 3 days later. Tibiae (+contralateral controls) were embedded; transverse confocal images from 5 diaphyseal sites were analysed histomorphometrically. Proximal tibial epiphyses were analysed by μCT scans, which extended 0.75 mm distally of the growth plate.

We found that murine tibia midshaft exhibited low physiological strains during normal locomotion (<300mE). Loading at 2000mE significantly increased periosteal bone formation at all sites. Increased endosteal formation was only evident at sites distal to the midshaft. In contrast, an increase in cortical bone formation was not observed in tibiae loaded at less than 1200mE. μCT scans showed that loading induced significant increases in trabecular bone thickness; SN-induced 'disuse' significantly decreased bone volume fraction and increased trabecular spacing, while loading after 'disuse' also increased trabecular thickness.

We show that: i) loading induces an osteogenic response of trabecular bone architecture and, at least partly, rescues the effects of SN, ii) tibial cortices, which encounter low strains during locomotion, require these to be exceeded during axial loading to induce bone formation. These results suggest that the murine tibia offers a novel model for studying the effects of loading on cortical and cancellous bone. To our knowledge this is the first evidence for direct load-induced changes in trabecular architecture in an animal model.

P038

OSTEOPETROSIS: CLINICAL ORAL IMPLICATION IN THE PREVENTION OF OSTEOMYELITIS

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Introduction: Alber's-schonberg's Osteopetrosis, an inherited disease with autosomal transmission, is characterized by defective osteoclast function causing generalized sclerosis of bone and reduction of marrow. Osteomyelitis of the jaws is a well documented complication of osteopetrosis. At present, there is little in the literature that address the prevention and management of osteomyelitis and osteopetrosis.

The aim of this report is to present an illustrated treatment protocol on the prevention and management of osteomyelitis of the jaw associated with osteopetrosis.

Methods: Hundred and sixty patients with osteopetrosis where treated at KFSH & RC in the period 1975–2003. Many of these patients underwent surgical and non-surgical dental treatment. All cases that require dental surgical treatment where treated with pre and post operative antibiotics. Group of cases that presented with osteomyelitis post dental extraction where used as a control.

Results: All the patients treated have no post-operative complications and the healing of the bone was established.

Conclusion: There is no generally recognized means of curing progressive osseous distraction. Therefore, the prevention of osteomyelitis is very important in patients with osteopetrosis that require oral surgical intervention. The treatment protocol proposed in this report was successful in preventing osteomyelitis in our population with osteopetrosis.

P039

FOREIGN BODY REACTIONS ASSOCIATED WITH THE USE OF BIOABSORBABLE PLDLLA-CAGES

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Introduction: The purpose of this study was to evaluate the mid-term osteointegration of two bioabsorbable cages in a sheep cervical spine fusion model.

Material and Method: 60 sheep underwent C3/C4 discectomy and fusion using following implants: group 1: autologous tricortical iliac crest bone graft (control, n=12); group 2: bioabsorbable Poly-(L,DL-lactid) 70/30 acid cage (PLDLLA cage) plus autologous cancellous bone grafts (n=24). Group 3: bioabsorbable polymer composite/CaP-cage (PCC cage) plus autologous cancellous bone grafts (n=24). After 12, 36 and 52 weeks 4 animals of group 1 and 8 animals of group 2 and 3 were sacrificed. Histomorphological and histomorphometrical analysis were performed. Fusion was assessed and foreign body reactions were evaluated using the score according to Hoffmann.

Results: Histological examination revealed that 2/8 of PCC-cages obtained interbody fusion within 12 weeks, 5/8 within 36 weeks and 7/8 within 52 weeks. PLDLLA-cages showed no interbody fusion during the complete follow-up. After 12 weeks all PLDLLA specimens demonstrated foreign body reactions grade I-III with osteolysis up to 7mm. Further, the PLDLLA-cages showed grade II-III foreign body reactions in all fusion areas after 36 and 52 weeks. In contrast, the PCC-cage group demonstrated only two animals with grade I after 36 weeks, and 2 grade I foreign body reactions after 52 weeks.

Conclusion: Although the fate of foreign body reactions in the PCC-cage group is currently unclear and the implant was not resorbed during the 1-year follow-up, similar fusion results to the tricortical iliac crest bone graft were documented. In contrast the PLDLLA cage showed no fusion and severe foreign body reactions during the complete follow-up. Based on this animal experimental study, the PLDLLA-cage can not be recommended for human use.

P040

NITRIC OXIDE ENHANCES CBFA1 EXPRESSION AND ACTIVITY

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We have previously shown that mice deficient in eNOS (eNOS-KO) have reduced bone density and that osteoblasts from these mice display reduced levels of alkaline phosphatase (ALP), as well as a reduced increase of ALP in response to PTH. However, the target for the eNOS-produced nitric oxide (NO) is at present unclear. As the transcription factor CBFA1 is essential for osteoblast differentiation, we studied the effect of NO on CBFA1 expression and activity.

Osteoblasts were isolated from the calvaria of 3-day-old mice by collagenase digestion. Cells were treated with either vehicle, the NO-donor SNAP or PTH for 18 h. For CBFA1 expression studies, RNA was extracted, reversed transcribed and analysed by real time PCR. For analysis of DNA binding activity nuclear extracts were prepared and binding activity was determined in gel shift assays. CBFA1 activity was assessed in osteoblasts transfected with a luciferase reporter construct.

CBFA1 mRNA expression levels were reduced (60% of WT) in eNOS-KO osteoblasts. The NO-donor SNAP (50 μM) stimulated CBFA1-expression levels about 2-fold in eNOS-KO osteoblasts and 1.5-fold in wild type cells. Overnight treatment of wild type osteoblast cultures with SNAP lead to a twofold increase in CBFA1 binding activity as determined in gel shift experiments. Treatment of nuclear extracts with the NO-donor SNAP had a biphasic effect on CBFA1-binding activity, resulting in a twofold increase at 50 μM, and a reduction to 50% of vehicle control at 500 μM. Treatment of the osteoblasts with PTH resulted in a twofold increase in CBFA1 activity, and this was further enhanced by treatment of the nuclear extract with SNAP, with a maximum eight-fold stimulation at 50 μM SNAP. In the reporter assays, stimulation of the cells with SNAP lead to a similar biphasic effect on luciferase activity, with optimum 2-fold stimulation at 10–50 μM. In these experiments treatment with PTH resulted in a modest (<50%) stimulation of luciferase activity, with a strong synergistic effect of SNAP with a maximal 6-fold stimulation at 50 μM.

Our results show that the transcription factor CBFA1 is a target for NO activation in osteoblasts. This finding could explain the decreased osteoblastic differentiation and the reduced response to PTH in eNOS-KO mice. The exact nature of the NO-mediated activation of CBFA1 is at present unclear, but the effect of the NO-donor SNAP on nuclear extracts suggests that direct nitrosylation of the CBFA1 protein is involved.

P041

ANAESTHETICS EFFECT THE OSTEOGENIC RESPONSE TO MECHANICAL LOADING

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It is well established that bone is capable of responding to changes in the mechanical strains placed upon it. Recent observations in our and other laboratories demonstrating the presence of functional glutamate signalling pathways in bone has led to the proposal that glutamate signalling contributes to the transmission of acute mechanical stimuli into chronic responses. As glutamatergic pathways are targets for a number of anaesthetic agents we hypothesised that antagonism of these pathways by general anaesthesia would perturb the osteogenic effects of mechanical loading. In addition, as many *in vivo* studies of bone responses involve anaesthesia they could also be affected. To test this hypothesis we applied osteogenic mechanical loads to the ulnae of rats under 3 different anaesthetic regimes fentanyl + fluanizone/diazepam, ketamine and xylazine, halothane and compared the osteogenic responses *in vivo* and osteogenic response of bone marrow cells *in vitro*.

Periosteal bone formation in loaded ulnae compared to contra-lateral controls increase 3 fold in fentanyl + fluanizone/diazepam treated animals, 2.5 fold in ketamine/xylazine and 1.5 fold in the halothane group. These observations demonstrate that although mechanical loading increased periosteal bone formation in all animals the magnitude of response was significantly lower in animals anaesthetised with halothane. To assess the cellular mechanism for the differences in osteogenic response we investigated the effects of the anaesthetics on the osteogenic differentiation of bone marrow cells *in vitro*.

In marrow cultures from non-anaesthetised animals treatment with fentanyl + fluanizone/diazepam or ketamine/xylazine dramatically depressed osteogenic colony formation.

In marrow cultures prepared from the anaesthetised groups, fewer osteogenic colonies were obtained from the unloaded ulnae of animals treated with fentanyl + fluanizone/diazepam or ketamine/xylazine compared to those treated with halothane. No additional effects on osteogenesis were observed in culture prepared from the loaded ulnae of fentanyl + fluanizone/diazepam treated animals whereas, halothane and ketamine/xylazine reduced the number of osteogenic colonies compared to unloaded controls.

Conclusions. We can conclude that anaesthetic agents effect bone formation and osteoblast differentiation from marrow progenitors differently. Fentanyl + fluanizone/diazepam and ketamine/xylazine inhibit marrow differentiation.

P042

EFFECTS OF VITAMIN K2 (MK-4) ON HUMAN OSTEOBLAST PROLIFERATION AND DIFFERENTIATION

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Vitamin K2 has been shown to affect the regulation of bone metabolism. MK-4 enhances gamma-carboxylation of bone glutamic acid residues and secretion of osteocalcin. Beneficial effects of MK-4 on lumbar spine BMD in post-menopausal women with osteoporosis have been shown. In culture, MK-4 inhibits the formation and activity of osteoclasts, induces osteoclast apoptosis and increases the number of CFU-F/ALP+ colonies, partly by inhibiting osteoblast apoptosis. Activation of the orphan nuclear receptor SXR, accompanied by induction of bone markers, by micromolar levels of MK-4 has been demonstrated in human cell lines and mouse osteocytes.

We have investigated the effects of 0.1 nM to 10 microM MK-4 on the proliferation of human bone-derived osteoblasts. Osteoblasts were cultured for 8 days in 96-well plates containing medium with increasing concentrations of MK-4. Proliferation of cells was measured using MTS tetrazolium compound. Conversion of MTS to formazan after one hour was measured at 490/600 nm in a plate reader. Cell proliferation progressively decreased at MK-4 concentrations from 0 to 0.1 microM but then increased to give significantly greater proliferation of cells cultured with 10 microM MK-4 than of control cells with no MK-4 ($P < 0.05$ at 10 nM to 10 microM).

We have also investigated the effects of 2 nM (about twice the normal plasma concentration of vitamin K2) and 0.1 microM MK-4 on the differentiation of human bone derived osteoblasts. Cells from small flasks were harvested into Trizol reagent for total RNA isolation. Levels of mRNAs for COL1A1, ALP, OC and GAPDH were measured using real-time RT-PCR. Samples were quantified by the comparative cycle threshold (Ct) method for relative quantification of gene expression, normalized to GAPDH. Levels of ALP and COL1A1 mRNAs but not OC mRNA were significantly increased by 0.1 microM MK-4 ($P < 0.05$) compared to controls with no MK-4. None of the mRNAs measured was significantly increased by 2 nM MK-4 compared to controls.

We have shown that 0.1 microM MK-4 significantly reduces the proliferation and enhances the differentiation of cultured osteoblasts compared to controls. Plasma levels of 0.1 microM vitamin K2 can be achieved by ingestion of foods containing high concentrations of this vitamin.

P043

COOPERATIVE INHIBITION OF OSTEOBLAST FUNCTION BY ACIDOSIS AND HYPOXIA

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Several lines of evidence indicate that osteoblast (OB) function is sensitive to extracellular pH. Bone formation *in vivo* normally occurs adjacent to blood vessels, suggesting an important role for oxygen. We investigated the effects of pH and pO_2 on OB using a number of quantitative and qualitative methods. OB were harvested from neonatal rat calvariae by trypsin/collagenase digestion and cultured up to 35d in DMEM / 10% FCS with 0.05 mg/ml ascorbate, 2 mM β-glycerophosphate and 10 nM dexamethasone. pH was manipulated by addition of 5-25 mmol/l HCl, and pO_2 was varied between 20% - 0.2% O_2 (balance N_2); 5% CO_2 was present in all experiments. We found that cell proliferation rate, assessed by ^{3}H-thymidine incorporation, decreased up to $\sim$12 fold from d1 to d15 but was unaffected by pH reduction from 7.4 to 6.9, except at d1, where a 2-fold reduction was seen. The same pH reduction caused modest decreases in collagen production (eg by 50% at d15), as assessed by ^{3}H-proline incorporation. Abundant matrix-containing nodules were formed in OB cultures at pH 6.9 but mineralisation of nodules was almost completely abolished, compared with pH 7.4; however TEM indicated that collagen ultrastructure and organisation was not significantly altered. These results suggest that low pH causes an "osteomalacic" condition due either to increased $CaPO_4$ solubility or to decreased alkaline phosphatase activity. In contrast, reducing pO_2 from 20% to 2% caused a 90% reduction in the area of bone nodules formed by OB after 35d but mineralisation itself did not appear to be affected. The inhibition of nodule formation in 2% O_2 was partly accounted for by reduction in cell proliferation (eg 70% after 5d). In 0.2% O_2, nodule formation was completely abolished. TEM studies indicated that collagen fibril organisation was impaired in 2% O_2. Tissue hypoxia is usually accompanied by acidosis. Thus, our results suggest that hypoxia and acidosis exert a powerful, cooperative inhibitory action on bone formation by reducing OB proliferation and the production and subsequent mineralisation of organised collagenous matrix. These effects will be compounded *in vivo* by the strong stimulatory action of hypoxia and acidosis on osteoclast formation and activity. Our findings emphasise the key role of the vasculature in bone.

P044

INITIAL VASCULARIZATION IS INFLUENCED BY FIXATION STABILITY IN BONE HEALING

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Fracture healing requires a certain degree of mechanical stability and an adequate blood supply. The hypothesis of the present study was that decreased stability leads to a reduced vascularization and prolonged healing. The aim of the study was to quantitatively analyze the vascularization with regard to different but defined biomechanical conditions at selected time points of fracture healing. A mid-shaft osteotomy of the tibia was performed in two groups of sheep (n = 32 each) and stabilized with either a hard (group I) or soft (group II) external fixator. The sheep were sacrificed at 2, 3, 6, and 9 weeks post-op (n = 8 each). The fracture callus was analyzed histomorphometrically to quantify various tissue types. Alpha-smooth muscle actin staining was performed to detect vascular smooth muscle cells and pericytes (blood vessels were differentiated in small, medium and large vessels). At 6 and 9 weeks group II showed a significantly larger callus area than group I ($P < 0.028$) with a trend towards a larger fraction of fibrocartilage. In both groups the number of blood vessels per mm^2 of callus area was greatest at the initial phase of fracture healing (2 weeks). The number of vessels per mm^2 of callus area in group I decreased from 2 to 6 weeks (2 to 3 weeks: $P = 0.006$) and then increased from 6 to 9 weeks. The increase was significant for the periosteal callus area ($P = 0.006$). Group II showed a relative constant number of vessels per mm^2 during the entire course of healing. At 2 weeks group I showed a distinct trend towards a greater number of vessels per mm^2 than group II. However, at 6 weeks a significantly lower number of vessels per mm^2 was determined in group I in comparison to group II ($P = 0.027$). Likewise, group I showed a significantly lower fraction of fibrous tissue than group II ($P = 0.014$) at this time point. This study quantitatively describes vascularization and tissue distribution of callus with respect to the mechanical stability in the course of fracture healing. Decreased fixation stability led to a lower initial vascularity and a larger callus formation. Despite the similar tissue distribution and vascularization reached in both groups at the final stage, the increase of blood vessels in the hard fixator group indicates an intensive remodeling of newly formed bone suggesting a faster healing. Therefore, in this study the less stable fixator led to a slower healing path, based on an initially reduced vascularity.

P045

GREATLY INCREASED FORMATION OF OSTEOBLASTS FROM PROLIFERATING PROGENITORS IN THE MATERNAL SKELETON AT THE END OF LACTATION

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Reproduction is accompanied by changes in maternal physiology to compensate for increased mineral requirements, especially during lactation for milk production. This increased requirement for calcium during lactation does result in substantial bone loss which has been documented in a variety of species as well as in humans. The maternal skeleton compensates for the bone loss with an anabolic recovery period associated with endocrine changes and reflected in cellular events. Osteoblasts may be derived from several sources including direct differentiation from a determined progenitor that does not require proliferation and inducible progenitors that proliferate prior to differentiation. The cells of origin may include the mesenchymal 'osteoprogenitor' cell, stromal cell or perhaps even bone lining cells. The purpose of this study was to determine the contribution of proliferating progenitors to the pool of functional osteoblasts that rapidly appear at or near weaning in the dam.

At weaning (day 21 of lactation) of a second reproductive cycle, 4 injections of bromodeoxyuridine (BrdU) were given at 8 hour intervals. The animals were necropsied at 1, 24, 48, 96 and 120 hours after the last BrdU injection. Age-matched controls that had completed one reproductive cycle were included in each group. Calcein and tetracycline labels were also given at 6 and 1 day prior to necropsy. The bones used for counting BrdU labeled cells were decalcified in EDTA at 4 °C and embedded in paraffin. Bones for histomorphometry were embedded in methyl methacrylate. The osteoblasts were counted on the trabeculae of the lumbar vertebra.

The percent BrdU-labeled osteoblasts at weaning reached a plateau at 24 hrs after the last injection ($23 \pm 7\%$) and remained at this level through 120 hours. This was substantially greater than the controls where the labeled osteoblasts peaked at about 3–4%. In the dams at weaning, most of the endosteal and endocortical surfaces were lined with osteoblasts. Bone formation rates measured histomorphometrically were greater in the dams than the controls. BrdU-labeled osteocytes began to appear at about 96 hours after the last injection in the dams. The results from this study demonstrated that there is a profound stimulation of new osteoblasts from proliferating progenitors to support the increase in bone formation following lactation.

P046

CELL BIOLOGY OF THE BONE AFTER CHLOROBENZENE TREATMENT

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The homeostasis of bone tissue is determined by endocrinological, neuronal and immunological regulation. The xenobiotics modify osteogenesis by cell-biological mechanisms.

We studied the effects of subtoxic doses of chlorobenzenes on bone formation (by measuring bone calcium content), and the collagene synthesis of the fibroblasts. These measurements were followed by the examination of the microscopic structure of bones and fibroblasts.

Wistar male rats (100–120 g bw.) were treated with chlorobenzene through gastric tubes, for 30–60 days (hexachlorobenzene: 2,4,6-trichlorobenzene = 1:1; 25μg/bw.kg.).

Control system were normal control (untreated); positive control (1.5% ethanol treated); negativ-1 control (only gastric tubes); negative-2 control (water treated).

After the treatment the calcium content of the bone were measured. Monolayer cell cultures were generated from the fibroblast of treated and control animals, and collagene synthesis (protein content), and membrane fluidity (fluorescent anisotropy) and structure (morphometry) were examined.

After treatment with chlorobenzenes, we detected a substantial morphological destruction of the bone matrix. The fibroblasts showed normal cell structure. High electrically mobilized calcium levels were measured in the femur samples as a result of the treatment. The fibroblasts synthesized collaganes showed normal amino acid sequences with degenerated three-dimensional structure. Fundamental matrix of the bone was changed by the chlorobenzene treatment as a result of the collagene degeneration processes in the different cells.

P047

A NOVEL ELEMENT SIMILAR TO THE TRIIODOTHYRONINE (T3) RESPONSIVE ELEMENT (TRE) REGULATES T3 INDUCED ACTIVITY OF THE MOUSE MMP13 PROMOTER

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The Matrix Metalloproteinase MMP13 (collagenase 3) is involved in endochondral ossification and bone remodelling by degrading components of the extracellular matrix. It is also a key regulator of calcium homeostasis in adult bone by solubilizing calcium through bone resorption. MMP13 primarily degrades collagen II, but it is also involved in degradation of type I, III and X. Its expression is dependent on several growth factors like Interleukin1 (IL1) or tumor necrosis factor alpha (TNF-alpha) and on hormones like 1,25 dihydroxyvitamin-D3 and Triiodothyronine (T3). Despite the fact that most T3 regulated processes act via direct interaction of T3 bound T3-receptor (T3R) with a distinct promoter element of the target gene, there is also evidence that T3 regulates MMP13 transcription via an indirect pathway but not through protein stability of the factor itself. The pronounced effect of T3 on MMP13 expression might reflect the general contribution of this hormone to bone remodelling. Although it has been clearly shown that T3 is involved in MMP13 gene expression, the mechanism of how T3 acts on the regulation of this gene still remains unclear.

We were able to identify a sequence around 1100 bp upstream of the mouse MMP13 gene that shows a high degree of homology to the consensus sequence of TRE (TAAGGTCA) through computational analysis. To show that this sequence is a functional TRE we cloned a 2kb fragment of the 5-prime region of the MMP13 gene into a mammalian expression vector and assayed for reporter expression before and after T3 treatment in MC3T3 cell cultures. N-terminally truncations of this construct revealed that its promoter activity significantly decreases whenever the region around the hypothetical TRE is deleted. Employing electrophoretic mobility shift assays (EMSA) we also showed that *in vitro* translated T3 receptor (T3R) efficiently binds to an oligonucleotide representing the sequence of the hypothetical TRE. Moreover, oligonucleotides harbouring point mutations within the TRE showed a considerable decrease of the binding signal compared to the wild type sequence. In summary, these results suggest that there is a direct interaction of T3R and a new TRE of the MMP13 promoter to regulate T3 induced MMp13 gene expression.

P048

BONE SIALOPROTEIN-ELICITED *IN VIVO* MINERALIZATION AND OSSIFICATION

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Bone sialoprotein (BSP) and osteopontin (OPN) are two major non-collagenous glycosylated phosphoproteins of the extracellular matrix in bone. Previous *in vitro* precipitation and cell culture studies have suggested that BSP may play a significant positive role in the initiation of calcification, whereas OPN may inhibit nucleation and/or crystal growth. This study was designed to explore the biological functions of BSP *in vivo*, especially in a bone defect model. We report for the first time that implantation of BSP-collagen complex into 8 mm rat cranial defects, which do not heal spontaneously, induces mineral deposition followed by osteoblast differentiation and ossification. The BSP-collagen complex first induced the proliferation of cells in the dura, followed by ectopic calcification at the junction areas between the dura and the BSP-collagen implant. Cell proliferation and calcification progressed further into the interior of the BSP-collagen implant at days 5–7. At this time there was no visual evidence that the proliferating cells are either chondroblasts or osteoblasts, which was confirmed by histological, histochemical, immunohistochemical and in situ hybridization. The proliferating cells in the BSP-collagen complex differentiated to osteoblasts at approximately 9–10 days followed by synthesis of bone. OPN-collagen complex or collagen alone implanted into 8 mm rat cranial defects did not stimulate the calcification or the differentiation of dural cells to either chondroblasts or osteoblasts within the same experimental period. BSP-collagen complex implanted into rat thoracic subcutaneous tissue did not induce mineralization or the differentiation of local mesenchymal stem cells to either chondroblasts or osteoblasts, which was distinctly different from the cellular response to demineralized bone matrix (DBM) or several of bone morphogenetic proteins (BMPs). These results suggest that BSP facilitates early biomineralization and the subsequent bone formation in calvarial defects, but not in subcutaneous tissues. The cellular response and matrix mineralization events occurred in the BSP-treated calvarial defects are distinctly different from those induced by DBM and BMPs both in calvarial defects and in subcutaneous tissue.

P049

GENISTEIN STIMULATES MINERALIZED BONE NODULE FORMATION IN HUMAN SAOS-2 CELLS

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Epidemiological studies revealed that consumption of soy and soy products has a protective effect against chronic diseases such as osteoporosis. This beneficial effect may be a result of phytoestrogen components of soy. The major phytoestrogen component of the soy products is genestein. The objective of this study was to investigate the effects of genistein on differentiation and mineralized bone nodule formation in cultures of human osteoblasts. SaOS-2 cells were cultured in HAM`s F-12 supplemented with 10 nM dexamethasone (Dex), 50 ug/ml ascorbic acid and 10 mM β-glycerophosphate. In parallel experiments, vehicle or varying concentrations of genistein were added on day 8 for the first 6 hours prior to replacing with fresh medium without the drug and continuing the cultures for another 42 hours. The 48-hr cycle was repeated until days 17-21. The cells were then fixed, stained with von Kossa and the mineralized nodule areas and numbers quantified by an image analyzer, or the cells were sonicated for an ALP assay. Results showed that genistein stimulated mineralized bone nodule formation in SaOS-2 cells in a time and dose-dependent manner. The stimulatory effect was seen from day 17 to day 21 and at concentrations of 1×10^{-7} M to 1×10^{-5} M. This was accompanied by stimulation of ALP. In conclusion, our results revealed that genistein has stimulatory effects on differentiation and mineralized bone nodule formation in SaOS-2 cells. This is the first report of the anabolic effect of genistein on human osteoblasts, a finding that may have important implications for the role of soy in the treatment and prevention of osteoporosis.

P050

CALCIUM IN MILK MINERALS IS BIOAVAILABLE UNDER STOMACH pH AND ENZYMATIC CONDITIONS WHILE STIMULATING MINERALIZED BONE NODULE FORMATION IN CULTURES OF HUMAN OSTEOBLAST-LIKE SaOS-2 CELLS

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Dietary calcium is essential during growth and development in achieving the genetically programmed peak of skeletal mass as well as in maintaining bone mass in the elderly, particularly in osteoporotic individuals. Although optimum calcium intake is generally obtained from dietary food sources, such as milk, many individuals cannot obtain the optimum calcium requirement from food for a variety of reasons. Therefore, calcium supplements are important sources of dietary calcium. One of the calcium sources commercially available is LactoCalcium® (milk minerals) that has 28% calcium, and a 2:1 ratio of calcium to phosphorus. The objectives of this study were (a) to examine whether calcium from milk minerals is digestible using digestive enzymes and (b) to determine the biological activity of the digested calcium from milk minerals by examining its ability to stimulate bone formation. Milk mineral (LactoCalcium®, Cyvex, Ca) was digested in vitro using (i) simulated gastric and intestinal fluids or (ii) porcine gastric, pancreatic and intestinal extracts. Calcium concentration was measured using a colorimetric calcium assay kit. To determine the effect of digested calcium on bone formation, different concentrations of calcium from digested milk minerals were added to the mineralizing cultures of human osteosarcoma SaOS-2 cells and the area of mineralized bone nodules of von Kossa-stained cells quantified by image analysis, as we have previously described. Our results indicated a role for enzymes or bile extract in digestion of the product. We showed that by decreasing the ratio of milk mineral (substrate) to enzyme concentration, the percentage of digestible calcium increased in a dose dependent manner, showing that at the right enzyme concentration as much as 100% of the calcium present in LactoCalcium® can be made available. The biological activity of the digested calcium was demonstrated by the stimulation of mineralized bone nodules in SaOS-2 cells in a dose dependent manner. Thus, 1 mM and 3 mM bioavailable calcium from the milk mineral increased the nodule area by $23.17 \text{ mm}^2 (P < 0.0001)$ and $77.78 \text{ mm}^2 (P < 0.0001)$, respectively, as compared to a value of 0.99 mm^2 at 0.5 mM calcium from milk mineral. On the basis of these results, it can be concluded that milk minerals are excellent sources of bioavailable calcium that can be used by individuals at risk for osteoporosis who are unable to get sufficient calcium from foods.

Cell Biology: Osteoclasts and Bone Resorption

P051

PAGET'S DISEASE MAY CONTRIBUTE TO THE HEART FAILURE

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Paget's disease is a common in which there is disorderly bony proliferation. Electron microscopic studies have demonstrated probable viral inclusions in the nuclei of osteoclasts, derived from measles. The main complaints of patients with paget's disease are bone pain, deformities or fractures. Serum calcium concentration is usually normal, but the alkaline phosphate is elevated reflecting the osteoblastic activity. A variety of histological changes occur and these flow a sequence of bone breakdown (lysis), new bone information and bony sclerosis. Large multinucleated osteoclasts are characteristic and florid bony proliferation. Osteoclasts are present around thick and vascular tuberculae of new bone and may be wide seams of uncalcified osteoid and this leads to dense bony sclerosis with trobeculae forming a mosaic pattern. This process is responsible for pagets's disease. The complications are deformities, bone pain, fractures, deafness, osteosarcoma, bone tumours and heart failure. The commonest complications are deformities and bone pain. The pain is the result of osteoarthritic degeneration of a related joint. The subperiosteal zone of bones is oedematous and highly vascular and there may be periosteal elevation. Pagetoid bone is susceptible to fracturing in initial lytic phase. Enlargement in the sclerotic stage can lead to nerve or spinal cord compression. Deafness is the result of both 8 cranial nerve compression and distortion of the middle ear cavity (paraplegia can result). The most sinister complication is osteosarcoma. There is also an increased incidence of fibrosarcomas, chondrosarcomas and giant cell tumors. Patients with paget's disease may also have heart failure. However, the bone in patients is extremely vascular and blood flow in these areas is markedly increases. This may contribute to the heart failure.

P052

THE OSTEOLYSE ASSAY A SENSITIVE HIGH-THROUGHPUT ASSAY FOR MEASUREMENT OF *IN VITRO* HUMAN BONE MATRIX RESORPTION

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In Vitro assays of bone resorption are invaluable to the discovery of drugs for the treatment of osteoporosis. Existing in vitro assays of bone resorption utilize either synthetic calcium phosphate-based matrices or slices of either dentin or bovine cortical bone, and require either image analysis or EIA assays for the generation of quantitative data. In the present study, the OsteoLyse™ assay was evaluated as an alternative to existing commercially available substrates for the analysis of inhibitors of bone resorption. The 96-well Osteo-Lyse™ plates, which contain fluorophore-labeled human bone matrix collagen type I, were seeded with 10,000 primary human Osteoclast Precursors (Cambrex) per well ± soluble RANK ligand. Cells were cultured at 37°C for 5 to 10 days ± various inhibitors. In some cases, media were changed on day 5 or 7 and the culture continued. At the appropriate times, cell culture supernatants (10 mul) were transferred to a 96-well plate of Fluorophore Releasing Reagent, which was then read in a time-resolved fluorescence-capable fluorimeter. The release of collagen degradation fragments was linear with time and signal-to-background ratio, which also increased with time, was as high as 38 after 10 days of culture. The coefficient of variation of the OsteoLyse assay was < 20% and the Z' value ranged from 0.5 to 0.7. Interferon υ-mediated inhibition of osteoclast precursor differentiation was assayed with both the OsteoLyse assay and the TRAP (tartrate-resistant acid phosphatase) cytochemical stain. Data from these two assays gave nearly identical results with IC50 values of approximately 0.1 ng/ml. Measurement of the *in vitro* inhibition of bone resorption by alendronate was also assayed with the OsteoLyse assay and gave an IC50 value of approximately 2 μM. In OsteoLyse assays of calcitonin, treatment of human osteoclasts with 1 nM calcitonin for 24 hours (days 5 to 6 of culture) inhibited bone matrix degradation by 88%. Treatment of the cells with calcitonin, after prior exposure to calcitonin on days 0 to 5, had little effect on the resorptive activity of the osteoclasts. In conclusion, the OsteoLyse assay 1) is a homogeneous assay – cell culture supernatant is simply transferred to a counting plate and the fluorescence immediately determined; 2) provides data that correlate well with existing assays and 3) provides a platform for rapid screening of large numbers of potential inhibitors of osteoclast differentiation and function.

P053

HYDROSTATIC PRESSURE AND UHMWPE PARTICLES MODULATE VITAMIN D SYNTHESIS BY MACROPHAGES

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Elevated hydrostatic pressure occurs during aseptic loosening, but the mechanisms by which pressure could enhance loosening are unclear. We have demonstrated that hydrostatic pressures increased MP synthesis of various factors implicated in bone resorption. 1,25-dihydroxy vitamin D3 (1,25D3)is pivotal to bone resorption, stimulating osteoclast activity. Macrophages (MP) are able to differentiate into osteoclasts and also synthesise 1,25D3. We therefore examined how hydrostatic pressure influenced synthesis of 1,25D3 by MP. Normal human peripheral blood MP (5×105/ml) were cultured for 7 days then exposed to physiological pressure (34.5×10-3MPa) and/or UHMWPE particles (8mg/ml) and the effect on synthesis of 1,25D3 was studied. MP were incubated with H3-25, hydroxy vitamin D and 1,25D3 synthesis was analysed by HPLC. 1,25D3 synthesis was increased in cells under pressure by an average of 17% compared to static controls. In situ hybridisation (ISH) was used to demonstrate expression of 1αOHase. Image analysis showed a small increase in 1αOHase mRNA in response to pressure (37%) and to particles (59%), and a larger increase to the two stimuli simultaneously (100%). These results demonstrate that MP synthesis of 1,25D3 was increased by exposure to either UHMWPE particles or pressurization at levels previously shown to increase synthesis of bone-resorbing factors. The effect of the two stimuli is additive, suggesting that 1,25D3 may be one of the factors which stimulates osteoclastic bone resorption in aseptic loosening. As both these stimuli are likely to be present *in vivo*, such synthesis could further exacerbate loosening.

P054

MODULATION OF MACROPHAGE ACTIVITY BY TNF

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The importance of elevated pressure in the development of aseptic loosening is now well documented. We have previously shown that hydrostatic pressure modulated synthesis by macrophages of several cytokines (TNF, IL-1b and IL-6), of the growth factor M-CSF, of chemokines (MCP and MIP-1a) and prostaglandin E2. This latest study further examines how pressure influences macrophage metabolism.

Human peripheral blood macrophages (MP) were exposed to pressure and/ or UHMWPE particles and the effect on cytokine synthesis of the addition of anti-TNFα antibody was studied. Our results showed that blocking TNFα production by MP reduced synthesis of IL-1 and IL-6 at both pressures and cell densities tested. Incubation of MP at 1×105/ml with anti-TNFα antibody reduced synthesis of all three cytokines at day 0 (40–63%) and day 7 (35–90%), compared to pressurised cultures (6.9×10-3MPa)incubated without antibody. TNFα concentration increased with increasing cell density (Table 1) and exposure to pressure (34.5×10-3MPa) increased synthesis of TNFα. This increase was greatest in the cultures incubated for 7 days, at the highest cell density of 5×105/ml. This density-dependent effect was not seen with cultures incubated overnight. Incubation of MP cultures at 5×105/ml with antibody reduced TNFα synthesis to < 1.0% of the control cultures without antibody.The antibody only reduced IL-1b to 81% and IL-6 to 69% of controls. Stimulation of cytokine synthesis by pressure did not overcome this reduction in TNFα and levels of IL-1b and IL-6 were similar to unpressurised cultures (88% and 73% of controls). A separate study looked at 1,25D3 and a positive correlation was demonstrated between TNFα and 1,25D3 concentrations (r2 = 0.73). These results show that TNFα may be an upstream initiator of IL-1b and IL-6. They also suggest an interrelationship between pressure-related increases in TNFα and 1,25D3 production, although further research is necessary to elucidate this complex relationship, which ahs implcations for the *in vivo* situation.

Table: Effect of cell density on TNF synthesis

Cell No. (X105)	D7 −P	D7 +P
1.0	42 (2)	44 (5)
2.5	96 (54)	128 (50)
5.0	381 (224)	825 (514)

P055

THE OSTEOASSAY PLATE: A 96-WELL FORMAT HUMAN BONE SUBSTRATE FOR ASSAYS OF *IN VITRO* BONE RESORPTION

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Existing *in vitro* assays of bone resorption utilize either synthetic calcium phosphate-based matrices or thin slices of either dentin or bovine cortical bone as substrates. While use of the former substrate requires morphometric image analysis for quantification, bone substrate-based assays can use either image analysis or the immunoassay of a specific product of the resorption process. In the present study, the OsteoAssay[TM] human bone plate was evaluated as an alternative to existing commercial substrates for use in assays of *in vitro* bone resorption. The 96-well OsteoAssay plates, which contain adherent fragments (< 0.5 mm diameter) of native human bone, were compared to dentin discs (ALPCO Diagnostics) in 96-well tissue culture plates. Wells were seeded with 10,000 primary human osteoclast precursors (Cambrex Bioscience Walkersville) per well ± soluble RANK ligand and cultured at 37°C for 5 days ± various inhibitors. Culture media were changed on day 5 and the cultures continued for another 1 to 4 days. To quantify results, the release of collagen type I peptides from dentin/bone slices was measured via EIA. Three different commercially available assay kits for the measurement of resorption-specific collagen degradation fragments were evaluated: The CrossLaps for Culture CTx (Nordic BioScience), Osteomark NTx (Ostex) and Metra Helical Peptide (Quidel) EIA kits. Significant differences relative to background levels and ease of use were observed. Release of the peptides was linear with time and the signal-to-background ratio (differentiated cells relative to undifferentiated precursors) was > 10 after 1 day. Data from assays with the two different substrates demonstrated that peptide generation from the dentin discs was > 20% of that released from the native human bone chips in the OsteoAssay plate. *In vitro* inhibition of bone resorption by alendronate, calcitonin and interferon γ were assayed with the OsteoAssay plate and gave IC_{50} values similar to those in the literature. The coefficient of variation of assays done with the OsteoAssay plate averaged 12% with a Z' value < 0.5. In conclusion, the OsteoAssay Plate provides a more physiologically relevant and less expensive substrate for assays of *in vitro* bone resorption. The OsteoAssay substrate is compatible with all three of the commercial EIA kits evaluated and was superior to dentin or bovine cortical bone slices in that results were obtained in days instead of weeks of culture.

P056

OPG, RANK-L, AND MARKERS OF BONE METABOLISM IN PATIENTS ON HAEMODIALYSIS AND CONTINUOUS AMBULATORY PERITONEAL DIALYSIS

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Patients with end-stage renal failure (ESRF) on dialysis may develop high or low bone turnover renal osteodystrophy, depending on PTH levels; PTH acts on bone resorption through the recently discovered cytokines osteoprotegerin (OPG) and Receptor Activator of Nuclear kB–Ligand (RANK-L).

Aim of our study: to evaluate the levels of serum OPG and RANK-L in patients with ESRF undergoing dialysis and to assess their relation with bone turnover rate estimated by biological markers.

Patients and methods: 21 patients were on continuous ambulatory peritoneal dialysis (CAPD), and 58 underwent haemodialysis (HD), all for longer than 2 months. 65 healthy control subjects matched for sex and age were studied for comparison.

Serum PTH was measured by IRMA (DiaSorin, USA); RANK-L and OPG by ELISA (Biomedica, Austria and protocol described by R & D Abington, UK); bCrossLaps (CL) by ELISA (Nordic Bioscience, Denmark); the carboxyterminal extension peptide of type I procollagen (PICP) by RIA (Orion Diagnostica, Finland).

Results: Control group: There was no sex difference for OPG nor RANK-L; OPG increased slightly with age, as described previously, but age did not affect significantly RANK-L nor the RANK-L/OPG ratio. Patients with ESRF: We observed a significant positive correlation between OPG levels and age ($P < 0.0001$), and OPG levels were higher in women than in men, significantly in patients undergoing HD ($P < 0.001$). RANK-L and the ratio RANK-L/OPG did not change with age nor sex. RANK-L, OPG and RANK-L/OPG ratio were significantly higher in HD group than in controls ($P < 0.001$ and < 0.05); RANK-L was higher in HD than in CAPD ($P < 0.001$) whilst OPG was equally increased; in CAPD, the median RANK-L level did not differ significantly from that of control group. None of the parameters varied with the duration of dialysis. OPG, RANK-L and RANK-L/OPG ratio did not depend on iPTH levels. Serum CL and PICP were significantly higher in ESRF than in controls ($P < 0.001$). No correlation was found between CL or PICP and OPG, RANK-L or RANK-L/OPG ratio.

Conclusions: 1) Serum OPG increases with age both in control subjects and in ESRF. 2) In ESRF, OPG and RANK-L serum levels are significantly increased and OPG is significantly higher in women than in men. 3) Serum OPG and RANK-L do not correlate with serum iPTH levels. 4) PICP and CL are significantly increased in ESRF, but they do not correlate with OPG, RANK-L or RANK-L/OPG ratio.

P057

REACTIVE OXYGEN SPECIES FUNCTION AS A SIGNALING MEDIATOR OF RANK IN OSTEOCLASTS

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RANKL, a member of tumor necrosis factor (TNF) superfamily, regulates the differentiation, activation, and survival of osteoclasts through binding with its cognate receptor, RANK. RANK can interact with several TNF receptor-associated factors (TRAFs) and activates signaling molecules including Akt, NF-κB, and MAPKs. However, the exact molecular mechanism of RANK signaling has not been fully elucidated. In the present work, we demonstrate that reactive oxygen species (ROS) act as a signaling mediator of RANK in osteoclasts. Stimulation with RANKL induced ROS generation in osteoclasts. Two antioxidants, N-acetyl-L-cysteine and glutathione, prevented RANKL-induced Akt, NF-κB, and ERK activation in osteoclasts. TNF-α also increased ROS production in osteoclasts. However, antioxidants did not prevent TNF-α-induced Akt and NF-κB activation, while slightly affecting ERK activation. These results indicate that RANK initiates signaling through Akt and NF-κB pathways in a way that is different from TNFR. Pretreatment with antioxidants also significantly reduced RANKL-induced actin-ring formation, required for bone resorbing activity, and osteoclast survival. Taken together, our results suggest that ROS play a critical role in RANK signaling and osteoclast function.

P058

INNOVATIVE STRATEGY FOR THE LOCAL TREATMENT OF PATHOLOGICAL BONE RESORPTION : IN VITRO BIOLOGICAL CHARACTERIZATION OF BISPHOSPHONATE-LOADED CALCIUM PHOSPHATE BIOMATERIAL.

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Calcium phosphate (CaP) biomaterials, which exhibit physicochemical properties closed to that of bone, have been successfully used as bone substitute and contemplated as carriers for therapeutic agents. Bisphosphonates have a strong affinity for CaP crystals that composed the bone biological apatite. We aimed at using this affinity to develop a bone drug delivery system (DDS) for bisphosphonates to locally prevent pathological bone resorption (osteoporosis and osteolytic tumors). This DDS, based on the association between an injectable CaP bone substitute and zoledronate was evaluated in vitro using osteoclasts, previously reported as one of the major targets of these therapeutic agents. Zoledronate was loaded on a calcium deficient apatite (CDA) according to a patented coating process (Patent No. WO03074098) that was characterized with phosphorus nuclear magnetic resonance. CDA was able to load zoledronate according to a chemical exchange between phosphonate groups of zoledronate and phosphate groups of CDA. Pharmacokinetic evaluation was performed with C14-labelled zoledronate. The in vitro release of zoledronate was sustained for several weeks. The antiresorptive effects of zoledronate were thereafter tested on multinucleated and resorbing osteoclasts obtained from a total rabbit bone cell culture model and from RANK-ligand treated RAW 264.7 macrophage-like cells. These effects have been determined by the inhibition of phenotypic markers and decrease of resorption activity. Results showed that zoledronate in solution (10–6 to 10–10 M) or zoledronate-released from CDA reduced in a dose dependent manner the number of osteoclasts (TRAP, VNR and actin ring positive cells) as well as the formation of resorption pits. Taken together, our data demonstrate that CDA is effective for local delivery of zoledronate and likely for preventing pathological bone resorption. This type of composite material would allow 1) to increase the efficiency of bisphosphonate by being locally released; 2) to decrease significantly secondary effects observed by systemic treatments. The clinical potential of this intra-osseous bisphosphonate-DDS is now under investigation in animal models of osteoporosis and osteolytic tumors. This work was supported by the French Ministry of Research (ACI technologie pour la santé 2001-2004), Fondation de l'avenir pour la recherche médicale appliquée (ET2-321), CNRS (Matériaux Nouveaux – Fonctionnalités Nouvelles), Novartis AG and Amgen Inc.

P059

MULTIPLE RESPONSES TO CATHEPSIN K OVEREXPRESSION IN MICE

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Bone modeling/remodeling is regulated by the delicate balance of bone resorption and bone formation. During growth and ageing, the sequence, proportion and location of bone resorption and formation appear to be differentially regulated. Osteoclastic bone resorption consists of cell attachment, polarisation, matrix demineralisation and degradation followed by cell detachment. Several recent reports indicate that cathepsin K, a lysosomal cysteine proteinase, is a major proteinase in bone resorption. We have recently described that overexpression of cathepsin K in mice results in osteopenia of metaphyseal trabecular bone. The purpose of the present study was to extend characterisation of this mouse model in vitro and in vivo.

Mice homozygous and negative for the transgene locus were selected for this study. Two-day-old mice were used as a source of osteoclasts, which were cultured on bovine cortical bone slices. The distribution of cathepsin K was studied using confocal immunofluorescence microscopy. Bone resorption capacity of osteoclasts was determined by bone slice and culture medium analyses. At the age of 1, 3, 7 and 12 months, the properties of trabecular and cortical bones were studied using peripheral quantitative computed tomography (pQCT) and histomorphometry.

In transgenic osteoclasts, confocal microscopy demonstrated localization of excess cathepsin K into various intracellular vesicles and into the resorption lacunae. Cathepsin K overexpression resulted in an enhanced bone resorption capacity. In transgenic mice, enhanced bone resorption resulted in osteopenia of metaphyseal trabecular bone during growth and ageing. However, an apparently opposing phenotype was observed in diaphyseal region of long bones, where cathepsin K overexpression resulted in increased thickness and density of cortical bone during growth and ageing, and in increased cortical porosity during growth.

The current data demonstrates that excessive cathepsin K in osteoclasts leads to an enhanced bone resorption capacity. On trabecular bone surface the overexpression is sufficient to cause an imbalance between bone resorption and bone formation towards bone loss. The strikingly different response seen in cortical bone demonstrates the different nature and reactivity of cancellous and compact bone, and emphasizes the complexities of skeletal biology and subsequent necessity to evaluate gene functions in living organism.

P060

ABNORMAL BONE REMODELING IN CATHEPSIN K DEFICIENT MICE

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Cathepsin K (Ctsk) has an important function in osteoclast-mediated degradation of organic bone matrix. Mutations in the human CTSK gene and Ctsk deficiency in mice have been shown to result in osteopetrosis due to impaired bone resorption by osteoclasts. However, osteoclastic bone resorption is partially preserved in Ctsk deficient mice as indicated by less severe phenotype of these mice compared to e.g. PU.1 knockout mice. The aim of this study was to characterize the mechanisms that partially compensate for the lack of cathepsin K in bone resorption of Ctsk deficient mice.

Long bones of control, Ctsk^{+/−} and Ctsk^{−/−} mice were studied using peripheral quantitative computed tomography (pQCT) at the ages of 2, 7 and 12 months. Histomorphometry, molecular biologic analyses and serum resorption marker measurements were carried out at the age of 2 months. Primary osteoclasts from newborn control and Ctsk^{−/−} mice were cultured on cortical bone slices and their structure was studied using confocal microscopy.

pQCT measurements indicated an osteopetrotic phenotype in Ctsk deficient mice. In metaphyseal region cortical thickness (Crt.Th) and total bone mineral density (BMD) were clearly increased in 2-, 7- and 12-month-old Ctsk^{−/−} mice. However, trabecular BMD was increased in 2-month-old mice but the difference from control mice decreased during ageing. Ctsk^{−/−} osteoclasts exhibited an altered sealing zone with disrupted actin ring and harboured large vesicles containing type I collagen. Histomorphometry verified trabecular osteopetrosis and decreased rate of bone turnover in Ctsk^{−/−} mice at the age of 2 months. Interestingly, the number of osteoclasts per bone perimeter was increased but there was no change in the number of osteoblasts. Northern analyses indicated up-regulation of mRNAs for matrix metalloproteinase (MMP) 9, 13, 14, TRACP and cathepsin L. Also the expression of receptor activator of NFkB ligand (RANKL) and osteoprotegerin (OPG) were increased but the RANKL/OPG ratio was also increased so that the net effect favoured osteoclastogenesis. Serum resorption markers CTX, ICTP and TRACP activity were paradoxically increased in Ctsk^{−/−} mice.

This data indicates that impaired bone resorption of Ctsk deficient osteoclasts leads to elevated RANKL expression and to increased RANKL/OPG ratio. This may stimulate osteoclast differentiation and contribute to the up-regulation of MMP, TRACP and cathepsin L expression to compensate for Ctsk deficiency.

P061

EFFECT OF GROWTH FACTORS INCORPORATED IN A POLY(D,L-LACTIDE) DRUG CARRIER ON OSTEOCLASTS *IN VITRO*

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Introduction: Previous *in vivo* studies were able to demonstrate the efficacy of locally released growth factors from a poly(D,L-lactide) implant coating on fracture healing in rat and pig [Schmidmaier 2001, Raschke 2002]. In order to get more information on the influence of the Lactide and the growth factors on individual cell types, we performed cell culture studies on osteoclasts. Human osteoclast like cells derived from monocyte/macrophage haematopoietic lineage were used.

Material and Methods: Titanium k-wires were coated with poly(D,L-lactide) (30 kDa) and incorporated growth factors.

1. control culture, 2. PDLLA coated wires, 3. PDLLA and IGF-I (30μg) & TGF-β1 (6μg)

The wires were added to the cultures in a non-contact manner. All test were performed in triplicate.

Cultures: Mononucleated cells were isolated from human peripheral blood. The cells were cultured with a-MEM plus glutamine. For stimulation of osteoclastogenesis RANKL and M-CSF were added.

Fusion-test: TRAP-stained and polynucleated cells were counted.

Resorption-test: The resorption lacunae on dentin were stained with Toluidine blue and counted.

Statistics: ANOVA, Bonferroni

Results: The cultures with the PDLLA wires revealed significantly less fused and TRAP positive cells compared to the control. The cultures with IGF-I & TGF-β1 showed a comparable fusion behaviour as the control group. On all dentin chips resorption lacunae and resorption traces were detectable. The dentin chips cultured with the PDLLA wires revealed significantly less lacunae. The cells treated with growth factors showed a less resorption activity than the control culture but more than the PDLLA group.

Discussion: This study examines the effect of a polylactide coating and growth factors on osteoclasts *in vitro*. The poly(D,L-lactide), used as a local drug carrier *in vivo*, inhibits osteoclast formation and resorption activity. The inhibiting effect of the PDLLA on osteoclasts fusion and resorption was abolished by the additional application of IGF-I & TGF-β1. No difference between the control and the GF group was detectable in the fusion of the monocytes to multicellular osteoclast like cells. The resorption activity was not completely rescued by to the growth factor application indicating a differentiation depending effect of growth factors on osteoclasts.

P062

BONE AFFINITY DIFFERENCES OF NITROGEN-CONTAINING BISPHOSPHONATES PREDICTED BY *IN VITRO* BINDING TO CARBONATED APATITE

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Bisphosphonates (BPs) are effective inhibitors of bone resorption. An important component in their ability to inhibit bone resorption is their affinity for calcium phosphate surfaces that allows them to target bone mineral. This can be predicted from *in vitro* studies of their effects on the dissolution and growth of calcium phosphates. The order of affinity predicted from recent *in vitro* growth inhibition studies on hydroxyapatite (HAP) at pH = 7.4 was Zol > Aln > Iban∼Ris > Etid > Clo. All binding affinities (K_L's) were significantly different ($P < 0.002$) with the exception of Risedronate (Ris) vs Ibandronate (Iban). We have now also studied the effects of several nitrogen-containing bisphosphonates (N-BP's) on carbonated apatite (CAP), which is more relevant to bone composition *in vivo*. These effects have been studied by a constant composition method at both physiological ionic strength and temperature, 0.15 M and 37°C. Adsorption affinity constants were calculated from the kinetics data. For HAP dissolution at pH = 5.50, the rank order of inhibition (high to low) was zoledronate > alendronate > risedronate, but was less marked than at pH 7.4. For CAP dissolution at pH = 5.50, the rank order of inhibition was as observed with HAP, but with a greater discrimination between zoledronate, alendronate, and risedronate. The degree of inhibition was dependent on the relative undersaturation, sigma, with respect to CAP. At a physiologically relevant undersaturation, $sigma_{CAP} = -.56$, K_L's for risedronate (3.90×10^5 M^{-1}) and alendronate (5.40×10^5 M^{-1}) were only 34% and 48% of that for zoledronate (1.12×10^6 M^{-1}), respectively. The inhibition of CAP dissolution by the these N-BPs was also related to the carbonate content of the crystallites. Greater dissolution rates were obtained with crystallites containing more carbonate. Thus, at $sigma_{CAP} = -0.56$ and pH = 5.50, K_L's for zoledronate, at a concentration of 1.0×10^{-6} 2795 M, were 1.11×10^6 and 3.56×10^5 M^{-1} for CAP containing $3.1 \pm 0.1\%$ and $8.0 \pm 0.1\%$ carbonate, respectively. These results confirm the importance of carbonate in distinguishing differences in the ability of BPs to target and affect bone mineral under physiological conditions. They further substantiate the significantly lower bone affinity of risedronate compared to alendronate and zoledronate. These differences in bone affinity may contribute to the observed differences in pharmacokinetics among these three BPs in the clinic.

P063

THE EFFECT OF TRYPSIN-CLEAVAGE AND REDUCTION ON THE PHOPHATASE ACTIVITY OF HUMAN RECOMBINANT TARTRATE-RESISTANT ACID PHOSPHATASE

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Tartrate-resistant acid phosphatase (TRACP) is an enzyme with unknown biological function. Two forms of TRACP circulate in human blood, a sialic acid-containing form TRACP 5a with a pH-optimum of 5.2 that is derived from macrophages and a non-sialylated form TRACP 5b with a pH-optimum of 5.8 that is derived from osteoclasts. The structure of TRACP contains at least three features that may be important for its activity, a cleavage site for proteases, a disulfide bridge in the polypeptide chain and a redox-active iron in a binuclear iron center. We produced human recombinant TRACP protein using baculovirus expression system, purified it using cation exchange and gel filtration chromatography, and cleaved the purified enzyme with trypsin. Trypsin cleaved the 35 kD monomeric native recombinant TRACP protein into two subunits of 23 and 16 kD that were linked together with the disulfide bridge. In Western analysis, a monoclonal antibody specific for serum TRACP 5a recognized the non-cleaved protein, but not the cleaved subunits. Kinetic parameters of the phosphatase activity of native and cleaved TRACP were studied in reduced and non-reduced conditions (see table). K_{cat} describes the capacity of the enzyme to catalyze the reaction, K_m the threshold of using the maximum capacity and k_{cat}/K_m how efficient the enzyme is in catalyzing the reaction. Beta-mercaptoethanol (BM) was used to reduce the disulfide bridge and ascorbate (ASC) to reduce the redox-active iron. The cleavage together with reduction changed the pH-optimum from 5.4 to 6.2. These results show that the cleavage and the reduction of both the disulfide bridge and the redox-active iron are all important regulatory systems for the phosphatase activity of TRACP. However, of these, only reduction of the redox-active iron affects k_{cat}/K_m alone, but cleavage and reduction together are powerful activators of TRACP. Antigenic properties and pH-optimum of the non-cleaved recombinant enzyme are similar to those of serum TRACP 5a, and after cleavage similar to those of serum TRACP 5b, suggesting that the osteoclast derived TRACP 5b may circulate in blood in an active cleaved form, and the macrophage-derived TRACP 5a in a less active non-cleaved form.

Table: Kinetic parameters of human recombinant TRACP

TRACP form	K_m (mM)	k_{cat} (sec^{-1})	k_{cat}/K_m (sec^{-1} M^{-1})
Native	1.47	9.11	6195
Native + BM	1.47	10.2	6965
Native + ASC	1.47	55.4	37658
Cleaved	3.85	17.9	4638
Cleaved + BM	3.85	111	28757
Cleaved + ASC	3.85	185	47928

P064

OSTEOPROTEGERIN IS BOUND, INTERNALISED AND DEGRADED BY CELLS STRONGLY EXPRESSING MEMBRANOUS RANKL

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Osteoprotegerin (OPG) is a decoy receptor for receptor activator of nuclear factor kB ligand (RANKL), an inducer of osteoclastogenesis via interaction with its receptor RANK. The balance between RANKL and OPG plays a central role in bone pathophysiology. Thus, the members of the triad OPG/RANK/RANKL, particularly RANKL, are implicated in a lot of osteolytic pathologies such as osseous tumors. The serum RANKL concentration is strongly increased in bone metastases, in primitive malignant bone tumors and in tumors associated with severe osteolysis resulting in an imbalance of the RANKL/OPG ratio in favor of RANKL. Human foetus kidney cells (293) expressing neither membranous RANKL nor OPG were transfected with the full length RANKL cDNA (293RL). Scatchard analysis has revealed a specific binding of OPG to these transfected cells. A time dependent decrease of OPG level was observed when OPG was incubated with 293RL cells. In contrast, for 293 cells, no binding of OPG was observed and the extracellular concentration of OPG was unaffected. This disappearance of OPG observed with the 293RL cells is however abolished in the presence of an antibody anti RANKL. Moreover, inhibitors of metalloprotease, serine, cystein and aspartic proteases did not prevent this decrease of OPG level, thereby excluding any enzymatic degradation of OPG in the culture medium. This study, based upon the becoming of OPG, has revealed that, after binding to the membranous RANKL, OPG is probably internalised and then degraded by the cells. This event explains that a high expression of RANKL is able to regulate the availability of OPG in the culture medium.

P065

EXPRESSION OF OSTEOCLASTOGENIC FACTORS BY MONOCYTES AFTER EXPOSURE TO METAL IONS

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Total hip joint replacement is a widely used and highly beneficial orthopaedic procedure. Despite its wide use, loosening of the implants is a frequent complication. Different processes may affect the longevity of the implant. One of these might be the sensitivity of the recipient to metal ions, which are dissolved from the implant. In the present study we investigated the release of inflammatory cytokines and the gene expression of monocytes treated with metal salts.

Monocytes isolated from peripheral blood from healthy donors were treated with metal salts [$KCr(SO_4)_2$; $TiCl_3$; $CoSO_4$; $NiCl_2$] at a concentration of 0.1mM for 2, 10, 18, 24, and 40 h at 37°C. The release of TNFα into the culture supernatant was measured by ELISA. Treatment of monocytes with Co^{2+} induced a significant increase of the release of TNFα as compared to unstimulated cells in 8 of 8 preparations at every time point. In further experiments using time point 40 h the release of TNFα was increased in 37 of 46 cell preparations. The effect of Ni^{2+} ions after 40 h of incubation on the release of TNFα was less pronounced (28/46), while Ti^{3+} and Cr^{3+} had virtually no effect. To further investigate the effects of Co^{2+} on monocytes, subtractive cDNA hybridization of cDNAs from monocytes treated for 40 h with Co^{2+} and from untreated control monocytes was performed. Differentially expressed cDNAs were subcloned into pGEM-T easy vector and the inserts were sequenced. From the characterized sequences, interleukin 8 (IL8), activin beta-A subunit, and chemokine ligand 5 (CXCL5) were chosen for further investigation. The regulated expression of the transcripts encoding these three proteins was confirmed by real time PCR. In three independent experiments, the expression of IL8, activin and CXCL5 transcripts was increased as compared to non-treated controls after stimulation for 40 h with Co^{2+}. The stimulation was 2–6 times for IL8, 370–1560 times for activin and 8–22 times for CXCL5. The levels of transcripts encoding TNFα were not changed under these experimental conditions.

The data demonstrate that exposure of monocytes to Co^{2+} induces a profound change in gene expression, leading to the release of factors that may eventually contribute to the haematopoietic microenvironment regulating the recruitment and activation of osteoclasts. This suggests that individual differences in the reaction of monocytes to metal ions may contribute to the focal resorption of periimplant bone.

P066

PREVENTION OF BONE LOSS BY CARTHAMUS TINCTORIUS L

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Carthamus tinctorius L. (Safflower) seeds are used in traditional Eastern folk medicine to promote bone healing and prevent osteoporosis. The aim of this study was to investigate how Carthamus seed extracts affect bone remodelling in vitro and to test if the extracts protect against bone loss in the ovariectomised rat model of postmenopausal osteoporosis. Hydrolyzed and non-hydrolyzed extracts of Carthamus seeds were tested for their inhibitory effect on in vitro bone resorbing activity in a rabbit mixed bone cell culture consisting of authentic osteoclasts in an environment of osteoblast and stromal cells and pit formation was assessed stereologically. Several different preparations of hydrolyzed Carthamus extracts (1–10 mg/ml) inhibited pit formation (29%–84%) in a dose-dependent manner when compared to the control. The Carthamus oil cake extract also inhibited pit formation by up to 85% at a similar concentration. No inhibitory effect on in vitro bone resorption was observed with non-hydrolyzed seed extracts, nor with extracts prepared from the flowers or the oil. The effect of the Carthamus seeds extracts on a key mediator of bone formation, BMP-2, was investigated using a murine BMP2-luciferase gene reporter assay. No effect of the Carthamus seed extract was observed on the expression of BMP-2. Whether or not Carthamus extracts affect other mediators of bone formation remains to be investigated. The estrogenicity of a seed extract was investigated in the human endometrial Ishikawa cells which express endogenous estrogen-inducible alkaline phosphatase activity. No estrogenic effect was detected with the extracts. Taken together, these results suggests that the postulated beneficial effect of Carthamus seeds on bone might be due to an inhibition of bone resorption acting by a non-estrogenic mechanism. The effect of different Carthamus seed extracts on in vivo bone resorption is presently being evaluated using the ovariectomised rat model of postmenopausal osteoporosis.

P067

RELEVANCE OF AN IN VITRO OSTEOCLASTOGENESIS SYSTEM TO STUDY RECEPTOR ACTIVATOR OF NF-KB LIGAND AND OSTEOPROTEGERIN BIOLOGICAL ACTIVITIES ?

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Receptor Activator of NF-kB Ligand (RANKL) is an essential requirement for osteoclastogenesis and its activity is neutralized by binding to the soluble decoy receptor osteoprotegerin (OPG). The purpose of this work was to study the effects of RANKL and OPG during osteoclastogenesis using the murine monocytic cell line RAW 264.7 that can differentiate into osteoclasts in vitro. RAW 264.7 cells plated at 104 cells/cm2 and cultured for 4 days in the presence of RANKL represent the optimal culture conditions for osteoclast differentiation, with an up-regulation of all parameters related to bone resorption: Tartrate Resistant Acid Phosphatase (TRAP), Calcitonin Receptor (CTR), RANK, cathepsin K, MatrixMetalloProteinase (MMP)-9 mRNA expressions. RANKL and OPG biological effects vary according to the differentiation state of the cells: in undifferentiated RAW 264.7 cells, TRAP expression was decreased by OPG and RANKL, RANK expression was inhibited by OPG, while MMP-9 and cathepsin K mRNA expressions were not modulated. In differentiated RAW 264.7 cells, RANKL and OPG both exert an overall inhibitory effect on the expression of all the parameters studied. In these experimental conditions, OPG-induced MMP-9 inhibition was abrogated in the presence of a blocking anti-RANKL antibody, suggesting that part of OPG effects are RANKL-dependent. The aim of the present study was also to compare the biological activities of RANKL and OPG between two cellular models (purified rabbit osteoclasts and the murine monocytic cell line RAW 264.7 that can differentiate into osteoclasts in vitro). This study confirmed evidence for a direct effect of OPG on osteoclast-like cells. However, results obtained demonstrate that the murine cell model of RAW 264.7 cells does not seem to be the best appropriate to study osteoclast differentiation.

P068

CHARACTERIZATION OF A BIPOTENT PRECURSOR POPULATION ASSOCIATED WITH HEMATOLOGICAL DEFECTS IN OSTEOPETROSIS

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Bone and bone marrow constitute a functional unit in which cell interactions are essential. These interactions have been confirmed by the analysis of various mice models. Among them, the spontaneous oc/oc mouse mutant develops an osteopetrosis, a disease characterized by a defect in bone resorption due to a loss of osteoclast function. In the oc/oc mouse, we have analyzed the consequences of the perturbed bone architecture resulting in the absence of normal bone marrow cavity on hematopoiesis. Our results show that hematopoiesis is altered in the oc/oc mouse with an increased myelopoiesis and a block in the B lymphoid differentiation at the pro-B to pre-B transition. Analysis of the oc/oc pro-B cells revealed that 85% of these cells coexpress myeloid and lymphoid markers and have a myeloid and a lymphoid differentiation potential *in vitro*. While this biphenotypic population is present at a very low level (0.5%) in the bone marrow of control littermates, it appears to be amplified (15%) in the osteopetrotic bone of the oc/oc mutants and associated with a down regulation of Pax5 and IL-7 expression levels, two factors essential for B cell differentiation. Thus, our results clearly indicate that perturbations in the bone microenvironment have important consequences in both myeloid and lymphoid hematopoiesis, and strongly suggest the existence of an *in vivo* plasticity between B lymphoid and myeloid pathways.

P069

THE EFFECT OF HYPOXIA AND PH ON OSTEOCLAST FUNCTION IN THE CAT

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Hypoxia has been implicated in the pathogenesis of a number of diseases associated with bone destruction e.g. arthritis, osteomyelitis, fracture and neoplasia. pH reductions also occur in hypoxic tissues, and there is *in vitro* evidence that hypoxia and H^+ strongly upregulate osteoclast (OC) function (*J Cell Physiol 196:2–8, 2003*). In domestic cats, OC resorption lesions of the root surface (FORL) are common yet the cause of the condition is not understood. Here we explore the hypothesis that FORL is associated with hypoxia and/or decreased pH in the oral microenvironment, leading to increased OC activity.

Methods: OCs were generated from feline PBMCs and cultured on bovine bone. The PO_2 of the cultures was varied from ambient (20% O_2) to hypoxic (2%) between d1–14. pH was adjusted from control (7.2) between d11–14 (range: pH 5.5–8.5) by addition of HCl or NaOH. Cells were then stained for TRAP. Parameters measured included: OC number and mean OC area. After wheat germ lectin-FITC staining, the number of resorption pits, mean pit area and percentage area resorbed were determined by image analysis (LEICA Qwin software). Confocal microscopy was used for semi-quantitative comparisons of the effect of decreased pH (days 7–14) and hypoxia on the expression of cathepsin K, the H^+-V-ATPse proton pump and TRAP.

Results: Reducing PO_2 from 20% to 2% increased the mean area of OC formed 9-fold from 0.01 ± 0.003 mm^2 to 0.09 ± 0.03 mm^2. In hypoxic cultures, very large OCs containing several hundred nuclei were evident. The area of bone surface resorbed increased 12.6-fold from $6.1 \pm 2.7\%$ in 20% O_2 to $76.7 \pm 13.9\%$ in 2% O_2. OCs exhibited maximal activity between pH 6.9–7.1. In low pH cultures OCs containing several hundred nuclei were also evident. The total area of bone surface resorbed increased 4.8-fold from $14.9 \pm 4.5\%$ at pH 7.25 to $71.5 \pm 13.1\%$ at pH 6.9, accompanied by a ~2-fold increase in the absolute numbers of resorption pits formed. Culturing cells at pH 6.8 and in 2% O_2 also upregulated the expression of cathepsin K, H^+-V-ATPse proton pump and TRAP.

Conclusion: These results show that decreased PO_2 and pH induces the formation of gigantic feline OCs and dramatically stimulates bone resorption *in vitro*. Our results imply that in inflammatory and ischaemic diseases, like FORL, the acid activation effect will amplify the osteoclastogenic effect of hypoxia, resulting in extensive mineralised tissue destruction.

P070

SIMULATING THE EFFECTS OF AGEING ON RATES OF RESORPTION VIA REDUCTION IN MATRIX TGF-BETA AND THE APOPTOSIS THRESHOLD

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The development of pharmaceutical treatments for bone disease can be enhanced by mathematical models that predict their effects on resorption during cancellous bone remodeling. The depth and duration of resorption at one microsite on the surface of cancellous bone can be simulated by Michaelis-Menten equations that describe cellular activity. During the first phase of resorption the substrate for osteoclastic activity is assumed to be limited by the ratio of RANKL:OPG ('effective RANKL'). The release of growth factors from the matrix during resorption causes two negative feedback effects: 1. TGF-beta1-induced OPG production by marrow stromal cells reduces osteoclast activity via a reduction in effective RANKL, and 2. apoptosis of osteoclast nuclei at high concentrations of TGF-beta1, assumed to occur above a 'threshold' level of TGF-beta1, which brings about the end of the first phase of resorption. The rate of collagen fibril removal during the second phase of resorption is simulated by the activity of mono-nucleated lining cells.

TGF-beta1 in cancellous bone matrix may be reduced by up to 30% in adults between the ages of 25 to 50 years. To test whether the changes in resorption observed in healthy adults between these ages can be simulated by the model, simulations were run with a 30% decrease in matrix TGF-beta1, both with and without a concurrent change in the apoptosis threshold.

A 30% decrease in matrix TGF-beta1 alone simulates a 41% increase in resorption depth, and a 41.8% increase in duration of resorption. However, with a concurrent 30% decrease in the apoptosis threshold, the decrease in matrix TGF-beta1 predicts less than 1% reduction in resorption depth, 2.3% decrease in duration, giving a slight increase in the overall rate of resorption of 1.5%. Thus, for the model to simulate the observed homeostatic control in bone resorption during ageing from 25 to 50 years, the variation in matrix TGF-beta1 must be linked with changes in the threshold of apoptosis. One possible mechanism of such linking would be via the control of both matrix TGF-beta1 and the apoptosis threshold by cells of the osteoblast lineage within the local bone/marrow microenvironment.

This is the first time that a mathematical model of cellular activity has been used to investigate the effects of ageing on the rate of resorption during remodeling, and forms a fundamental step in the process of modelling osteoporotic changes in the bone and potential treatment effects.

P071

INCREASED RANKL/OPG MRNA RATIO IN ILIAC BONE BIOPSIES FROM WOMEN WITH HIP FRACTURES: ASSESSMENT OF THE INFLUENCE OF UPSTREAM CYTOKINES IL-1, IL-6 AND IL-7

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Background: RANK-L is a potent physiological inducer of osteoclastogenesis. Its actions are blocked by the decoy receptor osteoprotegerin (OPG), and treatment with OPG potently blocks bone resorption in postmenopausal women. Both positive and negative associations between serum OPG and BMD have been reported in the literature, however. We hypothesized that decreased OPG production within bone itself-relative to RANKL-could be caused by increased expression of the upstream cytokines IL-1, IL-6 and/or IL-7, and lead to osteoporosis.

Study population: 24 women with osteoarthrosis of the hip (*age 72.8y $\pm$ 7.2, hip BMD 0.832 $\pm$ 1.1 g/cm^2*) and 10 women with hip fracture (*age 76.3y $\pm$ 8.0, n.s., hip BMD 0.686 $\pm$ 1.3 g/cm^2, P < 0.05*). Biopsies were obtained at the time of surgery after informed consent.

Methods: tRNA was extracted from 8 mm iliac bone biopsies, reverse transcribed and real time quantification relative to beta-actin mRNA was performed with a SYBR Green I real time PCR assay using the comparative CT method for calculating relative gene expression, with normalization of results for beta-actin message.

Results: Actin normalized mRNA levels for OPG and IL-6 were significantly lower in fracture patients (*median −58%, P < 0.05 and median & −77%, P < 0.05*). In contrast, there was no significant difference in RANKL (*−21%, P = 0.22*), IL-1beta (*−37%, P = 0.52*), IL-1ra (*−35%, P = 0.93*), or IL-7 (*−38%, P = 0.90*) expression. As for the derived ratios, there was no difference in IL-beta/IL-1ra ratio, but a significantly higher RANKL/OPG ratio in patients with fractures (*+ 133%, P < 0.01*).

Conclusions: This study suggests that lower OPG mRNA content within the bone microenvironment, both relative to RANKL mRNA and in absolute

terms, is involved in the pathogenesis of senile osteoporosis. Interestingly, the increased RANKL/OPG ratio is not explained by altered expression of any of the pro-inflammatory cytokines examined.

P072

CHARACTERIZATION OF OSTEOCLASTIC ACTIVITY DURING THE COURSE OF FRACTURE HEALING

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The continuos remodeling of bone is enabled by two specialized cell types, osteoblasts and osteoclasts. Fracture healing is a complex mechanism and therefore represents a special challenge for both these cell types. During fracture repair, osteoblasts synthethise new bone tissue, bridging the fracture gap, whereas osteoclasts resorb excess bone tissue, modifying the structure of the bone. In a study of fracture healing, Aro et al. (1990) described decreasing callus area and decreasing number of osteoclasts within 6 weeks. The aim of this study was to correlate the number of osteoclasts with histomorphometrical measures of the callus area and the density of callus and cortical bone. Four groups of sheep (n = 8 each) underwent an osteotomy of the tibia to be stabilized by an external fixator. Animals were sacrificed after 2, 3, 6, and 9 weeks. The fracture callus was analysed histologically. The mineralized bone area in the callus tissue increased from 2 to 6 weeks ($P \leq 0.038$). The density of callus and cortical bone decreased between 2 and 9 weeks (3 to 6 weeks, $P = 0.001$), while the number of osteoclasts increased steadily. The osteoclastic density (number of osteoclasts/mm^2 mineralised tissue) showed a similar development, the increase was significant for the total callus area between 2 and 9 weeks ($P = 0.002$), the endosteal callus between 2 and 3 weeks ($P = 0.01$) and for the cortical bone between 6 and 9 weeks ($P = 0.001$). These results contrast with the findings of Aro et al. (1990). This study indicates the important role of osteoclasts right from the very beginning of the fracture repair process. The periosteal callus was characterised by a constant density of osteoclasts, indicating a continuous remodeling within this main stabilising callus area. The endosteal callus however, showed an increase in osteoclastic density very early accompanied by decreasing amount and density of callus tissue suggesting recanalisation of the medullary canal begins in the very early phase of healing. The cortical bone remained unaltered up to 6 weeks, at which time bone density dropped drastically and osteoclasts increased in number and density. The structure of the cortical bone was visibly adapted to that of the callus, possibly to reduce strain rises at their interface. This study illustrated the complex role of osteoclasts during fracture healing. The remodeling process starts while the bony callus is still being generated.

P073

SIGNAL TRANSDUCTION PATHWAYS ACTIVATED BY PASTEURELLA MULTOCIDA TOXIN (PMT) DIFFERENTIALLY CONTROL OSTEOCLAST DIFFERENTIATION AND ACTIVATION: A ROLE FOR THE RHO GTPASE

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Rho family GTPases are well-known regulators of the cell cytoskeleton, cell migration and differentiation. We have recently demonstrated that the unique bacterial toxin, *Pasteurella Multocida* toxin (PMT), which activates the heterotrimeric G-protein, Gq, leading to stimulation of Rho and actin rearrangements, as well as activation of phospholipase C, protein kinase C and the Ras/MAP kinase pathway, inhibits osteoblast differentiation and bone nodule formation via activation of Rho and Rho kinase (ROK). However, PMT also targets osteoclastic cells, since the main *in vivo* effect of PMT is the porcine bone resorbing disease, atrophic rhinitis, resulting in pathological bone resorption. Since Rho proteins play an important role in the rapid cytoskeletal rearrangements during bone resorption, the effects of PMT on osteoclast differentiation and activity were investigated.

In RANKL- and MCSF-based cultures of murine bone marrow cells PMT dose-dependently inhibited osteoclast formation and resorption with a concomitant decrease in calcitonin receptor mRNA. This inhibition was manifested on osteoclast precursors, although molecular analysis demonstrated that PMT had no effect on RANK or *c-fms* expression. PMT also potently inhibited the differentiation and activity of both human and porcine PBMCs in a dose-dependent manner. Using specific pathway inhibitors, treatment of murine or human osteoclast precursors with the ROK inhibitor, Y-27632, partially rescued the inhibition of osteoclast differentiation, whereas Y-27632 alone had little or

no effect. In contrast, the MEK inhibitor, PD98059, failed to rescue the PMT effect. These data suggest an important inhibitory role for the Rho-ROK pathway in osteoclast differentiation. In contrast to the inhibitory effects on osteoclast precursors, PMT treatment of mature rabbit osteoclasts had no effect on resorption over 24 h, although preliminary studies showed that 72 h PMT treatment increased osteoclast number and resorption, suggesting a possible role for PMT in osteoclast survival. These stimulatory effects were also observed in human cultures, where treatment of mature human osteoclasts increased the proportion of F-actin ring-containing vitronectin receptor-positive osteoclasts, with a concomitant increase in resorption. These studies establish the relative importance of the pathways utilised by PMT in dissecting which signalling mechanisms are important for osteoclast differentiation vs. activation.

P074

SEVERE HYPOXIA INCREASES OSTEOCLAST FORMATION FROM HUMAN PERIPHERAL BLOOD: AMPLIFICATION OF RESORPTION BY ACIDOSIS

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Bone loss in humans frequently occurs in association with local or systemic hypoxia, e.g., in fractures, infections, inflammations, tumours, anaemias, airway and cardiovascular diseases (including smoking), excessive exercise or ageing. pO_2 as low as 0.2% increases osteoclast formation and resorption in mouse marrow cultures (J Cell Physiol 196:2–8, 2003). We have now studied the effects of severe and long-term hypoxia on the formation and function of human osteoclasts (hOC). Human PBMCs were cultured on ivory discs for up to 28d in MEM with 15% FCS, 5 ng/ml M-CSF and 0.5–2 ng/ml RANKL in 25 cm^2 flasks gassed with 0.2% to 20% O_2 (plus 5% CO_2; balance N_2) at pH 7.3. Cultures were acidified to pH 7.0 with HCl for the final 2d to increase resorption pit formation by OC generated. Discs were stained for TRAP or with toluidine blue for analysis of cell numbers and resorption. In 20%, 12%, 5%, 2% & 0.2% O_2, numbers of hOC formed per disc were 73.4 ± 22.8, 55.2 ± 23.4, 121.7 ± 31.6, $242.4 \pm 40.5^{**}$ & $202.6 \pm 48.9^*$, respectively. Numbers of nuclei per cell in 20% and 2% O_2 were 1.72 ± 0.15 and $3.32 \pm 0.40^{**}$; over the same range, surface resorption area was 0.69 ± 0.20 and $3.84 \pm 1.03^*$ (*$P < 0.05$; **$P < 0.01$). The results show that hOC formation is increased even when pO_2 is 1/100 of the atmospheric level (eg, corresponding to the most severe tumour hypoxia). The increased resorption in hypoxia was likely due both to increased number and size of hOC. We found that low levels of RANKL and M-CSF were necessary for effective hOC formation in hypoxia; in such conditions, low pO_2 stimulates hOC formation to the same extent as saturating doses of RANKL ($ > 10$ ng/ml). Whether hypoxia increases the expression of angiogenic, osteoclastogenic cytokines or endogenous RANKL remains to be determined. Hypoxia *in vivo* also causes tissue acidosis, and we investigated the effect of pH reductions on hOC formed in hypoxic cultures. We found that reducing pH from 7.4 to 6.8 resulted in a 12-fold increase in resorption pits per hOC, and that half maximal acid-activation of resorption occurred at pH ~7.3, as opposed to pH ~7.1 for rodents. Our results show that acidosis strongly amplifies the resorption resulting from increased hOC formation in hypoxia. Hypoxia and acidosis also cooperatively inhibit osteoblastic bone formation. These findings emphasise the key role of the vasculature in human bone loss disorders.

P075

THE MECHANISM OF ACTION OF M-CSF SUPPRESSING 1,25(OH)2D3 INDUCED OSTEOCLAST FORMATION

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Osteoclast differentiation and activation requires the presence of Osteoblast derived factors such as macrophage colony stimulating factor (M-CSF) and osteoprotegerin ligand (OPGL). OPGL is influenced by osteotropic hormones such as calcitriol (1,25(OH)2D3). In this study, we compared the *in vitro* effect of 1,25(OH)2D3 alone with the combination of 1,25(OH)2D3 and M-CSF on mouse bone marrow osteoclasts progenitors, as well as the difference on gene expression between these two groups. Osteoclast development was evaluated by examination and cell count of TRAP positive multinucleated cells at the day 10 of culture. Super array (Bioscience corporation super array Q series, original series and a probe synthesis kit) study was performed after RNA extraction of cells treated with 10-8 M 1,25(OH)2D3, and cells treated with 60 ng/ml M-CSF and 10-8 M 1,25(OH)2D3. When osteoclast precursors from mouse bone marrow were cultured with 10-8 M 1,25(OH)2D3, the osteoclast count was 24.25 ± 19.97 cells/well. When osteoclast precursors were cultured with 10-8 M

1,25(OH)2D3 and 60 ng/ml M-CSF, there was no osteoclast formation. In order to determine when the inhibitory effect of M-CSF is maximum, we cultured two groups of murine bone marrow cells with 1,25(OH)2D3. Afterwards we added M-CSF during the first 4 days to one group (23 ± 20.77) and during the last 6 days to the second group (2 ± 2.45). These results showed that the addition of M-CSF at the end of the culture inhibits significantly osteoclasts formation induced by 1,25(OH)2D3 ($P < 0.05$). Analysis of the super array in the group treated with 1,25(OH)2D3 showed a strong expression of FADD, Gro-1, IL-1β, IL-6, TGF-α and TNF-β. When apoptotic genes were analyzed, there was no difference between the group treated with 1,25(OH)2D3 and the group treated with 1,25(OH)2D3 and M-CSF. The expression of vitamin D receptor (VDR) was similar in both groups. We demonstrate that the addition of 1,25(OH)2D3 to M-CSF suppressed osteoclast formation. Since there was no difference in expression of VDR between the two group, the effect of 1,25(OH)2D3 could be mediated through an intracellular change in calcium concentration. Super array analysis in the group treated with 1,25(OH)2D3 alone compared to the group treated with 1,25(OH)2D3 and M-CSF showed a strong expression of FADD. Therefore we consider the possibility of FADD inducing cell proliferation and differentiation.

P076

VALIDATION OF A HISTOMORPHOMETRIC METHOD TO STUDY BONE FORMATION AND BONE RESORPTION IN MICE

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Bone histomorphometry is a widely used technique by which bone formation and bone resorption can be determined in a variety of *in vivo* systems, including in animal models of osteoporosis and other disorders of bone metabolism. Different methods can be applied in order to determine the indices of bone turnover, but few reports have addressed how to count resorptive and formative indices in sections of bone from mice. The aim of this study was therefore to establish and validate a standardized technique to measure bone formation and resorption on bone slices of the vertebral spine from mice. Five four-month old C57/BL mice were sacrificed, and bones were collected for histomorphometry. After methyl-methacrylat embedding, bones were sectioned and stained. From each individual, 6 slides were prepared, five for determination of resorptive indices and one for formative indices. On each slide, sections from 5–8 vertebrae were present. Mineralizing surface as percentage of bone surface (MS/BS) and eroded surface as percentage of bone surface (ES/BS) were determined. Confidence intervals of the mean and coefficient of variation (CV%) were calculated for each mouse between different vertebrae in the same section (inter-vertebral) and between the same vertebra in different sections (intra-vertebral). Mean MS/BS was found to be in the range of 45.8 to 56.3% in the different animals, while mean ES/BS was in the range of 7.8 to 14.5%. To compare intra- and inter-vertebral measurements, ES/BS was determined on all vertebrae in one slide, and on the same vertebra in all five slides. Confidence intervals and CV% were found to be almost identical whether intra- or inter-vertebral measurements were performed. CV% values ranged from 1.2 to 10.2. Further, to determine the number of vertebrae to count in order to minimize variation, we have used the least square principle. By using this method, we have determined that confidence interval of the mean and CV% is improved until three to five vertebrae evaluated. Increasing the number of included vertebrae beyond this does not improve the estimation of the mean nor does it improve the CV%. In conclusion, there is no difference whether resorptive indices are measured on one slide and several vertebrae or they are measured on the same vertebra but on several sections. Further, the optimal number of vertebrae to evaluate from each individual is (3-)5.

P077

LOSS OF CHAOTIC TRABECULAR STRUCTURE IN JUVENILE PAGETS DISEASE PATIENTS INDICATES A CONTROLLING ROLE FOR OSTEOPROTEGERIN IN NONLINEAR PATTERN FORMATION OF TRABECULAR BONE

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Why is trabecular bone trabecular? Evidence is presented that nonlinear pattern formation determines trabecular architecture and that the RANK-RANKL-OPG system of osteoclast regulation may play a key role in this pattern formation. In particular a role for OPG is suggested by the highly abnormal trabecular architecture found in iliac trabecular bone from Juvenile Pagets Disease (JPD) patients deficient in functional OPG. Evidence from experimental systems suggests that RANK-RANKL-OPG controls key nonlinear system parameters, which may include friction, forcing frequency, feedback and

boundary forcing. The Belousov-Zhabotinsky reaction-diffusion system, the catalytic oxidation of CO on platinum surfaces and thermal diffusion in liquid helium allow visualisation of nonlinear emergent patterns such as labyrinthine structures, cellular structures and turbulence, all of which have some similarity to trabecular bone. In JPD the gene for OPG (TNFRSF11B) is subject to an inactivating mutation, leading to greatly increased resorption and accelerated remodelling. Iliac crest trabecular bone from a teenage girl suffering from JPD shows a highly unusual array of parallel, regular trabecular plates, instead of the typical chaotic, fractal patterns of normal trabecular bone. Loss of OPG function is associated with a change from chaotic to regular structure, suggesting that the RANK-RANKL-OPG system is controlling at least one key nonlinear parameter. Nonlinear pattern formation implies higher order control of trabecular architecture over regions, as opposed to local "micromanagement" of remodelling. This perspective of nonlinear pattern formation establishes a direct link between biochemical regulation of bone remodelling and bone architecture, and may assist understanding of phenomena such as the marked dependence of trabecular bone's mechanical quality on remodelling rate, independent of trabecular bone mass.

P078

LITTLE EVIDENCE OF RESCUE OF OSTEOCLAST-POOR OSTEOPETROSIS FOLLOWING 'SUCCESSFUL ENGRAFTMENT' BY CORD BLOOD FROM AN UNRELATED DONOR

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Osteopetrosis (OP) is a rare genetic disorder characterised by severely reduced bone resorption resulting from a defect in either osteoclast (OC) development (OC-poor OP) or activation (OC-rich OP). The consequence is dense, fragile bones, a reduced bone marrow cavity associated with extramedullary haemopoiesis. Patients in which the defect results from OC activation can be rescued by bone marrow or cord blood transplantation. However, there is very little information concerning the success for transplantation as a treatment for OC-poor OP.

We report on a child with OC-poor OP, diagnosed at the age of 7 by histopathology and radiology. At presentation, the patient was blind, anaemic, thrombocytopenic and leucopenic and had evidence of extramedullary haemopoiesis. At age 8, the child received a cord blood transplant from an unrelated donor. Engraftment was considered successful on the basis that circulating cells were all of donor origin one year later. However, 2 years post-transplant, there was virtually no evidence of rescue of the skeletal disease and there was persistent anaemia and extramedullary haemopoiesis. Peripheral blood mononuclear cells (PBMC) from the patient prior to and post transplant were cultured on bone slices in the presence of M-CSF (25 ng/ml) and RANKL (30 ng/ml) for 2-3 weeks. PBMC cultures from healthy individuals were used as controls. PBMC from the OP child taken prior to transplantation, formed very small number of OCs (F-actin ring-positive cells which also expressed the vitronectin receptor and tartrate-resistant acid phosphatase) *in vitro*. The OCs were mono or binucleate and associated with small resorption lacunae (approx. 2% of the surface of the bone slices). Most of the cells generated in these cultures expressed macrophage markers including CD11c and CD18. Similar results were observed when the release of collagen fragments from these cultures, were assessed by ELISA. In contrast, OC formation in the PBMC cultures, generated from the child following the cord blood transplant, showed results similar to those observed in control cultures: large numbers of OCs were present and these contained numerous nuclei. Approximately 50% of the surface of the bone slices was resorbed.

Our findings indicate that not all forms of OC-poor OP can be rescued by haemopoietic transplantation. The *in vitro* result supports previous studies showing that neither M-CSF nor RANLK rescue OC-poor OP.

P079

EXPRESSION AND LOCALIZATION OF INTEGRINS, NOS AND CAVEOLIN-1 IN OSTEOBLASTS AND OSTEOCYTES

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During differentiation to osteocytes, osteoblasts change morphologically and functionally from cells producing bone matrix to mechanosensory cells. We studied whether this differentiation is accompanied by changes in the expression levels of beta 1 and beta 3 integrins known to play a role in osteoblast differentiation and implicated in the sensing of fluid shear stress. We also studied

whether nitric oxide synthases (NOS) are expressed and localized at the same sites as integrins in osteoblastic cells, as eNOS may be activated through integrin-linked-kinase in other cells types. Primary murine osteoblasts, the osteoblastic cell line MC3T3 and the osteocytic cell line MLO-Y4 were used. Cells were grown onto collagen-coated glass coverslips and stained with antibodies to integrin subunits, endothelial NOS (eNOS), neuronal NOS (nNOS) and caveolin-1 and analysed by confocal microscopy. In addition RT-PCR was carried out to obtain data on mRNA expression. All integrin units examined (a1, a2, av, b1 and b3) were expressed by RT-PCR. By immunostaining, b1 and a5 were highly expressed on all osteoblastic cells and there was little difference in expression levels between osteoblasts and osteocytes. Surprisingly a1 and a2 were undetectable; b3 was seen at very low levels and av had a perinuclear, rather than membrane-associated expression. We were unable to test whether av was associated with b5, due to the absence of a suitable reagent in the mouse and immunoprecipitation experiments or all integrins proved difficult with the reagents available for studies in the mouse. By immunostaining eNOS and nNOS were both detected in osteoblasts, confined to the perinuclear region and not colocalising with caveolin-1, or integrin on the cell membrane. In addition, b1 integrin was clearly excluded from the membrane regions staining for caveolin-1. qRT-PCR in all three cell types showed that the levels of nNOS were only 3% of those seen for eNOS, indicating that eNOS is the dominant NOS isoform in osteoblastic cells. These results indicate that a5b1 is the most abundant integrin in osteoblastic cells. nNOS is expressed in osteoblasts and osteocytes, but levels are very low compared to eNOS. The mechanisms of eNOS activation in osteocytes remains unclear as eNOS is not present at detectable levels in caveolae, or even near the plasma membrane, in resting cells. Further studies are ongoing to investigate relocalisation of eNOS and a5b1 integrin by mechanical stimulation.

Osteoporosis: Pathophysiology and Epidemiology

P080

NO EFFECT OF VITAMIN A INTAKE ON BONE MINERAL DENSITY AND FRACTURE RISK IN PERIMENOPAUSAL WOMEN

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Background: In recent studies from Sweden and USA, a high vitamin A intake has been associated with low bone mineral density (BMD) and increased fracture risk. In Sweden and USA, food items such as milk and breakfast cereals are fortified with vitamin A, whereas in Denmark there is no mandatory fortification with vitamin A.

Aim: We investigated relations between vitamin A intake and BMD and fracture risk in a Danish population consuming mostly unfortified food items. Within a population-based cohort study in 2016 perimenopausal women, associations between BMD and vitamin A intake was assessed at baseline and after 5-year follow-up. Moreover, associations between baseline vitamin A intake and 5-year changes in BMD were studied. Finally, fracture risk was assessed in relation to vitamin A intake.

Results: In our cohort, dietary retinol intake (0.53 mg/day) was lower than the intake reported in recent studies form Sweden (0.78 mg/day) and USA (1.66 mg/day). Cross-sectional and longitudinal analyses showed no associations between intake of vitamin A and BMD of the femoral neck or lumbar spine. Neither did BMD differ between those 5% who had the highest- and those 5% who had the lowest vitamin A intake. During the 5-year study period, 163 subjects sustained a fracture (cases). Compared to 978 controls, logistic regression analyses revealed no difference in vitamin A intake.

Conclusion: In a Danish population, average vitamin A intake is lower than in Sweden and USA and not associated with detrimental effects on bone. Further studies should explore whether fortification of food items (as in Sweden and USA) is harmful to bone health.

P081

HIP FRACTURE RISK IN STATIN USERS –A POPULATION BASED DANISH CASE-CONTROL STUDY

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Background: Statins have been suggested as potential agents in the treatment of osteoporosis. In some but not all previous epidemiological studies, treatment with statins has been associated with a reduced fracture risk.

Aim: To examine associations between statin treatment and hip fracture risk.

Subjects and methods: In a population-based case-control study design, a total of 6,660 subjects with hip fracture and 33,274 age-matched population controls were retrieved using the Hospital Patient Register in North Jutland County, Denmark and the Danish Central Personal Registry, respectively. Data on redeemed prescriptions for statins within the last five years before the index date were retrieved from a population-based prescription database. We used conditional logistic regression to estimate odds ratios (ORs) for hip fracture according to use of statin prescriptions adjusted for potential confounding factors, i.e., gender, diseases and use of other drugs known to affect bone metabolism and fracture risk.

Results: Risk of hip fracture decreased as number of statin prescriptions increased. After adjustment for potential confounders, statin treatment was associated with a reduced hip fracture risk (OR 0.68; 95% CI 0.49–0.92) for those who had redeemed more than three prescriptions for a statin drug. Stratified analyses on gender and age did not reveal any major differences between men and women or between different groups on the association between use of statins and hip fracture risk.

Conclusion: Our finding supports an effect of statin treatment on hip fracture risk. A reduced fracture risk may be a positive side effect of statin treatment. Further studies are needed to determien whether this association is causal.

P082

BONE RESORPTION RATE IS AN INDEPENDENT PREDICTOR OF OSTEOPOROTIC FRACTURES IN ELDERLY MEN. THE DUBBO STUDY

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Introduction. Approximately one third of osteoporotic fractures occur in men. Among many potential risk factors for fracture, bone turnover is considered an important determinant. In contrast to postmenopausal osteoporosis, however, their predictive value in men has not been well established. We examined the association between markers of bone turnover and fracture risk in elderly community-dwelling men.

Methods. A case-cohort control study involved 50 men with incident fractures and 101 men without fracture, aged 71 ± 5.2 yrs (mean $\pm$ SD), who have been prospectively followed in the Dubbo Osteoporosis Epidemiology Study for a median of 5.5 yrs. Serum carboxyterminal crosslinked telopeptide of type I collagen (S-ICTP) as a bone resorption marker, serum aminoterminal propeptide of type I procollagen (S-PINP) as a bone formation marker, and lumbar spine and femoral neck BMD (LS BMD, FN BMD) were measured at baseline.

Results. At baseline, men with subsequent fractures had lower BMD, lower dietary calcium intake, and higher S-ICTP levels than controls. Age, BMI, smoking habits, and S-PINP did not differ between groups. In multivariate logistic regression analyses, both S-ICTP (RR 2.6 per SD, 95% CI, 1.7–4.2) and FN BMD (RR 1.9 per SD, 95% CI, 1.2–2.9) were independent predictors of fracture risk. Men within the highest quartile of S-ICTP had a 2.8-fold (95% CI 1.4–5.4) increased risk of fracture compared with men with levels in the lowest quartile. Estimate of population attributable risk fraction indicated that 31% of the fracture risk in the population was explained by S-ICTP and FNBMD, to which S-ICTP contributed 24.3%.

Conclusion. High rates of bone resorption appear to be associated with increased risk of osteoporotic fracture in elderly men, independent of BMD. A combination of bone turnover markers and BMD may improve fracture prediction in elderly men over and above the sensitivity of either risk factor.

P083

BONE DENSITY IN YOUNG MALE PATIENTS WITH NEWLY DIAGNOSED INFLAMMATORY BOWEL DISEASE

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Background: A high prevalence of osteoporosis has been reported in inflammatory bowel disease (IBD). Development of osteoporosis in patients with IBD seems to be a phenomenon related to both disease process and steroid treatment.

Objectives: The purpose of this study was to evaluate prospectively the prevalence of osteoporosis and osteopenia in steroid naive young male patients with IBD at the time of initial diagnosis. Also, to determine whether bone density is associated with clinical variables (type of disease, age, body mass index (BMI), duration of disease).

Methods: 31 young male patients with newly diagnosed IBD, aged 18–37 years, participated in the study. 18 were with Crohn's disease (CD) and 13 with ulcerative colitis (UC). Calcaneal bone density was measured by a Lunar Achilles plus ultrasound bone densitometer using the T score (Tsc). All patients hadn't taken steroids or other treatment for IBD before ultrasound measurement. Osteoporosis was defined as Tsc < −2.5 SD, and osteopenia between −2.5 SD and −1 SD.

Results: 17 patients had duration of disease more than six months and the others less than six months. Mean age was 25.7 ± 4.9 years, BMI 23.9 ± 4.3 kg/m2 and Tsc -0.15 ± 1.54 (-0.11 ± 1.30 in UC patients, -0.19 ± 1.72 in CD patients and -0.67 ± 1.62 in patients with disease duration > 6 months, 0.47 ± 1.21 with disease duration < 6 months). Two patients, all of whom with CD, had osteoporosis (6.4%) and 7 patients, 4 with CD and 3 with UC, osteopenia (22.6%). There was a positive correlation between Tsc and BMI ($r = 0.481$ $P = 0.006$). A low Tsc was associated with disease duration > 6 months ($t = -2.260$ $P = 0.032$). No statistically significant difference in Tsc between patients with UC and CD was found.

Conclusion: About one third of young male patients (29%) with newly diagnosed IBD and without previous steroid use had osteoporosis or osteopenia. Bone mass was associated with BMI and duration of disease. Disease process seems to have a significant influence on bone loss. It is suggested that bone density measurement should be performed in all patients with IBD regardless of gender, age or previous steroid use.

P084

RISK FACTORS FOR LOW BONE DENSITY IN A GREEK YOUNG MALE POPULATION

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Background: Osteoporosis is a condition affecting not only women but also men. Peak bone mass is associated with genetic potential and environmental effects. Osteoporosis may be prevented by identifying and modifying risk factors in young age.

Objectives: To determine the risk factors associated with low bone density in young men.

Methods: 99 young males (mean age 20.32 ± 3.38 years) participated in the study. All subjects were asked to complete a questionnaire. Bone density was measured at the calcaneus by a Lunar Achilles plus ultrasound bone densitometer using the T score (Tsc).

Results: Mean height was 177.98 ± 7.17 cm and weight 73.47 ± 13.72 kg. 62.2% were smokers (16.70 ± 10.38 cigarettes per day, mean age at start of smoking 15.75 ± 2.68 years), 76.8% drank coffee (2.10 ± 1.09 cups per day) and 50.5% were alcohol drinkers (2.02 ± 1.27 glasses per week). Consumption of meat was found in 97% of young males (3.31 ± 2 times per week), milk in 72.7% (7.55 ± 6.54 glasses per week), yoghurt in 65.7% (3.52 ± 2.33 yoghurts per week), white cheese in 91.9% (5.69 ± 2.72 times per week), eggs in 71.7% (4 ± 3.77 eggs per week), fruits in 93.9% (5.19 ± 2.21 times per week) and vegetables in 88.9% (5.20 ± 2.14 times per week). Only 1% of subjects had thyroid disease, 22.2% had history of allergy, 40.4% with history of fracture, 16.2% had pain problems and 64.6% had regular physical exercise. There was a positive correlation between Tsc and consumption of white cheese ($r = 0.273$ $P = 0.017$) and a negative correlation between Tsc and intake of coffee ($r = -0.23$ $P = 0.07$). By t-test, Tsc was positively associated with milk consumption ($t = 2.124$ $P = 0.038$). Tsc was negatively associated with history of allergy (by Mann-Whitney U test).

Conclusion: Low bone density in young men is associated with low consumption of white cheese and milk, history of allergy and high intake of coffee.

P085

EPIDEMIOLOGY STUDY ABOUT RISK FACTORS FOR LOW BONE DENSITY IN OLD GREEK MEN AND WOMEN

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Background: Osteoporosis is frequent not only in women but also in men. The rate of bone loss could be reduced by modifying risk factors.

Objectives: The aim of the present study was to determine the risk factors associated with low bone density.

Methods: 639 subjects from the region of Macedonia (152 men and 487 women), mean age 67.85 ± 7.65 years, participated in our study. All subjects

were asked to complete a questionnaire. Bone density was measured at the calcaneus by a Lunar Achilles plus ultrasound bone densitometer using the T score (Tsc).

Results: Mean height was 156.57 ± 7.64 cm and weight 74.93 ± 12.08 kg. Of 639 subjects, 15.1% were smokers (16.96 ± 13.10 cigarettes per day, mean age at start of smoking 28.78 ± 13.59 years), 85.9% drank coffee (2.35 ± 3.39 cups per day), 15.1% were alcohol drinkers (1.78 ± 1.26 glasses per day), 11.5% had thyroid disease, 27% were allergic and 28.9% with history of fracture (1.2 ± 0.56 fractures). Consumption of meat was found in 88.5% of subjects (1.96 ± 1.00 times per week), milk in 60.4% (6.11 ± 2.96 times per week), yoghurt in 72% (3.54 ± 2.14 yoghurts per week), white cheese in 84.1% (4.77 ± 2.19 times per week), eggs in 47.5% (2.28 ± 1.72 eggs per week), fruits in 92.6% (2.00 ± 0.56 times per week) and vegetables in 95.4% (2.00 ± 0.60 times per week). Of women, 97.1% had menopause (mean age at menopause 47.54 ± 6.23 years) and 13.7% oopherectomy or hysterectomy (mean age at ectomy 49.85 ± 8.81 years). There was a negative correlation between Tsc and age ($r = -0.088$ $P = 0.026$) and a positive correlation between Tsc and height ($r = 0.275$ $P < 0.001$), and weight ($r = 0.202$ $P < 0.001$). By t-test, Tsc was positively associated with milk consumption ($t = 2.278$ $P = 0.023$). Tsc was positively associated with white cheese and vegetable consumption, and negatively with female gender, menopause and history of fracture (by Mann-Whitney U test).

Conclusion: Low bone density is associated with old age, female gender, low height, low weight, menopause, history of fracture, and low intake of milk, white cheese and vegetables.

P086

VITAMIN D AND BONE METABOLISM IN HEPARIN-INDUCED EXTRACORPORAL LDL PRECIPITATION

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Introduction: In patients with severe familiar hypercholesterolemia and coronary heart disease sometimes no sufficient decrease of serum concentrations of LDL cholesterol can be achieved by diet and medical treatment. In these cases a heparin-induced extracorporal LDL precipitation (H.E.L.P. apheresis) can be performed. Until now only few data about the possible influence of H.E.L.P. on Vitamin D and bone metabolism have been published. The aim of this study was to evaluate the influence of H.E.L.P. performed once weekly over a longer time of observation.

Patients: Investigated were 10 patients (7 men, 3 women), mean age 49 years. All patients had heterozygous familiar hypercholesterolemia and coronary heart disease.

Methods: H.E.L.P. apheresis was performed once a week. At the beginning and after 2 years of treatment the following laboratory parameters have been determined:

Serum: 25(OH)D3 (normal range 7–35 mcg/l), 1,25(OH)2D3 (20–67 ng/l), osteocalcin (3.5–11.8 mcg/l), bone specific alkaline phosphatase (8.0–16.8 mcg/l), calcium, inorganic phosphate. Urine: Deoxypyridinium crosslinks (2.5–5.5 nmol/mmol creatinin). Besides this parameters of lipid metabolism and blood coagulation were under control.

Results: 25(OH)D3 decreased significantly by −23% (23.7, after 2 years 17.4, $P = 0.037$) and 1.25(OH)2D3 by −42% (51.3, 28.7, $P = 0.005$). Osteocalcin also decreased by −36% (13.1, 6.3, $P = 0.037$) whereas bone specific alkaline phosphatase remained unchanged. Deoxypyridinium crosslinks were after 2 years of treatment within the normal range. Serum calcium and inorganic phosphate showed no variations.

Discussion: H.E.L.P. apheresis once weekly over a longer time of 2 years leads to a significant decrease of vitamin D metabolites, possibly caused by elimination during apheresis. It remains unknown whether the reduction of osteocalcin concentration is due to the same mechanism or due to an inhibition of osteoclast activity. Normal values of deoxypyridinium crosslinks argue against a significant influence of heparin that may increase bone resorption under certain circumstances.

It is concluded that in intermittent long term LDL apheresis 25(OH)D3 levels should be monitored every 6 months. If low levels occur vitmin D supplementation is indicated to prevent bone loss and osteoporosis.

P087

INCREASED BONE RESORPTION IN FEMALE ELITE ATHLETES - THE ROLE OF OPG/RANKL AND THE USE OF ORAL CONTRACEPTIVES

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Introduction: The genesis of osteoporosis in female endurance athletes is still under discussion. The Measurement of biochemical bone markers, osteoprotegerin (OPG) and soluble TNF-alpha receptor antagonist ligand (sRANKL) might help to understand the regulation of bone metabolism in these athletes.

Materials and Methods: We measured osteocalcin (OC), bone alkaline phosphatase (BAP), serum β-crosslaps (CTx), OPG, sRANKL, estradiol (E2) and LH in fastening blood samples from 25 female elite endurance athletes and 25 matched controls. Results are given as mean ± SD or median (25.–75. percentile) as appropriate.

Results: Athletes who did not use oral contraceptives [A-OCC(−)] had significantly higher CTx (0.82 ± 0.20 vs. 0.50 ± 0.14 ng/ml), BAP (37.3 (23.2–54.4) U/L vs. 25.2 (20.3–35.6) U/L) and OPG (3.4 ± 0.8 vs. 2.7 ± 0.8 ng/ml) levels than controls who did not use oral contraceptives [C-OCC(−)]. A-OCC(−) had also higher OC, CTx, BAP and E2 levels than athletes using oral contraceptives [A-OCC(+)]. E2 was slightly lower in controls using oral contraceptives (C-OCC(+)) than in A-OCC(+)(23.9 (16.9–44.0) ng/L vs. 12.3 (9.3–21.2) ng/L).

Conclusions: A-OCC(−) have an increased bone turnover with particular stimulation of bone resorption, which is not reflected by changes of E2 and LH. In these athletes the differentiation of osteoclasts is not stimulated. Increased OPG levels correspond probably to a negative feedback mechanism in response to the increased bone turnover. Since these effects were not present in A-OCC(+) it can be suggested that OCC use might be protective to preserve bone health in female athletes.

P088

CALCANEAL BROADBAND ULTRASOUND ATTENTUATION

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Introduction: In Singapore, as in the rest of Asia, osteoporosis will become an increasingly important public health problem. In the next 50 years, more than half of all hip fractures are projected to occur in Asia. The burden of osteoporosis lies not only in individuals but also with society. Population screening using Bone Densitometry is not cost effective (Advisory Group on Osteoporosis Report 1994) and clinical risk factors assessment is not sensitive (A Stewart, British Journal of Radiology 2000). Results from 4 prospective studies have shown that quantitative ultrasound (QUS) measurements of the heel can indeed predict the risk of fractures in elderly patients. However according to United States Food and Drug Administration's Guidance Document dated June 21 2001, Caucasian female normative reference databases cannot be used as a reference database for different ethnic group and genders. As there is currently no Asian normative value available for this purpose, we therefore embarked in this study to establish a normative reference database for Asian Male and Asian Female subjects. We have also adopted United States' Food and Drug Administration (FDA) guideline in establishing such a database. We also included a cost comparison table with regards to screening a group of 100 women age 50–59 years old for osteoporosis using Dual energy x-ray absorptiometry (DEXA) and QUS.

Method: Compilation of the local database is based on 366 healthy females and 236 healthy males. We measured the Broadband Ultrasonic Attenuation (BUA) of the left heel using the Contact Ultrasound Bone Analyser (CUBA) clinical system.

Results: Refer to table.

Conclusion: The study shows that the Asian population has a significant lower normative value than the Caucasian population. This BUA local reference database obtained will allow for more accurate determination of the at-risk group and thus not under-select them for BMD referral.

Table: Mean BUA score between Asian and Caucasian

Age	Male Asian	Male Caucasian	Female Asian	Female Caucasian
20–29	94.07	96.52	85.75	89.57
30–39	88.36	93.06	79.90	84.10
40–49	82.65	89.65	74.05	78.64
50–59	76.94	86.22	68.20	73.17
60–69	71.22	82.79	62.34	67.70
70–79	65.51	79.36	56.49	62.24
> 80	59.80	75.92	50.64	56.77

P089

OSTEOPROTEGERIN AND BONE MARKERS IN POSTMENOPAUSAL WOMEN

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The role of osteoprotegerin, an inhibitor of osteoclast differentiation and activation, in the postmenopausal osteoporosis is still not clarified. Association of osteoprotegerin and standard bone markers was investigated in 123 postmenopausal women. Fifteen patients received antiresorptive therapy for osteoporosis. The following parameters associated with bone metabolism were measured in serum by standard methods or commercial kits: total and bone alkaline phosphatase, telopeptide (Crosslaps serum, Osteometer), osteoprotegerin and RANKL (both Biomedica). Standard biochemical parameters were increased in less than 5% of patients. Telopeptide was lower ($P = 0.02$) in patients receiving antiresorptive therapy and also in those with menopause longer than 10 years. Relationships of both telopeptide ($P = 0.0005$) and osteoprotegerin ($P = 0.02$) were positive with menopause duration by second-degree equation. Osteoprotegerin correlated positively also with age ($P = 0.0005$), total ($P = 0.008$) and bone alkaline phosphatase ($P = 0.01$). No significant correlation was found between osteoprotegerin and telopeptide, and none with RANKL. These results indicate that bone turnover was higher in patients within the first 10 postmenopausal years and in those not receiving antiresorptive therapy. The observed relationships of osteoprotegerin, bone markers, age and duration of menopause suggest that higher bone turnover rate was associated with higher osteoprotegerin concentrations. Similar findings were reported by other investigators and interpreted as compensatory osteoprotegerin synthesis during postmenopausal bone loss. Clinical significance of osteoprotegerin and RANKL was not confirmed.

P090

DIFFERENCES IN CALCIUM METABOLISM BETWEEN MEN WITH UROLITHIASIS AND CONTROLS

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Investigations in the field of urolithiasis have revealed that the great number of patients with urolithiasis had some metabolic disorder. Stones develop from a wide variety of metabolic or environmental disturbances, including hypercalciuria, hypocitraturia, undue urinary acidity, hyperuricosuria, hyperoxaluria, infection with urease-producing organisms and cystinuria.

This paper describes a comprehensive metabolic evaluation of patients with recurrent stones from eastern part of Croatia. In order to assess possible metabolic disorders in those patients, the healthy control group was also evaluated.

In this study, 26 male patients with urolithiasis (mean age 39.1 + 6.2 years) underwent metabolic evaluation. Control group were 18 healthy male subjects (mean age 35.0 + 7.1), with no history of stone formation or renal diseases. Biochemical analysis included fasting blood and urine samples, 24-hour and 2-hour urine collections. The most common metabolic abnormalities in patients with urolithiasis were increased levels of urinary oxalate and calcium and uric acid in serum. There were ten patients with hyperoxaluria and six with hypercalciuria. Increased levels of alkaline phosphatase and serum calcium were found in three patients. Parathyroid hormone was inside the reference values in all patients and controls.

Our results showed that the most frequent metabolic abnormalities in male patients with urolithiasis, from eastern region of Croatia, were hyperoxaluria and hypercalciuria. However, there were no significant differences in mean values of metabolic parameters between patients and controls.

We may conclude that metabolic disorders are important characteristic of urolithiasis, but rarely the single cause of stone formation.

P091

ABNORMAL OSTEOPROTEGERIN AND RANKL LEVELS IN PRIMARY BILIARY CIRRHOSIS. LACK OF ASSOCIATION WITH OSTEOPOROSIS AND BIOCHEMICAL MARKERS OF BONE TURNOVER

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The pathogenesis of osteoporosis in primary biliary cirrhosis (PBC) is not well understood since both low or high bone turnover have been reported. Since

osteoprotegerin (OPG) and its ligand (RANKL) regulate osteoclastogenesis, and therefore may influence the development of osteoporosis, OPG and RANKL levels were assessed in 52 patients with PBC (age: 57 ± 1.5 years) and in an age-matched control group of healthy females. Besides liver function tests, serum bone gla-protein (BGP), as index of bone formation, and urinary amino-terminal telopeptide of collagen I (NTx), as index of bone resorption, were also measured. Bone mineral density of the lumbar spine and femoral neck were assessed for diagnosing osteoporosis (BMD below -2.5 T-score).

OPG levels (pM/l) were significantly higher in PBC (5.5 ± 0.2) as compared to controls (2.9 ± 0.2, $P < 0.0001$), whilst RANKL (pM/l) were lower in PBC (0.4 ± 0.1) with respect to controls (1.4 ± 0.3, $P < 0.0001$). 22 patients had osteoporosis. No significant differences in OPG and RANKL levels were observed between patients with and without osteoporosis. OPG was significantly higher in patients with advanced liver disease as defined by high bilirubin levels or by a Mayo score above 4. Moreover a direct correlation was observed between OPG and Mayo score ($P = 0.01$). No associations were found between OPG and RANKL with biochemical markers of bone remodeling.

In conclusion, OPG and RANKL are abnormal in patients with PBC, regardless of osteoporosis. The high OPG levels are associated with the severity of the liver disease.

P092

DIABETES MELLITUS INCREASES THE RISK FOR OSTEOPOROSIS

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The aim of this study was to examine the impact of diabetes mellitus on bone metabolism. In 105 patients with type-1, type-2a or type-2b diabetes mellitus (51 men, 54 women, mean age type-1 53 years, type-2a 64.3 years, type-2b 60.6 years) central (QCT) and peripheral (pQCT) bone density were measured and in addition further laboratory findings, anthropometric values and a questionnaire were analyzed. Statistical analysis: Chi-square test, Mann-Whitney U-test, Kruskal-Wallis test, Spearman rank correlation coefficient, SPSS. Mean bone density in the QCT-analysis (lumbar vertebra 1–3) was 98.5 mg/cm^3 in the type-2a group, 104 mg/cm^3 in the type-2b group and 111 mg/cm^3 in the type-1 group. A bone density of < -2 was seen in 10% of the type-2a group, 2.7% of the type-2b group and in 6.3% of the type-1 group. Females and males as equal shares an axial bone density of less the -2 SD. Mean peripheral spongiosa density (distal radius) was 160.1 mg/cm^3 in the type-2a group, 162.2 mg/cm^3 in the type-2b group and 160.8 mg/cm^3 in the type-1 group. A spongiosa density of less than -2 SD was observed in 20% of the type-2a group, 19% of the type-2b group and 19% of the type-1 group. 22.2% of the females showed spongiosa density values of less than -2 (males: 15.7). The correlation between duration of disease and bone density (radius) was slightly significant ($P = 0.02$). The measured values between QCT- and pQCT-measurement showed a significant correlation ($P = 0.0005$, n = 105). Osteocalcin was below normal limit in 53.3% of all patients; PTH-values above normal limit were observed in 7.6% of all patients. Type-2b diabetics showed significantly lower vitamin D-levels compared to type-2a and type-1 diabetics. The correlations between laboratory- and BMD-values were not significant. Calcium, Osteocalcin and PTH had the biggest influence concerning the classification into patients with normal or pathological QCT-values (correct classification of 79%). Of 105 patients enrolled in this study, 73 had completed the questionnaire. Only for the reduction of the mother's body size the association with the pathologic attenuation of bone density (pQCT) was significant ($P = 0.002$). Diabetics with long duration of disease have an increased risk for osteoporosis. Laborchemical and anthropometric parameters are suggestive for assessment of clinical course.

P093

LOW EXPOSURE TO CADMIUM AND FEMORAL NECK FRACTURE

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Bone lesion is one of the main unfavourable effects of chronic exposure to cadmium (Cd), but the critical level of the exposure leading to the skeletal injury is still unknown. However, recent epidemiological and experimental data suggest that Cd may promote skeletal demineralization and increase bone fragility at considerably lower exposure than previously anticipated. Thus, at present a

great attention has been focused to recognize whether environmental exposure to Cd occurring in industrialized countries may be a risk factor for osteoporosis and bone fractures. Femoral neck fracture belong to the most frequent osteoporotic fractures.

In the study, using a rat model of human environmental exposure to Cd in areas without excessive pollution with this heavy metal, we have investigated whether chronic low-level exposure to Cd can influence the mechanical properties of femoral neck and increase the risk of its fracture. For this purpose, young female Wistar rats were exposed to 1 mg Cd/dm^3 in drinking water for 12 and 24 months. Femur of control and Cd-exposed rats was assigned to densitometric measurements (Lunar DPX-L) of bone mineral content (BMC) and density (BMD) at the proximal end of the bone and biomechanical studies (Instron 4301 universal testing machine) of the femoral neck using a bending test with vertical loading of the head. Cd concentration in blood and urine was measured as a dose-estimate (AAS method, Hitachi Z-5000).

The exposure to Cd resulted in a dependent on the duration of treatment demineralization of the femur reflected in a decrease in BMC and BMD at its proximal end. After 12 months of the exposure to Cd, the bending strength (ultimate load) and stiffness of the femoral neck decreased, whereas its deformation at the yield and at fracture as well as the work to fracture were unchanged compared to control. After 24 months of the treatment, the Cd-induced changes were more seriously advanced and the femoral neck yield load and deformation at the fracture decreased as well.

The study clearly revealed that chronic even low-level exposure to Cd leads to disorders in the mineral status of the femur and in consequence weakens the biomechanical strength of the femoral neck. The results allow concluding that environmental exposure to Cd may increase the risk of femoral neck fracture.

P094

OSTEOCALCIN IN RELATION TO BONE MASS AND SODIUM INTAKE IN POSTMENOPAUSAL WOMEN

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Some research suggests a high sodium (Na) intake might be associated with bone loss, though the evidence is controversial. Our objective was to examine the relationship between Na intake (expressed as urinary Na) and markers of bone turnover, osteocalcin (OC) and undercarboxylated OC (UOC), in over 100 healthy postmenopausal women (68.6 ± 7.1 y, at enrollment) over a period of two years. After baseline evaluation, half of the subjects were instructed to follow a reduced Na diet ($\sim$1500 mg/day), group I, while the other half served as controls, group II, with habitual Na intake of $\sim$3000 mg/day. Bone mineral density (BMD), dietary intake and blood and 24-h urine samples were evaluated at baseline and at 24-month point. For all subjects at baseline, OC was a significant negative predictor of total body, hip, and forearm BMD in multiple regression models controlled for age, height, weight, total calcium intake and urinary Na. UOC was not significantly related to BMD of any skeletal site. ANCOVA controlled for age, height, weight, total hours of physical activity, calcium and Na intake, was used to check for group differences in OC and UOC at 24-month point. OC was significantly higher in group II (adjusted means, 11.02 vs. 6.53 ng/ml, $P = 0.02$), while there was no significant difference for UOC. In conclusion, our preliminary data show OC to be a significant negative predictor of BMD at whole body, hip and forearm sites at baseline. At 24-month OC was significantly higher in group II, indicating higher Na intakes are associated with greater bone turnover compared to lower Na intakes. Further studies, including other markers are needed to evaluate relationship between bone turnover and Na intake.

P095

THE EFFECT OF CADMIUM ON THE BONE COLLAGEN AND GLYCOSAMINOGLYCANS IN RATS

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Cadmium (Cd) is one of the most toxic heavy metals causing damage to the various tissues and organs, including bone. It has been reported that Cd has the direct and indirect effects on bone metabolism causing a decrease in both mineral and matrix content. These effects are probably accompanied by disturbances in collagen and proteoglycans metabolism. Previously, we have noted that exposure of rats to 5 and 50 mg Cd/dm^3 leads to bone demineralization and weakens their mechanical properties. Now we examined the effect of Cd on content of collagen and GAGs in femur bone of female rats intoxicated with 5

and 50 mg Cd/dm^3 for 6 months. The soluble collagen was extracted with 0.5 M acetic acid for 48 h. Collagen in the residue was successively extracted with pepsin (1 mg of pepsin/10 mg of decalcified bone) and 4 M guanidine hydrochloride. Collagen solubility was expressed as a percentage of the amount of acetic-soluble collagen to amount of total collagen. The contents of two types of collagen I and V were determined in each extracted fraction. In rats intoxicated with 5 and 50 mg Cd/dm^3, the solubility of bone type I collagen increased 2.7- and 2.9-times, whereas the solubility of type V collagen increased 2.1- and 2.9-times, respectively. Furthermore, exposure to 50 mg Cd/dm^3 caused reduction (by about 40%) in the total collagen content in bone which could result from a decrease in collagen synthesis. GAGs were released from core protein of proteoglycans by extensive digestion with papain. The purified GAGs were submitted to fractionation on a microcolumn with CF11 cellulose. The concentration of GAGs in the eluate from the column was determined using DMB (1,9-dimethylmethylene blue). In bone of the intoxicated rats the percentage of dermatan sulfate and chondroitin sulfate-4S to the total GAGs was increased, whereas percentage of keratan sulfate and chondroitin sulfate-6S was reduced as compared to control. The obtained results suggest that Cd can affect bone by increase in solubility of collagens I and V and decrease in the total collagen content as well as by disturbances of proportions of individual GAGs.

P096

DESCRIPTIVE STUDY OF BONE MINERAL DENSITY MEASURED BY PERIPHERAL DENSITOMETRY: ARGENTINE EXPERIENCE

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Aim: To describe the prevalence of osteopenia and osteoporosis by peripheral densitometry in women aged 50 years or older in Argentina.

Methods: Secondary analysis of data from a database with 41.118 ambulatory subjects (38.525 women) from 180 primary care centers from 7 regions of the Argentine Republic participating in a free campaign for osteoporosis detection. The examination was performed by Norland p-DXA equipment, in the no-dominant distal forearm.

Results: Demographics: Age: 63.9 ($\pm$9.2) years; Weight: 69 ($\pm$12.3) kg; Height: 158 ($\pm$0.07) cm; BMI (Body Mass Index): 27.6 ($\pm$4.8). Age distribution : 50–59 years: n = 14374 (37.3% of the sample); 60 to 69 years: n = 13194 (34.2%); 70 to 79 years: n = 9057 (23.5%); 80 years or older: n = 1899 (5%). Diagnosis (World Health Organization (WHO) criteria): Osteoporosis (T-score < −2.5): n = 6731, 17.47%; Osteopenia (T-score of −1 to −2.49): n = 17034, 44.22%; Normal (T-score > to −1): n = 14760, 38.3%. The prevalence of osteoporosis increased in accordance with the age progression and it was similar between different regions of the country. Using National Osteoporosis Foundation (NOF) criteria, the high-risk group (30.7%) was bigger than the osteoporotic group using WHO criteria (17.47%) (Table 1).

Conclusions: We observed a significant difference in the percentage of potential treated patients according to the criteria used. Our results presents some differences with the results of the National Osteoporosis Risk Assessment (NORA) study, performed with the same method, peripheral densitometry, and identical age distribution in the sample, but with different racial composition of the sample (we don't include black subjects). Given the lower costs and the accessibility of p-DXA, we are in agreement with other international authors that this method seems to be a useful screening tool to detect subjects with low bone mineral density.

Table: Diagnostic Classification by OMS/NOF Criteria

OMS CRITERIA		NOF CRITERIA	
NORMAL	38.3%	LOW RISK	38.3%
OSTEOPENIA	42.22%	MODERATE RISK	31%
OSTEOPOROSIS	17.47%	HIGH RISK	30.7%

P097

DOES THE PRESENCE OF OLIGO/AMENORRHOEA AND UNDER-NUTRITION IMPLY OSTEOPOROSIS IN YOUNG FEMALE DANCERS?

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Background and Objective: The athlete triad syndrome describes the occurrence of amenorrhoea, disordered eating/under-nutrition with osteoporosis. This study aims at measuring the bone mineral density (BMD) of the axial and appendicular skeleton of young dancers apparently suffering from the athlete triad syndrome and comparing with normal non-exercising controls

Methods: Full time dance students from a tertiary Performing Arts Institute were recruited. Athlete triad syndrome was suspected in the young dancers when oligo/amenorrhoea was present together with underweight (weight below 20th centile for height at specific age or body fat composition below 18%). The non-exercising controls consisted of eumenorrhoeic age matched patients presenting to an Adolescent Gynaecology Clinic. All dancers had regular weight-bearing exercises of at least 18 hours per week. All subjects and controls were between 17–20 years old, and all underwent full hormonal profile assay, pelvic ultrasound, bio-impedance estimation of body fat, and dual energy X-ray absorptiometry (DEXA) and quantitative peripheral CT scans (pQCT) to determine BMD.

Results: A total of 47 dancers were recruited, of which 14 (29.7%) fell within the criteria for suspected athlete triad syndrome. Comparing the normal dancers (n = 33) to 36 non-exercising controls showed that the dancers had lower body mass index (BMI) and body fat percentage, but significantly higher BMD values at axial skeletal sites as compared to the controls (lumbar spine 1.1 g/cm^3 Vs 0.93 g/cm^3; neck of femur 0.98 g/cm^3 Vs 0.83 g/cm^3, Ward's triangle 0.82 g/cm^3 Vs 0.72 g/cm^3; trochanter 0.78 g/cm^3 Vs 0.68 g/cm^3; $P < 0.05$). Comparing the dancers with suspected athlete triad syndrome to the same control group showed there were no significant BMD differences, though these dancers also had lower BMI and body fat percentage than controls.

Conclusion: Young dancers with oligo/amenorrhoea and apparent eating disorders that fit into the clinical diagnosis of athlete triad syndrome did not have lower BMD values in the axial and appendicular skeleton as compared to non-exercising eumenorrhoeic controls. The risk of osteoporosis was apparently offset by the benefits of regular intensive weight bearing exercises in these subjects. This was supported by the finding that young eumenorrhoeic dancers actually had higher BMD values at axial skeletal sites.

P098

REDUCED MATRIX MINERALISATION IN CASES OF HIP FRACTURE IS INDEPENDENT OF THE LEVEL OF NEW BONE FORMATION

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The traditional view of osteoporotic fractures is that they result from a reduction in bone mass combined with alterations in the micro-architecture. Apart from the effects of bone remodelling, the material properties of the remaining bone are thought to be unaffected. To test this we compared the degree of matrix mineralisation in femoral neck biopsies taken from cases of intracapsular hip fracture with age and sex-matched post-mortem controls.

Whole femoral neck biopsies from 7 female hip fracture cases (72–90y) and 9 controls (68–94y), were embedded in MMA, and sections stained with Solochrome Cyanin R for analysis of osteoid bearing canals. The blocks were then diamond micro-milled, carbon coated and analysed for the degree of matrix mineralisation using halogenated dimethacrylate standards for quantitative back-scattered electron (BSE) imaging (20kV, entire block face, sampling interval 5 μm). The BSE grey scale was adjusted such that 0 corresponds to an electron backscattering coefficient of 0.1159 ($\sim$1.70 gms/ml) and 255 to 0.1519, ($\sim$2.18 gms/ml). Remodelling and mineralization data were analysed for both the whole biopsy face and on a regional (anterior; inferior, posterior or superior) basis. Over the whole biopsy the level of mineralization was lower in the cases than the post-mortem controls (−2.8% $P < 0.05$). In both cases and controls mineralization was higher in the inferior (compressive) region compared with superior (tensile) region ($P < 0.05$). Mineralisation was lower in all regions of the cases (inferior: −3.3%, $P < 0.001$; posterior: −3.1%, anterior: −2.7%, $P < 0.001$; superior: −1.6%; $P < 0.05$) compared to the controls. New bone formation (%osteoid bearing canals) was higher in the anterior (+68%; $P < 0.01$), inferior (+74%; $P < 0.05$) and posterior (+44%; $P < 0.1$) regions of the cases. However there was no relationship between %osteoid canals and the mean level of cortical mineralization when assessed either over the whole biopsy ($P > 0.78$) or on a regional basis ($P > 0.17$).

In conclusion, this study has shown that in cases of intracapsular hip fracture matrix mineralization is reduced in the femoral neck. Unexpectedly, in view of the likely role of mild-moderate vitamin D deficiency osteopathy, this decreased mineralisation was independent of osteoid surface, allowing the possibility that alterations in the bone matrix [such as excessive glycation or changes in the composition of the collagen fibrils] might play a role in the aetiology of hip fracture.

P099

PREDICTORS OF BONE MINERAL DENSITY IN ETHNICALLY DIVERSE YOUNG WOMEN

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Genetic (Recker and Deng 2002), ethnic and lifestyle (Alekel et al. 2002) differences in bone density may cause some ethnic groups to be at risk for osteoporosis.

Purpose. The purpose of this study was to determine whether lower leg BMD was related to differences in ethnicity.

Methods. We recruited 110 young sedentary women (mean age: 24.7 ± 4.7 yr) whose self-reported ethnicity: Caucasian (CAU, N = 40), Hispanic (HIS, N = 35), and Asian (ASN, N = 35). The subjects were eumenorrheic (> 9 menstrual cycle/yr), currently not pregnant and non-smokers. BMD of distal forearm (WBMD) and Os Calcis (OCBMD) were measured with Lunar PIXI, and BMD of the leg (LBMD) and the entire arm (ABMD) with DXA (Hologic, model QDR 4500 W). Lean body mass (LBM), fat-mass (FM) and percent body fat (%BF) were assessed using whole-body DXA scans. Multivariate stepwise regression analysis was performed with LBMD as the dependent variable and all the others mentioned above as independent variables. For multiple group comparisons the Bonferroni t statistics was used (i.e., statistical significance was set with alpha = 0.05/2 or $P < 0.025$).

Results. LBMD was lower ($P < 0.019$) in the ASN and HIS than the CAU. OCBMD measurement was the only predictor that was significantly correlated to LBMD. The ASN and HIS strongest predictors for LBMD were OCBMD (R = .75 −.77, $P < 0.0001$) and WT (R = .66 −.72, $P < 0.012$). The CAU strongest predictors were OCBMD (R = .70, $P < 0.0001$) and HT (R = .60, $P < 0.004$). LBM, FM, and %BF were not different between ethnic groups. Bivariate analysis showed that BMI and OCBMD (r = .70, $P < 0.0001$) were correlated with LBMD in all groups, but WT was not correlated with LBMD in the CAU. All other BMD variables were not significantly correlated with LBMD.

Conclusion. Body weight remains the best morphological predictor of lower LBMD. ASN young women are at greater risk for lower level of LBMD than CAU or HIS. Identifying social and cultural factors, which influence healthy body weight, should be considered as part of an intervention strategy for impacting LBMD. (Supported by NIH Grant No. 5 S06 GM053933-06 and NASA No. SAA 2-401535).

P100

IMPACT OF A PUBLIC AWARENESS PROGRAM ON WOMEN'S LIFE-STYLE HABITS

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Objective: The purpose of this study was to assess the impact of a public awareness program on modifiable osteoporosis risk factors among French Canadian women. Between 1997 and 2002, awareness program was held in different working places, public events and in community pharmacies (n = 8,081). Each screening visit was done by a health professional team including a nurse, a nutritionist and a kinesiologist practitioner. Osteoporosis risk factors were evaluated by a validated questionnaire derived from the Mediterranean Osteoporosis Study (MEDOS) and the calcaneal bone measurements was determined using the Achilles ultrasound. Each subject received individual counseling according to results of their life-style habits such as smoking, calcium and alcohol intake, regular physical activity and to their risk fracture measurements. After a mean follow-up of 37.6 months, 1,354 women underwent the screening program.

Results: According to the results of the second visit, women reported a decrease of smoking, coffee and alcohol intake, and an increase in physical activity level and calcium supplement intake. No different change was observed between the two visits for crude ultrasound bone parameters (stiffness = 83.5 vs 83.8; $P = 0.07$). However, adjusted ultrasound bone parameter were significantly increased at the second visit (Z score = 0.42 vs 0.58; $P < 0.0001$ and T score = −1.50 vs −0.47; $P < 0.02$).

Conclusions: These results confirmed the importance of public awareness program to help women modify their osteoporosis risk factors.

P101

EFFECT OF AN ACIDIFYING DIET ON BONE MINERAL ACQUISITION IN LAMBS

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The age at which osteoporosis (OP) becomes a clinical problem is largely dependent upon peak bone mineral density. The most commonly consumed diets in Western societies are rich in protein and poor in fruits and vegetables. Such diets induce metabolic acidosis (MA). Dietary MA has been implicated as an important cofactor in the development of OP as the body uses Ca from bone to act as a buffer. Adolescents are increasingly consuming diets that induce MA. The purpose of this study was to examine the affect of dietary MA on the acquisition of bone mineral using a sheep model.

12-2 mo old lambs were divided into 2 dietary groups (3m, 3f) for this 6 mo study. Both diets (MA and ND) were analyzed according to the equation DCAD mEq = (Na + K + (0.15 Ca + (0.15 Mg) −(Cl + (0.2)S + (0.3)P) to determine the daily intake of ions, represented as the dietary cation-anion difference (DCAD), as a measure of their capacity to induce MA. Both diets had a majority of cations, the MA diet having 84% of the ND diet and both were relatively more alkalogenic than a typical human diet. Diets contained adequate levels of calories, protein, Ca and P for growth. Lambs were weighed monthly and the calories fed per day was increased according to NRC* recommendations. Arterial pH and Lumbar vertebrae (LV) and whole body (WB) DEXA occurred at 0 and 6 mos for measurement of bone mineral content (BMC). DEXA of the femora and radii was performed ex vivo at 6 mos. Analysis of variance with a significance of $P < 0.05$ was used. All sheep gained a significant amount of weight but weights did not differ between groups. Mean arterial pH was not different but tended to be lower in the MA group (pH MA = 7.325 ± 0.064, t = 0; 7.397 ± 0.031, t = 6mos; ND = 7.438 ± 0.106, t = 0, 7.438 ± 0.052, t = 6mos). There was no difference in the BMC of the LV or WB between groups at t = 0. Both groups had significantly more BMC at 6mos and the MA group had less BMC of LV and WB compared to the ND group at 6 mos (MA = 33.04 ± 10.48 g, ND = 46.509 ± 7.898 g for LV and MA = 633.00 ± 221.65 g, ND = 970.49 ± 127.40 g for WB). The MA group had less BMC of the femora (MA = 66.20 ± 6.89 g, ND = 82.97 ± 10.78 g) and the radii (MA = 88.54 ± 10.41 g, ND = $1 15.47 \pm 13.00$ g). This study demonstrates that consumption of a MA diet can significantly affect bone mineral acquisition and lower the peak bone mineral density in adolescents. *U.S. Board of Agriculture. Nutrient Requirements of Sheep. 6th edition. 1985. National Academy Press.

P102

ARE SCOLIOSIS AND OSTEOPOROSIS ALWAYS ASSOCIATED? EVALUATION OF A GROUP OF ADOLESCENTS

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Osteoporosis is characterized by the reduction of bone strength, which is due to microarchitectural alteration and to reduction of its density. Even if osteoporosis is typical of aging, scientists are trying to find some predictive or determinant factors during growth, in order to correct them for an optimal value of bone mass pick.

In this study we evaluated bone quality in a group of adolescents affected by scoliosis, in order to find out if there is a relationship between skeletal deformities and osteopenia.

Many Authors investigated the question, using dual X- ray absorptiometry (DXA) for determining bone mineral density at different skeletal sites.

We used an ultrasound device to determine speed of sound (SOS) at multiple skeletal sites, since the device isn't invasive.

In our Institute we examined 41 adolescents treated for spine disorders.

Everyone of them has been submitted to SOS measurement at radio and tibia, with OmnisenseTM (Sunlight Technologies).

According to most of Authors, more than a half of patients was affected by low values of SOS; the statistical analysis of results by multiple regression method allowed us to identify in scoliosis a predictive factor of alteration of SOS at radio site only; age, height and weight have been found other predictive factors.

Expressing SOS as Z- score, scoliosis didn't seem to predict osteoporosis.

No correlation has been experienced between SOS and site and severity of the curve and type of treatment, according to other Authors.

Low values of SOS in a high percentage of patients allowed us to consider patients affected by scoliosis at risk of osteopenia and to evaluate them periodically until skeletal maturity.

Modifying diet, paying attention to orthopaedic treatment (physical activity and brace) and planning accurately surgical treatment of scoliosis are the goals of evaluating bone quality during growth.

P103

NO ASSOCIATION BETWEEN 11-BETA-HYDROXY-STEROID DEHYDROGENASE ACTIVITY AND PEAK BONE MASS OR BONE TURNOVER IN YOUNG MEN

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Recent studies suggest that pre-receptor conversion of glucocorticoids may be important for their effect on bone *in vivo*. The enzyme 11-beta-hydroxysteroid dehydrogenase type-1 (11-beta-HSD-1) is expressed in bone and catalyzes the conversion of cortisone to cortisol. Osteoblastic expression of 11-beta-HSD-1 increases with age and has been implicated in age-related bone loss.

We hypothesized that pre-receptor metabolism of endogenous glucocorticoids may in part determine the peak bone mass.

The Odense Androgen Study (OAS) is a population based, prospective, observational study on endocrine status, body composition, muscle function, and bone metabolism in young men comprising 784 participants. The present cross-sectional study population comprised 68 participants aged 26.1 (20.1–30.0) years selected at random from the OAS population.

Bone mineral density (BMD) of the lumbar spine and hip, and whole body were measured using dual-energy X-ray absorptiometry. Bone turnover was assessed by measurement of serum osteocalcin, alkaline phosphatase, and type-1 collagen C-terminal telopeptide. Urinary 5-beta-tetrahydrocortisol (THF), 5-alpha-tetrahydrocortisol (allo-THF), and tetrahydrocortisone (THE) were measured by gas chromatography and 11-beta-HSD-1 activity was estimated from the (THF + allo-THF)/THE-ratio.

No significant relationship between BMD at any of the measured sites and age was found. No significant relationship between BMD at any of the sites and (THF + allo-THF)/THE-ratio could be detected (R = −0.02 to R = 0.08, NS). Significant bi-variate correlation between body height, body weight, and BMI on the one hand and BMD of the lumbar spine, hip, and whole body on the other hand was seen (R = 0.09 to R = 0.34; $P < 0.05$ to $P < 0.001$). No significant differences in (THF + allo-THF)/THE-ratio or urinary cortisol between smokers and non-smokers was found. In multiple regression analysis with BMI, (THF + allo-THF)/THE-ratio, and urinary cortisol as independent variables and bone mineral measurements and biochemical markers of bone turnover as dependent variables no significant relationship between (THF + allo-THF)/THE-ratio or urinary cortisol and bone mineral measurements or turnover could be demonstrated.

We conclude that 11-beta-HSD-1 activity is of minor or no importance for peak bone mass and bone turnover in young men.

P104

THE CHANGE OF MEASUREMENTS OF BODY BUILD AS PREDICTORS FOR BONE LOSS THE MIYAMA STUDY

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The aim of the present study was to assess the association between rate of bone loss and the change of anthropometric factors such as height, body weight, BMI, arm span, circumstances of both wrists and grip power among general inhabitants of Miyama, a rural Japanese community. A cohort of 1543 inhabitants aged 40–79 years was established using resident registration in 1989. Fifty men and 50 women each in 4 age strata between 40 and 79 years, totaling 400 participants, were selected and completed a self-administered questionnaire and anthropometric measurements included height, weight, arm span, circumstances of both wrists and grip power. In 1990, the baseline BMD of lumbar spine and proximal femur was measured using Dual energy X-ray absorptiometry (DXA). BMD was measured on the same participants in 1993, 1996 and 2000. The rates of changes of lumbar spine BMD during 10 years in men in their 40s, 50s, 60s and 70s were 1.7%, 5.5%, 0.1% and −1.6%, respectively, and those in women were −8.7%, −8.4%, −4.8% and −4.8%, respectively. The change of height during 10 years in men in their 40s, 50s, 60s and 70s were −0.7 cm, −0.5 cm, −1.2 cm and −1.5 cm, respectively, and those in women were −0.7 cm, −1.4 cm, −2.1 cm and −3.6 cm, respectively. The change of weight during 10 years in men in their 40s, 50s, 60s and 70s were −0.2 kg, −0.8 kg, −3.0 kg and −3.0 kg, respectively, and those in women were −0.3 kg, −1.7 kg, −2.4 kg and −3.1 kg, respectively.

Among men, there was significant positive relation between weight change and the rate of change of BMD at the lumbar spine after adjustment for age and height change ($P < 0.01$). By contrast, among women, there was significant positive association between the change rate of BMD at lumbar spine and height and weight change after adjustment for age ($P < 0.05$). Other anthropometric measurements were also assessed. These result suggest that body build are important determinants of bone loss.

P105

LACK OF ALTERATION IN BONE DENSITY AND BONE TURNOVER IN PSORIATIC ARTHRITIS

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Introduction: Impaired bone turnover is well-known in several chronic polyarticular inflammatory diseases. In psoriatic arthritis (PsA), however, studies of bone involvement have led to conflicting results. In the present study, we were examining whether bone density and biochemical markers of bone turnover show any alteration in PsA when compared to controls.

Materials and methods: 60 patients with PsA (20 men, 20 premenopausal women and 20 postmenopausal women) and 120 healthy controls (30 men, 34 premenopausal women and 56 postmenopausal women) were included in our study. PsA was diagnosed according to the criteria of Wright & Moll, and neither patients, nor controls had any disease or medication known to alter bone density. To assess bone characteristics in patients and controls, serum calcium, phosphate, alkaline phosphatase, parathyroid hormone, osteocalcin (OC), beta-crosslaps (CTX), and bone densitometry (DEXA) of lumbar spine and femoral neck were measured, and body mass index and years since menopause were marked. To characterise the activity of PsA, the Health Assessment Questionnaire score (HAQ), number of swollen and tender joints, erythrocyte sedimentation rate (We) and C-reactive protein (CRP) were measured. Statistical analysis included Kruskall-Wallis test, Spearman rank correlation test, Mann-Whitney U-test and analysis of covariance.

Results: Disease characteristics of PsA patients showed an inflammation of moderate activity (HAQ = 1.04 ± 0.8, number of swollen joints = 5.2 ± 5, We = 32 ± 26 mm/h, CRP = 15.6 ± 18 mg/l), and no significant difference was found between the three subgroups. We found positive correlation between the different parameters characterising disease activity, but we found no correlation between disease duration and any of the measured parameters characterising disease activity and bone. We found no correlation between disease activity and DEXA results or OC, CTX concentrations. Femur neck density correlated with age and years since menopause, and lumbar spine density correlated with years since menopause in PsA patients. When comparing patients and controls, we found no significant difference in lumbar spine/femoral neck density, OC and CTX concentrations in any of the three subgroups.

Conclusion: Psoriatic arthritis of moderate activity does not seem to impair bone density and bone turnover, thus these patients are not at higher risk of osteoporosis when compared to normal population.

P106

ALCOHOL INTAKE AS A RISK FACTOR FOR FRACTURE

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High intakes of alcohol have adverse effects on skeletal health. The aim of this study was to quantify this risk on an international basis and explore the relationship of this risk with age, sex and bone mineral density (BMD).

We studied 5,939 men and 11,032 women from 4 prospectively studied cohorts comprising CaMos, DOES, and the Rotterdam study. Cohorts were followed for a total of 75,433 person-years. The effect of reported alcohol intake on the risk of any fracture, any osteoporotic fracture and hip fracture alone was examined using a Poisson model for each sex from each cohort. Covariates examined included age and BMD. The results of the different studies were merged using weighted b-coefficients in a fixed effect model.

Alcohol intake was associated with a significant increase in osteoporotic and hip fracture risk but the effect was non-linear. No significant increase in risk was observed at intakes of 2 units or less daily. Above this threshold alcohol intake was associated with an increased risk of any fracture (RR = 1.23; 95% CI = 1.06–1.43), any osteoporotic fracture (RR = 1.38; 95% CI = 1.16–1.65) or hip fracture (RR = 1.68; 95% CI = 1.19–2.36). There was no significant interaction with age, BMD, or time since baseline assessment. Risk ratios were moderately but not significantly higher in men than in women, and there was no evidence for a different threshold for effect by gender. Results were comparable using a variable effects model.

We conclude that reported intake of alcohol confers a risk of some importance beyond that explained by BMD. The validation of this risk factor on an international basis permits its use in case finding strategies.

P107

THE GLOBAL BURDEN OF HIP FRACTURE

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The aim of this study was to quantify the global burden of osteoporosis as judged by hip fracture and the burden in different socio-economic regions of the world. The population mortality in 1990 and the incidence of hip fracture in different regions were identified, where possible in 1990. Excess mortality from hip fracture used data for Sweden, and disability weights were assigned to survivors from hip fracture.

In 1990 there were 1.31 million new hip fractures and the prevalence of hip fractures with disability was 4.48 millions. There were 738,116 deaths associated with hip fracture and 1.7 million life-years lost reduced to 951 thousands with weighting for age. Disability adjusted life years lost accounted for 1.75 million disability adjusted life years, representing 0.1% of the global burden of disease world wide and 1.4% of the burden amongst women from the established market economies. We conclude that hip fracture is a significant cause of morbidity and mortality world-wide.

P108

BONE MINERAL DENSITY AND ITS RELATION TO PLASMA LEPTIN LEVELS AND MENSTRUAL DYSFUNCTION IN COLLEGIATE DANCE STUDENTS

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Objective: To evaluate whether low plasma leptin levels are indicative of a higher incidence of exercise induced oligo-amenorrhoea in a group of collegiate dance students undergoing intensive training, as well as lower bone mineral density (BMD) of the axial and appendicular skeleton in these adolescents.

Methods: 46 full time dancers (mean age of 20.3 years, SD 2.23) were recruited from a tertiary Performing Arts Institute. All were healthy with no significant medical diseases. Basic anthropometric assessment, a full hormonal profile, bio-impedance estimation of body fat, and dual energy X-ray absorptiometry (DEXA) and quantitative peripheral CT scans (pQCT) were done. Fasting plasma leptin levels were measured using a human leptin RIA kit (Linco Research).

Results: The incidence of exercise-induced oligo/ amenorrhoea in this series was 19.5%. The mean plasma leptin level was 10.7 ng/ml (range 6–16.7, SD 10.75). Using the mean minus 1 SD as an arbitrary cut-off, 9 were ascribed to the low leptin level (LL) group and the rest 35 the normal leptin (NL) group for analysis. There were 3/9 (33%) subjects with oligo/amenorrhoea in the LL group compared to 6/35 (17.1%) in the NL group ($P < 0.01$). The LL group had lower weight (43.9 kg Vs 48.3 kg, $P < 0.01$), body mass index (17.25 kg/m^2 Vs 18.9 kg/m^2, $P < 0.01$) and body fat percentage (14.3% Vs 17.1%, $P < 0.01$), as well as lower BMD values for the lumbar spine (0.958 g/cm^2 Vs 1.055 g/cm^2, $P = 0.04$) as compared to the rest. BMD of the hip sites as well as the total (cortical + trabecular) BMD of the distal tibia and radius were also lower in LL group, though these differences did not reach statistical significance.

Conclusion: Low leptin levels were associated with lower body mass index, body fat content and BMD in this group of intensively exercising adolescents. Whether the lower BMD values were related to the higher incidence of oligo/amenorrhoea or directly to the undernutrition and lower metabolic states associated low leptin levels require further evaluation.

P109

BONE LOSS IN RENAL TRANSPLANT RECIPIENTS - DIETARY HABITS AND NUTRITIONAL STATUS

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Background: Incresed rate of bone loss continues long-term following renal transplantation caused by hyperparathyroidism and steroid therapy and hypovitaminosis D has been found to be common in this population.

Methods: In a group of 115 stable renal transplant patients (mean duration of transplantation 7.3 years) body composition was measured by DEXA scan and the dietary intakes estimated from a 3-day dietary record. Energy, protein, fat, carbohydrates, vitamins and minerals were considered. Valid information allowing reliable calculations of intakes, however, were obtained in only 79.

Results: Energy intake was sufficient. The patients were often overweight with a higher than normal proportion of body fat. The diets were characterized by a high fat content and insufficient amounts of folic acid, vitamin D, thiamine, iodine, selenium and iron according to local recommendations.

Conclusions: The main nutritional problem of this group of patients is obesity. Supplements with folic acid and vitamin D are generally indicated.

P110

BONE MINERAL DENSITY IN ADOLESCENT GIRLS

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The Actuality. Recently, the data of osteopenia occurrence in healthy adolescents girls is produced much more regularly. The correlation of adolescents osteopenia with transient decrease of sex steroids is expected. However, the clinical significance of this fact is still unclear.

The objective. To assess bone mineral density and the correlation of its characteristics (BMC, BMD, Z-score) with body height, body mass, body mass index, Tanner puberty stages, sexual development and serum estradiol level.

Primary data and methods applied. In total, 95 healthy children at the age of 13–16 years had been examined. The bone mineral density had been determined using Lunar osteodensitometer DPX-MD+. In all children, their physical growth, overall score of sexual development and Tanner puberty stages had been assessed. To estimate serum estradiol levels immune enzyme analysis method had been applied.

Results. The examined children had no abnormalities of physical growth nor puberty. Mean age of menarche was of 12.7 years. In Russia, osteopenia had been detected in 22% of children and this does not exceed mean population data for given age. Independently of Z-score, bone mineral density characteristics had been correlated with body height (r = 0.6, $P < 0.01$), body mass (r = 0.7, $P < 0.05$), body mass index (r = 0.4, $P < 0.04$), overall score of sexual development (r = 0.5, $P < 0.01$) and Tanner puberty stages (r = 0.6, $P < 0.01$). It had been determined that serum estradiol levels significantly increased at the age from 15 to 16 years. In girls at the age of 13–15 years, significant increase in body height paralleled with intensive skeleton mineralization, had been detected. At the age from 15 to 16 years as the level of body estradiol saturation had been increased rates of growth and mineralization had been minimized.

Conclusions. In healthy girls at the age of 13–16 years osteopenia rate was 22% and did not exceeded its population level. In adolescent girls, estradiol impact on bone mineral density had been caused by correlation of estradiol with skeleton growth and mineralization rate.

P111

BONE MINERAL DENSITY IN IDIOPATHIC SHORT STATURE

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Aim: To examine differences in risks of osteoporosis, we measured the bone mineral density (BMD), bone mineral apparent density (BMAD) and serum osteocalcin of four groups young adults with different growth patterns as part of the PROGRAM study. The PROGRAM study (PROgramming factors of

Growth And Metabolism) is unique in determining risk profiles for diabetes, cardiovascular disease and osteoporosis of four groups, distinguishing weight and height at birth, and weight and height in young adulthood.

Methods: Our four study groups: 1. Small for Gestational Age (SGA; birth length and/or birth weight <-2 SDS) with no catch up (current height <-2 SDS) 2. SGA with catch up (current height >-2 SDS) 3. Idiopathic Short Stature (ISS; birth length and/or birth weight >-2 SDS and current height <-2 SDS) 4. control group (birth length and/or birth weight as well as current height >-2 SDS). Up to now, we have completed group 3 and group 4. 27 young adults (13 girls, 14 boys) with ISS were included. Our control group consisted of 31 young adults (17 girls, 14 boys). All included young adults were 18–23 years old, healthy, Caucasian and born after 37 weeks of gestation. BMD (g/cm^2) measurements of lumbar spine (LS) and total body (TB) were performed, using dual energy X-ray absorptiometry. LS BMAD was calculated as 4/pixwidth vertebral body. Serum osteocalcin was measured using radioimmuno-assay.

Results: The mean BMDs of the LS and TB were significantly lower in girls of group 3 than in girls of group 4 ($P=0.005$, $P=0.004$ respectively). Boys of group 3 had significantly lower TB BMD ($P=0.015$) and serum osteocalcin ($P=0.026$) than boys of group 4. After adjustment for birth length, birth weight, age, BMI, cigarette smoking, alcohol consumption and sports participation, these differences remained significant. No significant differences were found in mean BMAD between boys of group 3 and 4 and between girls of group 3 and 4.

Conclusions: Our results indicate that girls and boys with ISS have a significant reduction in TB BMD. Girls with ISS also have a significant reduction in LS BMD. These differences may be explained by the difference in current height between ISS young adults and controls, as is suggested by a non-significant difference in BMAD. Serum osteocalcin is significantly lower in boys with ISS than in controls.

P112

RELATIONSHIP BETWEEN RENAL FUNCTION AND THE PREVALENCE OF VERTEBRAL FRACTURES IN OLDER HOSPITAL ATTENDEES

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Introduction: The prevalence of moderate and severe renal failure in elderly osteoporotic women has been documented (Klawansky, Osteoporosis International, Vol 14 No 7). However the prevalence of vertebral fractures in these populations is unknown. We aimed to determine if the severity of renal failure affects the prevalence of vertebral fractures in patients with osteoporosis.

Methods: Data was gathered on sequential referrals from all hospital departments to a DEXA screening service. Bone densitometry and lateral vertebral morphometry were performed on all patients, and Creatinine Clearance (CrCl) was estimated using Cockroft and Gault's method.

Results: 342 patients were included, of whom 188 were over 65 (mean 80.17, range 65–95.87, M = 32, F = 156). Renal function was end-stage or severely impaired in 19% of patients; a further 66.4% had moderate renal impairment. (Table)

A significantly greater proportion of patients with end stage or severe renal disease had vertebral fractures compared to patients with mildly impaired or normal renal function, independent of age (47.2% vs 24%, $P<0.001$, Table). Significantly, 19.4% of patients in severe or end-stage renal failure were taking bisphosphonates at time of referral.

Conclusion: The prevalence of renal failure in this population of hospital attendees is higher than that previously reported for community dwellers. Renal failure was associated with an increased risk of vertebral fracture. An estimation of CrCl is obligatory in hospital attendees referred for DEXA, as usual therapy is contra-indicated in up to 20% of these patients.

Table: Vertebral fractures and therapy by renal disease

Renal Functio(CrCl)	Total	Number on Bisphosphon	Vertebral Fractures (%)
End Stage (<15 mls/min)	2	1 (50)	2 (100)
Severe (15–29)	34	6 (17.6)	15 (44.1)
Moderate (30–59)	125	18 (14.4)	38 (30.4)
Mild (60–89)	21	2 (9.5)	6 (28.6)
Normal (>90)	4	0 (0)	0 (0)

P113

CORRELATION BETWEEN SERUM OSTEOPROTEGERIN LEVELS AND BONE METABOLISM, INSULIN RESISTANCE IN DIABETIC PATIENTS

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Introduction: Osteoprotegerin (OPG) is known to prevent bone resorption through binding to RANKL and inhibit the differentiation of osteoclasts. Recently, OPG is reported to be associated in the pathogenesis of atherosclerosis. Diabetes is the representative model of on-going atherosclerosis, but the correlation between diabetes and OPG has not been confirmed yet. The aim of this study is to find out the relationship between serum OPG levels and bone metabolism, insulin resistance and cardiovascular risk factors in diabetic patients.

Methods: Blood pressure, body mass index, fasting blood glucose, fasting insulin, total cholesterol, HDL-C, LDL-C, triglyceride and urine microalbumin were measured in 84 patients (33 males, 51 females, mean age 56.7 yrs) being treated for type 2 diabetes. BMD and cardiac echocardiogram were also checked. Serum osteocalcin and urine deoxypyridinoline levels were measured as bone turnover markers. HOMA and QUICKI were calculated and the presence of calcification was confirmed in the aortic arch in simple chest X-ray by trained radiologist. Serum OPG levels were measured with sandwich ELISA method.

Results: Serum OPG levels showed no gender difference and showed positive correlation with age, left ventricular mass index, left atrial size and HOMA and negative correlation with lumbar spine BMD (g/cm^2), QUICKI and serum calcium levels ($P<0.05$). There were no differences in mean serum OPG levels between 3 groups divided by urine microalbumin levels and serum OPG levels showed no differences between groups according to the presence of aortic calcification.

Conclusion: OPG is negatively correlated with L-spine BMD in diabetic patients and positively correlated with insulin resistant status and LV mass index which is thought as an independent risk factor for cardiovascular mortality. Further studies are warranted to discover the exact mechanism existing between atherosclerosis and OPG in diabetic patients.

P114

ANTI-EPILEPTIC DRUG USE AND BONE HEALTH: A STUDY OF EXPOSURE DISCORDANT TWINS AND SISTER PAIRS

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Anti-epileptic drug (AED) use is associated strongly with fracture risk, but the underlying mechanism is unclear. Studies of the long-term effects of AED use on bone mineral density (BMD) and other indices related to bone health are limited by small sample sizes, inadequate controls, the use of cross-sectional design, and lack of adjustment for potential confounders.

We identified participants with epilepsy or AED use from the twin and sister research databases of the collaborating institutions. BMD was measured at the lumbar spine (LS), total hip (TH), femoral neck (FN) and total forearm (FA). Total body bone mineral content (TB BMC) was determined. Results were expressed as the within-pair percentage difference, relative to the non-user. All data were adjusted for age, height and weight.

Thirty-three (15 monozygous and 18 DZ dizygous) twin pairs and 5 sibling pairs (<3 years age difference) with a mean (SD) age 46.4 (16.2) years (range 18–75 years) were assessed. Pairs were discordant for AED use, with one having >12 months AED use (user) and the other no exposure (non-user). There were marginal AED-associated bone mineral deficits in the whole group: TH (-3.2%, $P=0.08$) and FA (-2.3%, $P=0.09$). There were significant within-pair differences in the following subgroups: (1) Those with more than 24 months of AED discordance ($24+$) (n=30): TH (-4.5%, $P=0.036$) and FA (-3.4%, $P=0.024$). (2) AED of the inducer class (AEDI) (n=30): TH (-4.5%, $P=0.036$) and FA (-3.4%, $P=0.020$). (3) Women aged $40+$ years and $24+$ (n=18): TH (-4.5%, $P=0.05$) and FA (-4.7%, $P=0.021$). (4) Women aged $40+$ years, $24+$ and AEDI (n=16): TH (-5.8%, $P=0.009$) and FA (-5.2%, $P=0.011$). (5) Women aged $40 +$ years, $24+$, and current users for at least 5 years prior to their study visit (n=15): TH (-8.3%, $P=0.010$), FA (-6.2%, $P=0.015$), LS (-8.3%, $P=0.03$) and TB BMC (-5.6%, $P=0.02$); 13/15 users were taking AEDI.

These findings using a matched twin/sister, discordant-pair approach indicate that patients using AEDs have significantly lower BMD at clinically-rele-

vant sites. This bone deficit helps to explain the increased fracture risk among AED users. Mechanisms underlying this important association warrant further investigation. Monitoring of BMD may be important in patients taking long-term AED therapy.

P115

ASSOCIATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPAR GAMMA) EXON 6 C/T POLYMORPHISM WITH BONE MINERAL DENSITY AND SERUM OSTEOPROTEGERIN LEVELS IN HEALTHY KOREAN WOMEN

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Introduction: Since adipocytes and osteoblasts derive from same mesen-chymal progenitor cell line, the possibility that PPAR gamma activation might involve in the pathogenesis of osteoporosis was suggested. Furthermore, recent evidences suggested the PPARgamma gene as a candidate gene for osteoporosis. Osteoprotegerin (OPG) is a decoy receptor for RANKL and prevents bone resorption through inhibition of osteoclastogenesis. To analyze the role of PPARgamma gene in the bone metabolism, we examined the association of a PPARgamma C/T substitution at exon 6 with bone mineral density, bone turnover markers and OPG levels in healthy Korean women.

Methods: Blood samples were obtained from 263 healthy Korean women (mean age 51.5yrs, 37–73 yrs) and DNAs were extracted from leukocytes. PCR-RFLP done in all samples with restriction enzyme Pml I. Anthropometric data, bone turnover markers, and serum OPG levels were measured.

Results: The frequencies of the CC, CT and TT genotypes were 65%, 30.8% and 4.2% and the T allele frequency was 0.804, which were in Hardy-Weinberg equilibrium. Mean serum OPG level was higher in CC genotype group (1361.03 ± 398.61 vs. 1213.55 ± 358.43 pg/mL, $P < 0.01$) and BMD and other bone turnover markers showed no differences between the groups. When the subjects are divided in two groups with and without osteoporosis, subjects with T allele showed higher serum OPG levels than those with CC genotypes only in non-osteoporotic group ($P < 0.01$). When the subjects are divided in two groups according to the menopausal status, in postmenopausal group, subjects with CC genotype showed higher mean serum OPG level than those with T alleles ($P < 0.05$) and other variables showed no significant differences between groups.

Conclusion: C/T substitution in exon 6 of PPARgamma gene was associated with higher serum OPG levels in female subjects, which implicates the possibility of involvement of this gene in the bone mineral metabolism.

P116

FTIR IMAGING (FTIRI) ANALYSIS OF FEMORAL NECK BIOPSIES IN HIP FRACTURE CASES

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Osteoporosis has been defined as a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, whereas the mineral and matrix properties have sometimes been assumed to remain unchanged.

The aim of this pilot study was to analyse these properties in the female femoral neck cortex biopsies of hip fracture cases (n = 3, 79–86 years), and post-mortem controls (n = 4, 78–82 years). FTIRI provides quantitative data on crystal size and composition, and on matrix structure and composition at 6–10 μm spatial resolution. The spectra were acquired from 400×400 mum areas (6–8/biopsy). Crystallinity was calculated from the ratio of the peak intensities at 1030 and 1020 cm^{-1}, corresponding to the mineral phosphate vibrations PO$_4^{3-}$ in a stochiometric and non-stochiometric apatite environment respectively. Collagen maturity ("cross-linking") was calculated using the amide I band from the ratio of the peak intensities at 1660 and 1690 cm^{-1}, corresponding to vibrations in non-reducible and reducible collagen cross-links, respectively. Mineral to matrix ratio was calculated from the integrated area of the phosphate and amide I bands. All calculations were performed using ISYS (Spectral Dimensons, MD).

The bone mineral in the femoral neck fracture cases was more crystalline than that in controls (controls: 1.32 ± 0.01, cases: 1.39 ± 0.02, $P = 0.002$). The mineral to matrix ratio and collagen maturity of fractures cases tended to exceed

that in controls (min/matrix: controls: 4.0 ± 0.1, cases: 4.3 ± 0.1, $P = 0.101$). (maturity: controls: 3.37 ± 0.10, cases: 3.58 ± 0.13, $P = 0.205$).

Our results are concordant with previous findings by Kent et al. (JBJS 65-B:189 1983) who found that, close to the fracture site, the mineral crystals were enlarged compared those in control specimens. This, according to Gao's recent theoretical study (PNAS 100:5597 2003), would make them more fragile if they (as is likely) contain imperfections. Paschalis et al (CTI 61:487 1997) found that osteoporotic iliac crest biopsies also showed a higher crystallinity than controls.

Our study suggests that bone mass and its distribution might not be the only determinants of fracture risk. The properties of mineralised bone as a composite material that contribute to determining its material toughness appear to show differences between fracture cases and controls. These warrant more detailed and systematic investigation.

P117

INFLUENCE OF VARIOUS PROTEIN INTAKES ON INTRINSIC BONE TISSUE PROPERTIES OF RAT VERTEBRAE

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We previously demonstrated the marked decrease in bone strength induced by isocaloric protein deficiency in rats, and its reversal by essential aminoacids supplements. The effects of the latter treatment on bone strength could not be entirely explained by changes in BMD and/or in micro-architecture. We hypothesized that protein dietary intake would influence intrinsic properties of bone tissue and thus play an important role in the determination of macroscopic mechanical properties. To test this hypothesis, we performed nanoindentation tests on the lateral, anterior and posterior site of L5 vertebral bodies of 17 Sprague-Dawley rats fed normal or isocaloric low protein diet or treated with isocaloric essential amino acids supplements. Ex vivo DXA measurements and axial compression tests of adjacent vertebral bodies were correlated with the indentation results. The tissue properties (indentation modulus, hardness, dissipated energy) varied significantly between the different sites (anterior, posterior, lateral), suggesting heterogeneity of bone within the vertebrae. Isocaloric low protein intake associated with an ovariectomy led to significant decreases of tissue properties on the posterior vertex ($P < 0.05$), which were corrected by treatment with essential aminoacids. Correlations between macroscopic data (axial compression of vertebral body) and tissue properties as assessed by nanoindentation technique suggest that postelastic behaviour strongly varied with material fragility detected at the tissue level. Macroscopic stiffness however was mainly determined by bone geometry changes and less by variations of tissue properties. Combining parameters of tissue properties (indentation modulus, hardness) and bone mineral density, prediction of ultimate strength reached 95%. These data indicate that besides geometry and micro-architecture, intrinsic bone tissue property is an important determinant of the mechanical competence of rat vertebrae. Changes in intrinsic tissue competence could thus contribute to the pathogenesis of osteoporosis in response to a low protein diet.

P118

OSTEOCYTIC EXPRESSION OF NITRIC OXIDE SYNTHASE ISOFORMS IN THE FEMORAL NECK CORTEX: A CASE-CONTROL STUDY OF INTRACAPSULAR HIP FRACTURE

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Osteocytes are considered to control the response to mechanical stimuli in bone through the expression of local signalling molecules such as nitic oxide (NO) derived from the mechanically sensitive endothelial isoform of nitric oxide synthase (eNOS). As NO is inhibitory to osteoclastic resorption it has been suggested that osteocytes expressing eNOS act as sentinels confining resorption within single osteons (Bone 30:866, 2002). Recently the neuronal isoform of NOS has been shown to be present in adult human bone osteocytes (Caballero-Alías et al. CTI in press).

This study was designed to determine the osteocytic expression of both NOS isoforms in the femoral neck cortex of a further 8 female cases of intracapsular hip fracture and 7 post-mortem controls (female aged 68–91 years). Cross-sections of the femoral neck were analysed by immnuno-cytochemistry using well characterised antisera for nNOS and eNOS that showed no cross-reactivity with the other NOS isoform. Methyl green was

used as a counter stain. The data were analysed on the basis of the percentage of all osteocytes expressing each isoform along with the distance of the closest NOS expressing osteocyte to the canal surface.

The percentage of osteonal osteocytes positive for nNOS was lower in the fracture cases compared to the controls (nNOS: cases: 43.1 ± 1.5, controls: 56.7 ± 1.5, $P < 0.0001$). Compared to nNOS, eNOS expression was further reduced ($P = 0.009$) in the cases but was not different in the controls (cases: 36.4 ± 1.5, controls: 56.5 ± 2.4, $P < 0.0001$). The minimum distance of an osteocyte expressing either eNOS or nNOS to the canal surface was higher in the cases compared to controls (eNOS: controls: 44.4 ± 2.2 mum, cases: 61.7 ± 2.0 mum, $P < 0.0001$; nNOS: controls: 52.4 ± 1.7 mum, cases: 60.2 ± 2.1 mum, $P = 0.0039$) with the eNOS expressing osteocytes being closer than those expressing nNOS in the controls (-14.98 ± 4.02, $P = 0.0012$).

In conclusion, the expression of both nNOS and eNOS is reduced in the fracture cases suggesting that the capacity to generate NO is reduced. Furthermore, the reduction in NOS expression occurs in those osteocytes closest to the canal surface so that the ability of NO to limit resorption depth is likely to be impaired. Further studies into the regulation and the possible effects of NO production from the separate NOS isoforms, including the possible role of different intracellular locations, are essential to determine their role in bone turnover and hip fracture.

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PROTECTIVE EFFECTS OF BONE LOSS BY OPG AND TNF-ALPHA ANTIBODIES IN A COLLAGEN-INDUCED ARTHRITIS MODEL: DISTINCT ROLE ON BONE RESORPTION AND FORMATION

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Focal bone erosions in rheumatoid arthritis (RA) involves RANKL and TNF-alpha, these cytokines regulating bone turnover as well. Osteoprotegerin (OPG) is a potent inhibitor of bone resorption in different models of enhanced resorption. Anti-TNF-alpha decreases joint inflammation and bone erosions, but its effect on systemic bone loss remains not clearly demonstrated. Our aim is to investigate the respective and combined effects of OPG and anti-TNF-alpha on systemic bone remodeling in inflammatory conditions. Collagen-induced arthritis (CIA) was induced in DBA/1 mice immunized with bovine type II collagen. Immunized mice were treated at the onset of arthritis with: 1) OPG-Fc (10 mg/kg SC 3 times/week); 2) TNF-alpha antibodies (anti-TNF-alpha, 10 mg/kg IP 2 times/week); 3) both OPG-Fc + anti-TNF-alpha or with; 4) saline solution; and 5) one group of mice remained naive. At baseline before immunisation and at sacrifice, we measured bone mineral density (BMD, Piximus Lunar) and urinary deoxypyridinolin (D-Pyr) excretion. Histomorphometric indices were measured at the femur metaphysis.

Clinical arthritic scores significantly improved in anti-TNF-alpha-treated group compared with saline ($P < 0.02$), but no effect of OPG. BMD gain was lower in CIA mice treated with saline compared with naives (15 ± 2 vs $30 \pm 1\%$, $P < 0.001$) confirming that CIA promotes bone loss. Both OPG and anti-TNF-alpha increased BMD gain compared with saline ($39 \pm 2\%$ and 251% vs $15 \pm 3\%$ respectively, $P < 0.001$ and 0.05). However, OPG was more efficiency to increase BMD than anti-TNF-alpha ($P < 0.003$) and there was no additive effect of combined OPG and TNF-alpha on BMD ($40 \pm 3\%$). In addition, D-Pyr decreased by 65% with OPG compared to 7% in saline mice ($P < 0.001$) and 13% in anti-TNF-alpha treated mice ($P = $ NS). Histomorphometric data showed a lower bone volume (BV/TV) in saline mice than in naives (8.4 ± 1.1 vs 14.9 ± 0.6, $P < 0.05$). Compared to saline, OPG induced increased BV/TV (8.4 ± 1.1 vs 13.4 ± 0.9, $P < 0.02$) and decreased Tb spacing (345 ± 91 vs 190 ± 13 um, $P < 0.02$) indicating an inhibition of bone resorption, but completely decreased the BFR/BS (45 ± 8 um 2/d vs 0, $P < 0.01$). In contrast, anti-TNF-alpha increased Tb thickness compared to saline (30.4 ± 0.8 vs 23.9 ± 1.4 um, $P < 0.02$) which was close to those of naive mice (32.4 ± 2.3, p NS), suggesting a protective effect on bone formation.

In conclusion, systemic administration of OPG and anti-TNF-alpha prevented bone loss in CIA-mice through distinct mechanisms affecting bone resorption and formation.

P120

UNDER-RECOGNITION OF VERTEBRAL FRACTURES IS IMPROVED BY AN EDUCATIONAL INTERVENTION PROGRAMME AMONG GENERAL INTERNIST RESIDENTS

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Background: Vertebral fractures (VFx) are strong but largely under-recognized predictors of subsequent fracture risk. Thus, spinal osteoporosis remains very often under-treated. To evaluate the impact of an educational programme among residents on the recognition of VFx, we prospectively collected X-rays and assessed the presence of VFx during 2 consecutive phases separated by specific teaching on VFx recognition.

Methods: During an 3.5-month initial phase, we analysed lateral spinal or chest X-rays of 405 patients (pat.) 60 yrs or older. X-ray were reviewed by two independent and trained physicians as investigators and VFx graded according to Genant's semiquantitative method (SQ1-2-3). The results were compared with the radiologist's report and the resident's discharge summary. All residents, but not radiologists, were actively educated to diagnose VFx using a broad range of lectures, posters and flyers. In the second 2-month phase, 284 inpatients were included.

Results: Phase 1: 405 pat.; 54% men, 46% women; age mean $\pm$ SD:76 ± 9; range: 60–97 yrs; 88 pat. with VFx (SQ1-2-3) were reported by investigators as compared to 26 (29%) by radiologists and 17 (19%) by residents; 59 pat. with VFx (SQ2-3) were reported by investigators as compared to 23 (39%) by radiologists and 13 (20%) by residents.

Phase 2: 284 pat.; 56% men, 44% women; age mean $\pm$ SD:76 ± 8; range: 60–100 yrs; 54 pat. with VFx (SQ1-2-3) were reported by investigators as compared to 11 (20%) by radiologists and 20 (37%) by residents; 41 pat. with VFx (SQ2-3) were reported by investigators as compared to 10 (24%) by radiologists and 20 (49%) by residents. In phase 1 recognition rate was significantly higher among radiologists as compared with residents ($P = 0.05$ for SQ2-3). In phase 2 recognition rate among residents significantly improved as compared with phase 1 (19 vs 37%, $P = 0.02$ for SQ1-2-3; 20 vs 49%, $P = 0.005$ for SQ2-3) and was significantly better than among radiologists (49 vs 24%, $P = 0.02$ for SQ2-3).

Conclusions: The results of our prospective survey confirm the large under-recognition of VFx in hospitalized pat. even with moderate to severe VFx. The educational programme did significantly improve the VFx detection by the residents as compared to the absence of any change among radiologists who did not benefit from the programme. Our study demonstrates the efficacy of such an information campaign, which should be broadly implemented in order to improve both the detection of VFx and the therapy of osteoporosis.

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BONE MINERAL DENSITY AT THE SPINE AND HIP AND ITS DETERMINANTS IN PERIPUBERTAL JAPANESE CHILDREN

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Aims: Achieving greater peak bone mass is one of the most important strategies in preventing osteoporosis later in life. However, the annual rate of fracture in pupils and students in Japan has increased steadily and doubled in the last quarter of 20th century. The aim of the present study is to clarify the axial bone mineral density (BMD) in peripubertal Japanese children and factors promoting higher BMD which have never been investigated fully in Japan.

Methods: We measured height, weight, and BMD at the spine (LS) and hip (TH) by DXA (Hologic QDR4500A, USA) in 585 elementary school pupils (the grade 4 to 6: abbreviated as G4 to G6, respectively) and junior high school students (the grade 1 to 3: G7 to G9) in Shiokawa Town, Fukushima Prefecture, Japan. Detailed interviews were conducted by trained nurses to obtain past history of illness, pubertal status and lifestyle factors such as exercise and diet habits. Pubertal onset was defined as the time of pubic hair appearance in boys and of menarche in girls.

Results: Among the subjects, 579 without any history affecting bone metabolism were analyzed (283 boys and 296 girls, mean age 12.7 ± 1.7 with range of 9.7–15.7 years). Mean BMD increased linearly from G4 to G9 in boys and reached 87% and 97% of the young adult mean BMD (YAM) at LS and TH, respectively, in G9. However, BMD in girls plateaued in their junior high school age with 94% and 101% of YAM at LS and TH, respectively. Puberty showed a great effect on BMD at both skeletal sites in both genders. Height and weight correlated with BMD at LS and TH in every grade in both sexes. The greatest correlation coefficient was observed in G6 in girls and G9 in boys. To know independent effects of lifestyle factors on BMD, the analysis of covariances allowing for age (prepubertal subjects) or years since puberty onset (postpubertal subjects), height and weight was applied. The subjects participating in sport

clubs at school showed higher BMD than those who did not irrespective of gender, skeletal site or pubertal status. Similar beneficial effects on BMD were observed for milk consumption.

Conclusions: Increase in BMD of Japanese girls may stall in their mid-teens before reaching the adult level. Sporting activities and dairy food intakes should be promoted more than ever in early teenage Japanese girls.

P122

BISPHOSPHONATES INFLUENCE BONE BLOOD FLOW

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Cessation of estrogen production in a biological system cause enhanced bone remodelation and simultaneous weakening of bone. This action can be attenuated by bisphosphonates.

Estrogen depletion (and higher bone remodelation rate) is connected also with enhancement of bone blood flow (BBF).

Aim: The aim of this animal experiment was to answer the question, if bisphosphonate administration influence enhanced BBF after estrogen depletion.

Material and methods: Female rats were 4 week old (VUFB Konarovice). Oophorectomy was performed by standard approach from the dorsum of animal. Aminobisphosphonate pamidronate (Aredia,CIBA Geigy) was administred 3 mg/kg i.p. every other day in volume 0.2 ml. BBF was determined by use of radioactive (Sr-labeled) microspheres (1.2).

Experiment arrangement: 60 female rats were alloted in 4 groups, 15 animals in each group: I. Control group. II. Oophorectomised group. III. Pamidronate + sham operation. IV. Oophorectomised + sham operation.

Results of BBF are in ml/min/gr.bone tissue.

Results: (mean ± SD):

Group: I. II. III. IV.

BBF tibia: 0.13 ± 0.02 0.15 ± 0.01 $0.09 \pm 0.01^*$ $0.11 \pm 0.01^*$

*statistically significant 0,05 in relation to group II,

Conclusion: Aminobisphosphonate pamidronate, given according the scheme mentioned above restrict BBF, which is enhanced after oophorectomy.

Reference:

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P123

RENAL TUBULAR IMPAIRMENT IN CHILDREN WITH IDIOPATHIC HYPERCALCIURIA

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Idiopathic hypercalciuria (IH) is defined as hypercalciuria that persists after correction of dietary inbalances and has no detectable cause. Renal tubular dysfunction has been described in patients with IH. The excretion of urinary N-acetyl-beta-D-glucosaminidase (U-NAG), a marker of proximal tubular damage, has been previously reported as either increased or normal in children with IH. We evaluated U-NAG in 20 children (13 boys and 7 girls, mean age 10.3 years ± 5.7 SD) with IH (urinary calcium excretion above 0.1 mmol/kg/24 hours, with no detectable cause) and with otherwise normal renal function tests. Ultrasound examination revealed urolithiasis (n = 4) and nephrocalcinosis (n = 1). The U-NAG values were evaluated in the spot urine collected from the second morning void and calculated as the urinary NAG/creatinine ratio (U-NAG/Cr) and expressed in nkat/mmol. The 24-hour urinary calcium excretion (U-Ca/24 h) was assessed in a urinary sample from 24-hour collected urine and calculated in mmol/kg. The obtained results of U-Ca/24 h and U-NAG/Cr were expressed as Z-scores. When compared to the reference data, the U-Ca/24 h and U-NAG/Cr were significantly higher ($P < 0.0004$ and $P < 0.006$, respectively) There was no correlation between the U-NAG/Cr and U-Ca/24 h (r = 0.18, $P = 0.20$). The U-NAG/Cr values were significantly higher in the 5 patients with urolithiasis/nephrocalcinosis, whether compared to the rest of the group ($P < 0.02$), or to the reference data ($P < 0.01$). The U-NAG/Cr activity was higher in 15 children without urolithiasis/nephrocalcinosis when compared to reference data ($P < 0.01$). There was no difference in U-Ca/24 h between the children with and without urolithiasis/nephrocalcinosis ($P = 0.58$). These findings suggest that tubular impairment, as reflected by U-NAG/Cr, might occur in children with IH, especially in patients with urolithiasis/nephrocalcinosis. There doesn't seem to be a direct relationship between the U-NAG/Cr activity and the degree of calcium leakage.

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BONE QUALITY IN RECOMBINANT INBRED STRAINS OF MICE

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Previous studies have demonstrated genetic variability in adult bone density, biomechanical properties and microstructure among inbred strains of mice. In particular, C3H/HeJ (C3H) has been shown to have thicker femoral and vertebral cortices and fewer trabeculae in the vertebral body compared with C57BL/6 J (B6), despite having similar vertebral bone strength. Eleven recombinant inbred (RI) mice strains have been generated from B6 and C3H to isolate genetic regulation of many different traits. Our objective was to examine relationships between bone mineral density (BMD), bone architecture, bone mineralization, bone microhardness, and overall bone strength in the femurs and vertebrae of these 11 RI strains. Dual energy x-ray absorptiometry (DEXA) was used to examine femoral and vertebral BMD and the values were used to assign a BMD ranking to each mouse strain. Femoral BMD rankings correlated positively with vertebral BMD ranking in all mice strains (both progenitor and RI strains) except for BXH-9 and BXH-10. Biomechanical test results of the femur (three-point bending) and the vertebrae (compression) showed positive correlations between BMD to ultimate force and work to failure ($P < 0.001$) but not to stiffness. Image analysis of the vertebrae showed a large variation in trabecular bone volume (TBV) between RI strains, where the largest TBV is 3.5 times the smallest TBV and the extremes of the TBV spectrum is beyond the values of the progenitor strains. Within the vertebrae, backscattered electron (BSE) imaging showed that the mineralization profile, as represented by the logit function, was significantly higher in vertebral cortical bone than trabecular bone, ($P < 0.01$). However, microhardness testing did not show a significant difference between cortical and trabecular microhardness of the vertebrae, except in BXH-8 ($P < 0.01$). Microhardness testing was also performed on the femurs and results indicate that cortical bone of the femur was 50% harder than cortical bone of the vertebrae ($P < 0.001$). Furthermore, BSE imaging data showed that femoral cortical bone had a much higher bone mineralization profile than cortical bone in the vertebrae. In conclusion we found that cortical bone is not the same throughout the body. Cortical mineralization and microhardness are higher in the femur than in the vertebrae, and the genetic control of such bone properties may be site dependent.

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BETABLOCKER USE, BONE MINERAL DENSITY AND FRACTURE RISK IN OLDER WOMEN RESULTS FROM THE EPIDOS PROSPECTIVE STUDY*

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Recent experimental studies in mice suggest that beta-blocker use may be associated with a reduced risk of osteoporosis. To verify this hypothesis, we assessed the association between non cardio selective beta-blocker use and both bone mineral density and fracture risk in a large cohort of elderly French women who participated to the EPIDOS (Epidemiology de l'Ostéoporose) study. Out of the 7598 women (mean age 80.5 ± 3.8 years) included in the cohort, 283 (3.7%) were taking a beta-blocker at baseline. The average duration of use was 13.9 (± 10.1) years. After adjustment for age and subjective health status, there was no significant difference in hip or total body BMD between beta-blocker users and non users. During an average of 3.6 (± 1.2) years of follow-up, 1311 women suffered at least one non-vertebral osteoporotic fracture. After adjustment for age and health status, there was no significant association between beta-blocker use and risk of fracture (hazard ratio = 1.2; 95% CI: 0.9–1.5). We conclude that beta-blocker use is not associated with a reduced risk of osteoporosis in older women.

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BONE ARCHITECTURE AND DENSITY IN MALE HYPOGONADISM

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Low bone mass and alterations of bone microarchitecture are considered as important determinants of bone fragility and fractures in post-menopausal females. We assessed 2D and 3D architectural descriptors of cancellous bone in male patients suffering from hypogonadism in order to describe the pattern observed in this condition.

Patients and methods. Trans-iliac bone biopsies were obtained in 16 male patients (31–75 years old) suffering from hypogonadism and secondary osteoporosis (with vertebral in 12/16 cases). Descriptors of cancellous architecture were obtained by histomorphometric analysis (2 D sections) and by micro-computed tomography (3 D reconstruction, Skyscan 1072 X-ray computed system). Trabecular bone volume and parameters describing the trabeculae (Tb.N, Tb.Th, Tb.Sp) and their spatial distribution and connectivity (Euler-Poincaré's number, ICI, NN, NF) were analyzed in order to characterize the main abnormalities and to describe the structural profile of trabecular bone. Lumbar and femoral bone mineral density was assessed by dual photon absorptiometry.

Results. In association with a marked reduction of lumbar and femoral bone density, we found several alterations of the trabecular bone microarchitecture: (a) a decrease in the number of trabeculae without significant change of their size; (b) a 50% decrease in the number of plates, replaced by rods; (c) the 3D demonstration of large perforations defects leading to a disorganization of the spatial distribution of trabeculae. A good correlation was found between 2 D and 3 D analysis.

Conclusions. In male, the profile of secondary osteoporosis due to hypogonadism is characterized by a severe disorganization of the trabecular network due to large perforations defects and to a conversion of plates into rods. In association with low bone mineral density, these alterations might explain the increased bone fragility and the increased risk of fractures observed in this condition.
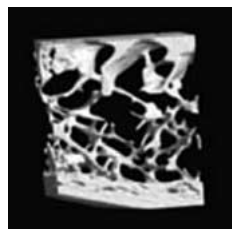
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A TWO-YEAR LONGITUDINAL STUDY ON THE EFFECTS OF LIFESTYLE FACTORS TO BONE MASS GAIN IN JAPANESE BOYS AND GIRLS: KYOTO KIDS BONE HEALTH STUDY

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Aim: Puberty is considered to be the important period in the acquisition of peak bone mass. At present, there is a lack of information regarding this topic in Japanese children and adolescents. The purpose of present study was to clarify associations between lifestyle factors such as calcium intake and exercise habits, and bone mineral density (BMD) and the rate of change in BMD at the lumbar spine (L2-L4) [LS] and total hip [TH].

Methods: For 412 first grade students aged 12 or 13 years old of 3 junior high schools in Kyoto, Japan, we measured BMD by DXA (QDR4500, Hologic) at LS and TH, height, weight and grip strength at baseline. Past and present history of illness, lifestyle factors such as dietary calcium intake and exercise habits, and information on maturity were obtained from detailed interviews. We examined the same variables in 346 of the students again after 2 years. We also analyzed 336 third grade students (122 boys: mean age 14.9 ± 0.26 years and 214 girls: 14.8 ± 0.30) who had no disease and were not on medication affecting bone metabolism of them.

Results: The boys showed significantly greater BMD than did the girls at TH, but LS did not differ between the genders. There was significant increase in BMD at LS (boys: 10.4%/yr, girls: 6.0%/yr) and TH (boys: 8.6%/yr, girls: 5.3%/yr) in both genders, and the gender differences in the rate of change in BMD at both sites have observed. The more mature students had the higher BMD at LS and TH in both sexes, but an inversed relationship was observed between the maturation stage and the annual change rates in BMD. BMD at LS and TH increased with weight in both sexes. In girls, the annual change rates in BMD decreased significantly as the weight rose. On the other hand, there was no significant decrease of the change rates between the weight groups of boys. When

BMD was adjusted for weight and maturity, and the annual change rates in BMD were adjusted for BMD at baseline, weight and maturity by the analysis of covariance, the boys and girls with more active lifestyle at their junior high school age and those with greater intake of milk at follow-up showed greater BMD and the change rates at LS and TH.

Conclusions: BMD at LS and TH increased with maturity in both genders and the annual change rates in BMD decreased with progression of pubertal development. The physical activity and the intake of milk at junior high school age were inferred to influence bone development in adolescents positively.

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COGNITIVE IMPAIRMENT AND OSTEOPOROSIS IN PERSONS 50 + YEARS OLD A POPULATION-BASED STUDY

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Background. There is limited information to date concerning the association of cognitive impairment with osteoporosis. Case control and cohort studies have revealed a number of risk factor for osteoporosis including cognitive impairment. However, large cohort studies on this issue are lacking.

Aims and methods. To evaluate the association between cognitive impairment and osteoporosis, during the II phase of the baseline collection of an epidemiological study in a rural Italian population aged 50 years or older, 594 subjects (408 women and 186 men) were screened for osteoporosis. Ultrasound measurement of the calcaneus of the non dominant heel was obtained for all subjects. Speed of sound (SOS), broadband ultrasound attenuation (BUA), and the T-score stiffness index (SI – a composite parameter of SOS and BUA) were measured using the Achilles system (Lunar, Madison WI). A threshold of −2.5 SD for T-score SI was used for identification of osteoporosis. To screen for cognitive status, subjects underwent to the Mini-Mental State Examination (MMSE). A sex- and education-corrected cut-off of 23.8 was choose to considered subjects as cognitive impaired. The association between cognitive impairment and osteoporosis was analyzed by using odds ratios (ORs) with 95% confidence intervals (CI) from logistic regression models.

Results. The prevalence of osteoporosis in our population was 23%, being higher in women than men (29.4% vs 8.6% respectively, $P = <0.001$) and in subject aged 65 + than those 50–64 years old (31.5% vs 14.4% respectively, $P = < 0.001$). There was a higher prevalence of cognitive impairment in subjects with osteoporosis respect to those without (40.7% vs 20.6% respectively, $P = < 0.003$). Adjusted ORs for osteoporosis-related cognitive impairment was 1.4 (95% CI, 1.04–1.8) for the total sample and 2.3 (95% CI, 1.2–3.2) for women, whereas men did not show this association.

Conclusion. Our data confirm recent findings detailing an association between cognitive impairment and osteoporosis in adult-to-elderly subjects. The strong association between the two conditions in women is possibly due to post-menopausal hormonal changes common to both syndromes.

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URINARY CREATININE AS A PREDICTOR OF BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN

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Urinary creatinine (U-Cre) is a by-product of muscle breakdown and can be used for estimates of muscle mass. We examined its relationship with bone mineral density (BMD) of various skeletal sites in over 100 healthy, postmenopausal women over a period of 2 years. BMD, and body composition (by DEXA), dietary intake, and 24-h urine samples were evaluated at baseline, 6,12,18 and 24-month points. The 24-h U-Cre values for each visit were in the normal range with the baseline and 24-month averages of 0.97 ± 0.18 and 0.96 ± 0.21 g/day, respectively. The Pearson correlation, r, between U-Cre and total body lean tissue (by DEXA) was above 0.6 ($P < 0.0001$) at each visit, indicating good 24-h urine collections. The regression equations for total hip and spine (L1-L4) BMD, respectively, at baseline were: BMD $(g/cm^2) = 0.23*$U-Cre (g/day) + 0.63 and BMD $(g/cm^2) = 0.30*$U-Cre (g/day) + 0.75. Similar equations were obtained at each subsequent visit. In multiple regression models with BMD of various skeletal sites, constructed at each time-point and controlled for age, height, protein intake, and recreational/sports activities, U-Cre contributed from 1.5% to 10% of the variance for each model, $P < 0.05$. We conclude that in healthy postmenopausal women with normal kidney functioning and proper 24-h urine collection, U-Cre could be a useful and significant predictor of BMD for most of the measurable skeletal sites, even when physical activity and protein intake, both known to alter U-Cre, are taken into consideration. The positive relationship between U-Cre and BMD of various skeletal sites found in this study is too strong to be explainable

solely by the U-Cre acting as a marker of muscle mass, the latter proven to positively influence BMD. More research is warranted to investigate this relationship and its actual mechanism.

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BMD AND FRACTURE RATES IN PATIENTS ON ORAL GLUCOCORTICOID TREATMENT: RESULTS OF THE POPULATION-BASED PSIO-D STUDY

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Background: Glucocorticoid (GC) induced osteoporosis is a widespread skeletal disease. Still there are few population-based data. The PSIO-D study (''Prevalence of steroid induced osteoporosis in Germany'') is a population-based study. In a first phase (Survey Phase) among 22944 randomly selected inhabitants of Kiel, Germany, we observed a point prevalence of 1.4% for patients that take oral GC (>2.5 mg/d) for at least the last three months. Here we report data from the second phase of the study (Clinic Phase) in which patients on GC treatment contacted in the survey phase were examined for fracture and bone mineral density (BMD) status during a visit at the clinic. We investigated how fracture prevalence and BMD status compare to the general population.

Methods: A subset of 118 women on oral GC (>2.5 mg/d for at least 3 months) was examined with regard to vertebral fracture and BMD spine and hip status. A central standardized analysis of vertebral fracture (reduction in vertebral height $>=20\%$) was performed and non-osteoporotic fractures were excluded. Standardized BMD (sBMD) of the spine and hip were measured by DXA (Hologic QDR-4500). Postmenopausal women from the Kiel subset of the OPUS study (OPUS-Kiel) served as population-based reference sample of the general population.

Results: The 118 patients were 69.3 ± 7.0 years old (compared to 67.2 ± 7.0, n = 533 for OPUS Kiel). The sBMD of the lumbar spine in patients on GC was 958.2 ± 179.9 mg/cm^2 (T-score $>-1.54 \pm 1.3$; Z-score -0.15 ± 1.0) compared to 1047.6 ± 182.5 mg/cm^2 (T-score -0.86 ± 1.4; Z-score 0.30 ± 1.1) for OPUS Kiel. The sBMD of the total hip in patients on GC was 827.5 ± 142.9 mg/cm^2 (T-score -1.21 ± 1.1; Z-score -0.15 ± 1.0) compared to 886.2 ± 141.2 mg/cm^2 (T-score -0.76 ± 1.1; Z-score 0.16 ± 1.0) for OPUS Kiel (all differences $P < 0.01$). The prevalence of osteoporotic vertebral fractures among patients on GC was significantly larger (25.0% compared to 15.6% in OPUS Kiel, $P < 0.05$).

Discussion: Women on oral GC treatment have a 60% higher prevalence of fractures and moderately reduced sBMD of the spine and the hip compared to women selected from the general population in Kiel (Z-score differences of -0.45 and -0.31, respectively). Our data support the observation that fracture risk in patients on GC treatment is high and prevention or treatment of fracture risk need to be considered.

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EVALUATION OF OSTEOPOROSIS RISK ASSESSMENT TOOLS TO DETECT LOW BONE MASS DENSITY IN FRENCH CANADIAN WOMEN

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Objective: The aim of this study was to evaluate the potential of osteoporosis risk's questionnaires and quantitative ultrasound sonography (QUS) as screening tools to detect low bone mass in French Canadian women.

Methods: Three index scoring systems, including the Simple Calculated Osteoporosis Risk Estimation (SCORE), the Osteoporosis Risk Assessment Instrument (ORAI) and ACTI-MENU questionnaire, were studied among women aged 21 to 87 years. We compared the performance of these indices with Achilles quantitative ultrasound (QUS) in predicting low bone density. Femoral neck and/or lumbar spine DXA (T-scores ≤ -2.5) were used as outcome thresholds. Values of cutoff point for each index were those already used by the scoring system.

Results: Sensitivity of the screening alternatives in predicting low femoral and/or lumbar spine bone density was 47.0% for ACTI-MENU, 79.1%

for ORAI, 82.2% for SCORE and 86.8% for QUS. Specificity was 77.8% for ACTI-MENU, 54.7% for ORAI, 52.1% for SCORE and 48.9% for QUS.

Conclusion: Our results show similar capacities of prediction of low bone density for the SCORE and ORAI questionnaires and quantitative ultrasound measurement. Therefore, in public awareness program, physicians could use one of these three methods for evaluation of women osteoporosis risk.

P132

PREVALENCE OF OSTEOPOROSIS AND OSTEOPENIA AT LUMBAR SPINE IN A SPANISH EARLY POSTMENOPAUSAL WOMEN POPULATION

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Background: For the design of optimal strategies to prevent osteoporotic factures, adequate knowledge of the problem in the early menopause is essential. The other hand there are differences among published data about different populations.

Objective: The aim of this study was to evaluate the prevalence of osteoporosis and osteopenia in spanish women aged 51 to 56 years.

Methods: 183 women aged 51 to 56 years (mean 53.6 yr, 95% CI: 53.4–53.8 yr) were randomly selected in the province of Albacete (Spain). All women were postmenopausal, from 1,5 to 4 years(mean 2.6 yr, 95% CI: 2.5–2.7 yr) and they did not have diseases or taking drugs known to affect bone metabolism. It was collected the osteoporosis risk factors: calcium intake, physical activity, sun exposure, smoking, alcohol consumption, family history of osteoporosis, menarche age and body mass index (BMI). The mean of BMI was 28.1 Kg/m^2 (95% CI: 28.4–28.7 Kg/m^2). Bone mineral density (BMD) was measured by DXA at the lumbar spine (anteroposterior measurement from L2 to L4) with the use of Norland XR 26 Mark II densitometer. The coefficient of variation assessed by de phantom was 0.64%. Osteoporosis was defined as a BMD < -2.5 standard deviations (SD) and osteopenia as a BMD between -1 and -2.5 SD.

Results: The mean of BMD was 0.942 g/cm^2 (95% CI: 0.922–0.963). There were 18 women with osteoporosis (9.8%, 95% CI: 6.1–15.3%) and 70 with osteopenia (38.2%, 95% CI: 31.2–45.7 %). Among the assessed osteoporosis risk factors, we only found association with the BMI ($P < 0.005$, r = 0.222).

Conclusion: The prevalence of osteoporosis in spanish early postmenopausal women was similar to the some european and american populations and lower than others with different ethnicity and/or geographic situation. Only BMI was associated with bone mineral density.

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DOES HISTORY OF FALLING PREDICT FRACTURES IN POSTMENOPAUSAL WOMEN?

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Falling tendency and decreased bone strength are main causes of fractures among the elderly. The purpose of this study was to evaluate if falling tendency predicts fractures of postmenopausal women before old age.

The study population consisted of those OSTPRE cohort (Kuopio area) women (born in 1932–41) who responded to postal enquiries in May 1989 (baseline), May 1994 and May 1999 (N = 11074). Falls during the preceding 12 months were asked in May 1994 and fractures during the preceding 5 years in May 1999. Fractures were validated with patient record perusal. A total of 1103 women recorded a follow-up fracture. In all, 3283 women reported a fall from standing height. These falls were calssified as falls due to slipping (SF) (N = 2009), due to other cause (OF) (N = 1094) and due to both causes (N = 180). Fractures were classified as distal forearm fracture (DFF) (N = 417) and other fractures (NDFF) (N = 686).

Falls from standing height did not predict DFF but predicted NDFF with odds ratios (OR) of 1.05 (ns) for women reporting 1–2 falls but 1.44 ($P = 0.005$) for women reporting three or more falls compared to women who did not report any fall. Falls due to slipping did not predict DFF or NDFF, whereas other falls (3+) predicted NDFF with OR of 1.73 ($P = 0.001$) but did not predict DFF. Adjusting for height, weight, age, menopausal age, calcium intake, HRT, smoking, use of alcohol, number of health disorders, work disability, number of prescribed drugs and leisure physical activity did not affect the associations between falls and fractures.

We conclude that, in women aged 53–67, falling tendency does not predict distal forearm fracture but is a moderate predictor of other fractures.

Osteoporosis: Evaluation and Treatment

P134

TREATMENT OF OVARIECTOMIZED RATS WITH STRONTIUM RANELATE IMPROVES BONE STRENGTH AND BONE QUALITY

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The efficacy of strontium ranelate (SR), a new compound which has shown anti-fracture activity in postmenopausal osteoporosis, was assessed on ovariectomy-induced bone loss using mechanical strength testing of the lumbar vertebra. Six-month old Sprague-Dawley rats were either ovariectomized (OVX) or received sham (SHAM) surgeries. Beginning 1 day post-ovariectomy, 3 groups of OVX rats were treated daily for 52 weeks with 125, 250, or 625 mg/kg of SR and one received vehicle. Vehicle-treated OVX and SHAM animals served as controls. After 12 months treatment, bone loss in the OVX rats was substantiated by a 12.7% lower lumbar spine BMD compared to SHAM ($P < 0.01$). Furthermore, in 3rd lumbar vertebra (LV3), OVX rats had 49.0% lower cancellous bone volume (BV/TV), a 36.3% decrease in trabecular number (Tb.N) and a 107.7% increase in trabecular spacing (Tb.Sp; $P < 0.01$ for all parameters). The changes in bone mass and architecture led to declines of 31.9%, 33.3% and 34.9% in the maximum load (load necessary to break the bone), stiffness (bone elasticity) and ultimate strength (maximum stress the bone can sustain) of the 5th lumbar vertebra (LV5; $P < 0.01$ for each parameter). SR treatment of OVX rats dose-dependently increased the mechanical properties of LV5 with maximum load, energy to failure and ultimate strength increased up to 24.7%, 74.5% and 26.4% with 625 mg/kg/d ($P < 0.01$). At this dose, the values for biomechanical parameters in OVX animals were nearly equivalent to those in SHAM animals. These changes occurred without any modification of bone stiffness. Furthermore, bone histomorphometry also showed positive, dose-dependent effects of SR on LV3 with increases in BV/TV and Tb.N (39.6% and 28.0%; $P < 0.05$), and decrease in Tb.Sp (30.8%; $P < 0.01$). However, as the improvements in bone mass and architecture were intermediate between SHAM and OVX values, the magnitude of improvement in mechanical strength can also be explained by an improvement in bone quality synergistic with SR's effects on bone architecture. In conclusion, these results indicate that treatment with SR in OVX rats improves bone strength and quality and support SR efficacy and safety as an anti-osteoporotic therapy.

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CALCIUM INTAKE IN EARLY POSTMENOPAUSAL (OSTEOPOROTIC) WOMEN IN ZUERICH

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All the international EBM studies investigating the effects of antiresorptive agents in the prevention and therapy of osteoporosis are performed with supplementation of Calcium (Vitamin D) in the control and verum group. In our experience the general practitioner often disregards this supplementation.

The question is whether at least the Calcium supplementation is really necessary.

To enable us to answer this question we calculated the daily Calcium intake in 500 early postmenopausal and osteoporotic (osteoporotic according to the WHO definition) women (mean age: 58 y).

Daily Calcium-Intake
in Osteoporotic Women (Zurich 1 + 2 Da/Ne) n = 500
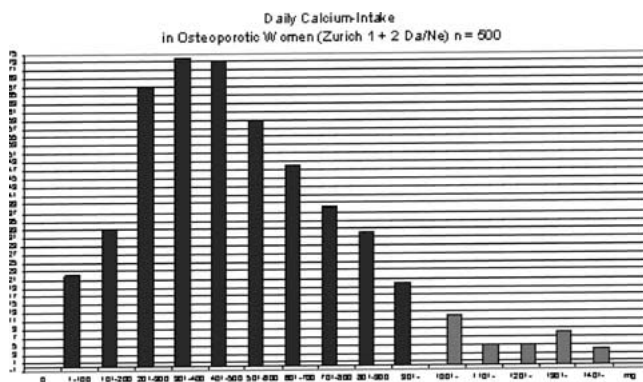
The Calcium intake was determined by direct inquiry and calculated according tables from Novartis and Roche.

None of these 500 women reached the recommended daily intake of (more than) 1'500 mg Calcium. The mean Calcium intake was only 590 mg (see graph).

At least in our (small big) town of Zürich a Calcium supplementation additional to e.g. Bisphosphonates or SERMS is ''mandatory'' to obtain the same positive results as in the extended international studies.

P136

EFFECT OF RISEDRONATE TREATMENT DISCONTINUATION ON BONE TURNOVER AND BMD

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!?twb = .28w? > Treatment with risedronate is safe and effective through 7 years. It is unknown how long the effects of treatment would persist after therapy is stopped. It might not be desirable to have prolonged suppression of bone turnover after discontinuation of bisphosphonate treatment. Patients in the present study received risedronate 5 mg daily (N = 398) or placebo (N = 361) for 3 years during the VERT-NA study followed by open-label one year extension with calcium 1000 mg/d and vitamin D, if baseline levels were low, while risedronate treatment was discontinued. Patients were assessed at the end of the 4th year. In the absence of risedronate treatment, urinary NTX increased significantly, from a median of 30.3 nMol/uMol creatinine at the end of 3 years of treatment ($P < 0.05$ vs placebo) to 50.9 nMol/uMol creatinine after 1 year off treatment (n.s. vs placebo). Bone alkaline phosphatase returned to pretreatment levels after stopping treatment and was no different from placebo. Lumbar spine BMD decreased by 0.9% in the year off treatment but remained 4.4% higher than baseline ($P <= 0.001$) and higher than placebo ($P < 0.001$). Similar results were seen at the femoral neck and trochanter.

In conclusion, the effects of 3 years of risedronate treatment on bone turnover show reversibility within 12 months after stopping treatment. BMD decreases, but remains higher than in placebo patients. Risedronate appears to differ from some other nitrogen-containing bisphosphonates both in early onset of effect (as shown previously) and in reversibility of effect (shown here) possibly due to differences in binding affinity to bone or in skeletal retention, or both. This reversibility of risedronate's effect may have relevance to long term bone safety as well as when other therapeutic options are considered.

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VERTEBRAL FRACTURE RISK REDUCTION IS INDEPENDENT OF THE MAGNITUDE OF BMD CHANGE

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The objective of this analysis was to explore the relationship between treatment-related change in BMD and fracture risk.

The analysis included patients from the three pivotal risedronate fracture endpoint trials (VERT-NA, VERT-MN and HIP) in postmenopausal women with osteoporosis. Women received risedronate (2.5 mg or 5 mg daily) or placebo daily for up to 3 years. All patients received calcium 1000 mg/day, and if baseline vitamin D levels were low, up to 500 IU vitamin D/day.

For patients taking risedronate, the 3-year fracture incidence was estimated for patients who did not show an increase in BMD from baseline (i.e. < 0%) and for patients who did show increases (0 to < median%, > = median%). Patients who had a post-baseline BMD measurement and a known vertebral fracture status during 0–3 years were included (N = 2047 for lumbar spine; N = 2255 for femoral neck).

The figures below show, that patients who experienced increases in BMD from baseline were at lower risk of fracture than those whose BMD declined. Importantly for patients with BMD increases, the fracture incidence was independent of the magnitude of the increase.

This analysis shows that larger increases in BMD do not translate into larger decreases in fracture incidence in patients on active therapy. It follows that BMD differences between established therapies cannot be interpreted as differences in fracture efficacy.

New Vertebral Fracture Incidence vs. Lumber Spine BMD % Change From Baseline

New Vertebral Fracture Incidence vs. Femoral Neck BMD % Change From Baseline
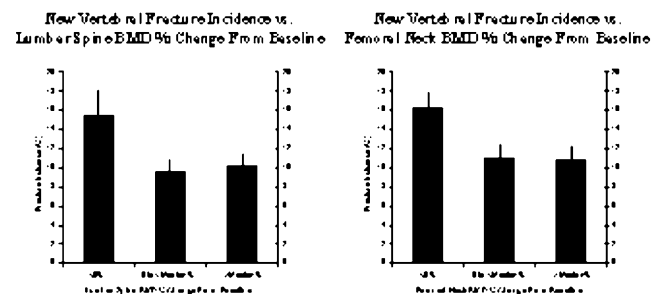
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FIVE YEARS OF TREATMENT WITH RISEDRONATE AND ITS EFFECTS ON BONE SAFETY IN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS.

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Recently, concerns have been raised about the long term safety due to oversuppression of bone turnover with some bisphosphonates1,2. We report the results of 5 year treatment with risedronate (Ris) on bone safety, showing a moderate suppression of bone turnover without negative effects. A 3-year double blind, placebo-controlled study that had significant decreases in fracture incidence in postmenopausal osteoporotic women was extended to 5 years for the bone biopsy subset. There were 42 placebo (mean age 69.2 yrs, femoral neck T-score −2.3) and 44 Ris women (mean age 69.5 yrs, femoral neck T-score −2.8) in the extension. We present biopsy data from 21 placebo and 27 Ris patients who were evaluable for histomorphometric and/or histological analyses at baseline and 5 years.

For Ris patients, histology showed normal lamellar bone without pathological findings. Bone formation and mineralization were normal as assessed by Osteoid Thickness, Mineral Apposition Rate, Mineralization Lag Time, and wall thickness. Bone turnover decreased in both treatment arms, with statistically significant changes in the Ris arm only. All Ris biopsies had double tetracycline labels indicating continuous turnover. Cortical thickness decreased in both groups, but the Ris group decrease was about 3-fold less than the placebo group.

Ostase and N-telepeptide/creatinine in the Ris group showed significant changes at month 60 of −33% and −47%, respectively. Lumbar spine BMD in the Ris group at month 60 increased by 9.2% compared to no significant change in the placebo group.

In conclusion, these unique data from paired biopsy samples show effective moderate and sustained decrease in bone turnover with normal bone formation after 5 years of risedronate treatment. These data demonstrate the long-term bone safety of risedronate.

Table:

	Placebo Baseline	Placebo 5 years	Risedronate Baseline	Risedronate 5 years
Osteoid Th. (µm)	8.6	10.1	8.9	9.4
Mineraliz. lag time (d)	28	27	19	31
Mineral. apo rate (µd)	0.55	0.61	0.53	0.58
Mineraliz. surface (%)	6.8	4.0	6.3	1.2*
Activation freq. (1/yr)	0.44	0.21	0.42	0.09*
Trabecular bone vol. (%)	19	19	17	15
Wall Th. (µm)	39.0	42.2	41.5	42.6
Cortical Th. (µm)	1321	915*	1017	885*

*$P < 0.05$ vs. baseline; data shown are mean values

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LONG-TERM TREATMENT WITH RISEDRONATE PRESERVES BONE MINERAL CRYSTALLINITY AND COLLAGEN CROSS-LINKS RATIO

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In this study we analyzed the effects of 5-yr treatment with risedronate on two parameters of bone quality, mineral crystallinity (crystallite size) and collagen cross-links ratio (pyridinoline/dehydro-dihydroxylysinonorleucine or pyr/deH-DHLNL) via Fourier transform infrared microscopic imaging (FTIRI). These parameters are indicators of the maturity/tissue age of the bone composite (pyr and deH-DHLNL cross links are abundant in mature and young collagen, respectively). Paired iliac crest biopsies were obtained from postmenopausal osteoporotic subjects at baseline and after 3-yr treatment with placebo (n = 8) or risedronate (5 mg/day po; n = 11). Biopsies were also obtained after 5-yr treatment with risedronate from 8 of these 11 subjects. Biopsies were embedded in methylmethacrylate, and the trabecular bone region was analyzed by FTIRI in ∼4 um thick sections. Spectroscopic images were obtained via a step-scanning FTIR spectrometer with an MCT array detector placed at an image focal plane of an IR microscope. Analysis was focused on trabeculae devoid of resorbing surfaces. Three images per section were acquired (each image 400 × 400 um[1]area or > 2000 pixels with a spatial resolution of 7 um). Mineral crystallinity and collagen cross-link ratio were compared before and after treatment.

Subjects treated with placebo had significant increases in both mineral crystallinity and collagen cross-link ratio, a pattern consistent with untreated osteoporosis and representing maturation of the bone matrix. In contrast, 5-yr treatment with risedronate preserved mineral crystallinity and collagen cross-link ratio of trabecular bone. This lack of an increase in both mineral crystallinity and collagen cross-link ratio coupled with increased BMD and preservation of microarchitecture suggests that risedronate suppresses osteoclastic activity relatively more than osteoblastic activity.

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PRESENCE OF OSTEOPOROSIS RISK FACTORS IN POSEMNOAPUSAL WOMEN ATTENDEN IN PRIMARY CARE CENTERS IN SPAIN COMPARISON OF 3 GROUPS:
50–55. 55–60 Y 60–65

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OBJETIVE: To compare the prevalence of osteoporosis risk factors (ORF) in a sample of postmenopausal women attended in primary care centers belonging to three age groups: 50–55 years old, 56–59 and 60–65.

DESIGN: This is an observational, descriptive, transversal study in which 97 Primary Care Centers in Spain and 5000 women have participated.

MEASUREMENTS: Demographic, anthropometrical and ORF data were collected. Quantitative bone ultrasound scan of the right-foot calcaneus and another one of the left-foot calcaneus were performed.

Qualitative variables are described by percentage and analysed by Chi square test or, if non-applicable, by Fisher exact test.

RESULTS: Data of 2985 women are available. They have been classified in three age groups for the analysis: A 1118 (50–55 years), B 837 (56–59 years) and C 1030 (60–65 years).

With the increasing age, in group C, there is a significant increase of risk in: falls suffered in the last year (29.32%), inability to stand up of a chair, height decrease (39.8%), personal fracture history (15.2%) and higher use of medication associated to osteoporosis risk (42.2%).

CONCLUSION: Even though we compared data between very close age groups, significant differences in the presence of osteoporosis risk factors were found in the group of 60–65 years.

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PREVENTION OF SUBSEQUENT OSTEOPOROTIC FRACTURES AFTER A HIP FRACTURE THE HORIZON RFT, STUDY DESIGN AND BASELINE DATA OF SUBJECTS

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Hip fractures represent the most serious consequence of osteoporosis and more than 1.7 million individuals in the world are affected annually. The risk of subsequent osteoporotic fractures after a hip fracture is dramatically increased, despite this fact no treatment for osteoporosis is offered to the vast majority of hip fracture patients. No current osteoporosis therapy has been shown to reduce subsequent fractures in this population.

A multinational, multicenter, randomized, placebo-controlled double blind study is currently ongoing in patients with a recent hip fracture. The primary aim of this study is to investigate if treatment with zoledronic acid (annual infusion of 5 mg over 15 minutes) compared to placebo will significantly reduce the incidence of clinical fractures in a population of men and women at high risk of fracture. Approximately 1714 subjects will be randomized to zoledronic acid or placebo. All patients will receive adequate vitamin D and calcium supplementation. Clinical fractures occurring after randomization will be centrally collected and adjudicated by a Clinical End-point Committee at Duke University. This is an event driven trial. It has been estimated that 211 clinical fractures will be required to show a 35% reduction relative to placebo with 90% power.

Subjects entering the study will be: Male or female aged 50 years or older, have had surgical repair of a low trauma hip fracture within 90 days; are ambulatory with or without assistive device and must have intact both lower appendages. Patients will be excluded: if a bisphosphonate was used within two years of randomization, calculated creatinine clearance < 30 ml/min, hypo- or hypercalcemia, any metabolic bone disease other than osteoporosis, active cancer, any prior use of PTH, sodium fluoride or strontium, and non-osteoporotic hip fractures.

These are the baseline characteristics of the first 301 randomized patients with hip fracture: A total of 220 female and 81 male subjects were recruited. The mean age is 77.6 years with a mean height of 163.8 cm and mean weight of 65.5 kg. 38.2% of the subjects had intertrochanteric and 32.2% had femoral neck, 20.6% had subcapital and 8.9% had other fractures. The vitamin D level (25 OH vitamin D) was measured in a subset of 312 consecutive patients and a total of 51% (n = 159) of subjects were vitamin D deficient (25 OH vitamin D below 15 ng/ml).

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EFFECT OF RALOXIFENE ON SERUM LEVELS OF OSTEOPROTEGERIN IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS: 3 MONTHS RESULTS

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INTRODUCTION: *In vitro* studies have suggested that the antiresorptive effect of raloxifene could be mediated by the changes in several cytokines involved in bone remodeling. Osteoprotegerin (OPG)-RANKL system is well recognized as essential in the regulation of osteoclastogenesis. However, the effect of raloxifene on the system are not well established in postmenopausal women. OBJECTIVE: to determine the effect of raloxifene (60 mg/day) on serum levels of OPG, bone markers and serum hormonal profile in postmenopausal women with osteoporosis.

SUBJECTS AND METHODS: We studied 48 postmenopausal women (mean age 63 ± 7), who had osteoporosis, defined as bone mineral density (BMD) T- score below −2.5 SD. Anthropometrics parameters, serum biochemical markers of bone turnover, sex hormone, serum levels of OPG (OPG ELISA BI-20402, BIOMEDICA-GRUPPE, Wien, Austria) and BMD by dual X-ray absorptiometry (DXA; Hologic QDR4500) at lumbar spine (LS) and femoral neck (FN) were measured at baseline visit and at 3 months.

RESULTS: Serum concentration of OPG had a significant decreased (−10.4%) during the first three months of raloxifene treatment (83.5 + 35.3 vs 73.4 + 32.8 pg/ml; P = 0.003). Also, we found a significant decreased of serum concentrations of alkaline phosphatase (86.6 + 41 vs. 66.9 + 17.6 UI/L; P = 0.001) and tartrate resistant acid phosphatase (3.1 + 0.4 vs. 2.7 + 0.6 UI/L; P = 0.002). We did not found significant changes in estradiol, testosterone, FSH, SHBG, DHEAS and PTH levels. Serum levels of LH increase significantly (26.1 + 14.2 vs. 28.3 + 14.7 mUI/L; P = 0.031).

CONCLUSION: Raloxifene treatment in postmenopausal women decrease serum levels of OPG. This effect could be associated with the antiresorptive effect on bone.

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OSTEOPOROSIS BY CALCANEUS ECOGRAPHY IN 2985 POSTMENOPAUSAL WOMEN BETWEEN 50 AND 65 YEARS OF AGE ATTENDED IN PRIMARY CARE CENTERS.

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OBJECTIVE: To describe the prevalence of osteoporosis in postmenopausal women between 50 and 65 years of age in a sample of population attended in primary care centers using calcaneus ecography

To evaluate the risk factors related to osteoporosis in those women.

DESIGN: This is an observational, descriptive, transversal study taken place in Spain, in which 5000 women have participated throughout 97 Primary Care Centers.

EASUREMENTS: Demographic, anthropometrical, osteoporosis and fractures risk factors were collected.

Quantitative bone ultrasound scan was made to both the right and the left foot calcareous using Sahara equipment.

Quantitative variables: mean and median values, typical deviation, first and third quartile, and range (maximum and minimum values) are analysed using ANOVA model.

Qualitative variables: frequency and percentage are analysed using Chi square test or, if non-applicable, by Fisher exact test. Confidence intervals of 95% are used.

T-score £−1.5 in any feet has been considered as osteoporosis criteria.

We used logistic regression analysis to determine the most important risk factors

RESULTS: Data of 2985 women are currently available.

34.5% of them showed T-score £−1.5.

Mean BUA value is 68.75, SOS 1542.09 and QUI/Stiffness 89.29.

The osteoporosis risk increases as age increases (C.I. 95% 1056–1098)

Osteoporosis is 2.12 in women with previous personal fractures and 1.42 in women with previous familiar fractures.

When the B.M.I. increases 1 point, the risk of osteoporosis decreases in 4.7% (0.937–0.970)

CONCLUSION: More than 1/3 of women participating in the study with an age between 50 and 65 suffer from osteoporosis according to the calcaneus ecography criteria of Sahara equipment.

In relation with risk factors, some should be considered with more importance than others. Special attention to these factors should be taken during the evaluation of these women.

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DESCRIPTIVE ANALYSIS OF OSTEOPOROSIS RISK FACTORS IN 2985 POSTMENOPAUSAL WOMEN FROM 50 TO 65 YEARS OLD ATTENDED IN PRIMARY CARE CENTERS

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OBJECTIVE: To describe the prevalence of osteoporosis risk factors in a sample of postmenopausal women from 50 to 65 years old attended in Primary Care Centers in Spain.

DESIGN: This is an observational, descriptive, transversal study.

5000 women have participated in the study. Data of 2985 are currently available–they have been distributed in three age groups: 1118 women 50–55 years, 837 women 56–59 years and 1030 women 60–65 years.

Demographic, anthropometrical, osteoporosis and fracture risk factors were collected. Quantitative bone ultrasound scan was made to both the right and the left foot calcareous using Sahara equipment.

Quantitative variables are described by the mean, median values and typical deviation, first and third quartile, and range (maximum and minimum values) are analysed using ANOVA model.

Qualitative variables are described by frequency and percentage and analysed by Chi square test or, if non-applicable, by Fisher exact test. Confidence intervals of 95% are used.

RESULTS: Amongst the analysed risk-factors 14.8% of women have had surgical menopause, mean BMI is 28.4, height increase has been reported by 32.4% of the patients, 9.2% are unable to stand up from a chair, 79.8% have never smoked, 10.65% have a history of fractures: forearm is the most frequent one. Decrease of height is 32.6%.

Usage of Calcium of less than 600 mg/day is 43%. Benzodiazepines are the most commonly used drugs in 25.2%. History of familiar fractures is 23.4%

CONCLUSIONS: The evaluation of FR in Primary Care centers can be of great importance in the early diagnose of OP and in the identification of women with high risk with DEXA indication.

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MORPHOMETRIC CHARACTERIZATION OF BONE SPECIMENS: MICROCT VS. HISTOLOGY

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Histology has been over years the common method for the morphometric characterization of bone specimens. With this technique bone biopsies are included in PolyMethaMethylAcrylate (PMMA), sectioned to thin slices, stained and then observed at the microscope. The morphometric parameters (e.g. Bone Volume (BV), Tissue Volume (TV), Bone Surface (BS), Trabecular Thickness (Tb.Th), Trabecular Spacing (Tb.Sp), etc...) are extracted to characterize the examined bone. In the last ten years a new examination technique, X-ray microtomography (microCT), came up. It permits the non destructive examination of bone specimens. This procedure is faster than histology, does not require laborious preparation as the embedding in PMMA, preserves the integrity of the examined bone sample, permits the visualisation of virtual sections of the whole specimen (while in histology some slices can be lost by the mechanical cutting), permits the calculation of the morphometric parameters over the whole sample's volume, the creation and visualisation of a 3D-model also to be used for Finite Element Analysis (FEA). In this work a quantitative comparison of these two techniques is presented. An amount of 19 bone biopsies taken from the human femoral neck of different patients have been examined by microCT (model 1072, Skyscan, Belgium) and by histology. From the single cross section images obtained by microCT and their corresponding slices obtained by histology the morphometric parameters (BV/TV, BS/TV, Tb.Th, Tb.N, Tb.Sp) were calculated and then compared. The correspondences in the outcomes were excellent. The parameters calculated by the two techniques were highly correlated ($r > 0.97$ for all parameters). The paired t-test showed for BV/TV no statistically significant differences ($P = 0.995$) between microCT and histology. MicroCT underestimated the parameters BS/TV and Tb.N by 6.9% ($P < 0.05$), while it overestimated Tb.Th by 9.4% ($P < 0.05$) and Tb.Sp by 6.9% ($P < 0.05$). Although microCT presents small systematic differences when compared to histology, it has the advantage of being a non destructive, fast, three dimensional examination technique. These results confirm that microCT permits a reliable morphometric characterization of bone specimens.

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COMPARATIVE EVALUATION OF MARKERS OF BONE TURNOVER IN HEALTHY MEN, PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN

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The interest for bone markers is increasing for the management of several bone diseases. However, comparative evaluations between the available markers in large series of untreated healthy subjects are scanty. In this context, we collected serum and fasting second void urine specimens in a total of 380 healthy individuals, divided into three groups: 141 men (median age 49 years, range 22–65), 130 premenopausal women (PreMP; median age 40 years, range 21–53) and 109 postmenopausal women not taking HRT (PostMP; median age 57 years, range 40–82). We measured the urinary excretion of crosslinks (PYD, DPD) by HPLC using the new assay of Bio-Rad calibrated against new CDC reference material, the telopeptide NTx (Ostex) and the serum concentrations of total Alk Phos, of its bone isoenzyme (BAP, Hybritech) and of intact osteocalcin (BGP, Biosource). The upper limits of the normal range (97.5 th percentiles) and the comparative means ± SD are tabulated below. Values were significantly ($P < 0.0001$) different in the three groups for all markers. There were significant correlations between the six markers (Spearman rank correlations: $r = 0.186$ −0.871; $P < 0.01$). We compared the percentages of increased values («sensitivity») of these markers in PostMP as compared with the upper limits of normal in PreMP women. PYD and DPD were more often elevated (36.5 and 40.0%, respectively) than NTx (21.5%) ($P < 0.01$). On the other hand, BGP was more often elevated than Alk Phos and BAP: (27.7%, 20.4%, and 14.6%, respectively; $P < 0.01$). These data indicate that crosslinks are more often elevated than NTX, and BGP more often than BAP in PostMP women as compared to PreMP women.

Table:

Parameters	Men	PreMP	PostMP
PYD, nM/mM Cr	61 (31 ± 13)	47 (25 ± 11)	66 (40 ± 14)
DPD, nM/mM Cr	16 (5 ± 3)	10 (5 ± 3)	15 (9 ± 4)
Ntx, ECO/mM C	97 (38 ± 21)	90 (47 ± 21)	148 (70 ± 34)
Alk Phos, mU/	105 (69 ± 21)	104 (60 ± 21)	126 (78 ± 26)
BAP, µg/ml	18 (11 ± 3)	14 (7 ± 3)	19 (10 ± 4)
BGP, ng/ml	17 (10 ± 3)	15 (8 ± 4)	24 (12 ± 7)

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EFFECT OF RALOXIFENE ON BONE MINERAL DENSITY AND MARKERS OF BONE REMODELING FOLLOWING ALENDRONATE THERAPY–TWO YEAR FOLLOW-UP

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Aim: To compare the BMD and marker effects of two-year treatment with raloxifene (R) or placebo (P) following alendronate (A) therapy versus continuous treatment with A.

Methods: 99 women with PM osteoporosis (65 ± 7 yr, femoral neck NHANES T, −2.8 ± 0.8) who were treated with A (10 mg/d) for 43 ± 7 months were randomized to double-blinded R 60 mg/day (N = 33), P (N = 33) or continuing open label A (N = 33) for another 12 months. On the second year, all patients were treated open label. All patients received Ca (500 mg/day) and vitamin D (800 IU/day). BMD was assessed using Hologic Delphi. N-propeptide of type I collagen (S-PINP, Orion Diagnostica), and type 1 collagen C telopeptide (S-CTX, Elecsys, Roche) were measured in the serum.

Results: No baseline differences were observed between the groups. Continuing A or switching to R results in similar BMD changes. S-PINP increased significantly at year 1 and 2 after discontinuation of A treatment in both P and R group. S-CTX increased significantly only in the P group. Both markers remained suppressed in the A group.

Conclusion: R is superior to P in preventing decrease in BMD at the lumbar spine and increasing BMD in the total body after discontinuation of long-term A treatment. This can be explained by a prevalence of bone formation over bone resorption in the R treated patients.
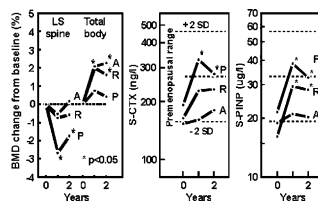
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SUBSEQUENT FRACTURE AFTER VERTEBROPLASTY

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Vertebroplasty procedure has often been shown to decrease pain related to acute vertebral fracture and osteoporosis. We present a case series of six patients who had been treated with vertebroplasty for acute osteoporotic compression fracture with subsequent significant pain relief in most cases. These patients were referred to Rehabilitation of Osteoporosis Program-Exercise (ROPE) for management of their recurrent pain that had initiated within one to three months after the vertebroplasty procedure. Similarities among these patients included new vertebral fracture and pain within 1 to 3 months of the vertebroplasty, subsequent fracture(s) occurred without significant mechanical stress/strain, and all but one of the new vertebral fractures occurred above the vertebroplasty site.

The patients who developed subsequent fractures demonstrated common characteristics which can guide their post-fracture management. Review of the literature finds that new fractures after vertebroplasty can occur in a significant number of patients. Because many of these fractures occurred within a few weeks after the initial vertebroplasty, instruction in exercise and lifestyle modification should be initiated as soon as possible after the initial compression fracture. An emphasis on stretches and proper biomechanics in activities of daily living in the initial portion of their training program may be more helpful since the effect of strengthening exercises will take at least 8 weeks to be detectable.

Patients may have a false sense of confidence regarding their risk of further complications because their bone has been cemented. The adage "an unbreakable toy is useful for breaking other toys" often provides them with an epiphany regarding this risk.

We conclude that patients who undergo vertebroplasty are at risk for developing subsequent vertebral compression fractures. This has important considerations when implementing preventive measures and educating patients and providers about prognosis and management.

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RALOXIFENE TREATMENT IMPROVES THE MECHANICAL STRENGTH AND STABILITY OF THE PROXIMAL FEMUR: RESULTS FROM THE MULTIPLE OUTCOMES OF RALOXIFENE EVALUATION (MORE) TRIAL

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Raloxifene treatment increases lumbar spine and femoral neck bone mineral density (BMD) and reduces the risk of vertebral fractures in postmenopausal women with osteoporosis. In this study our objective was to determine the effect of raloxifene treatment on the structural geometry of the proximal femur. A subset of postmenopausal women with osteoporosis enrolled in the MORE trial (n = 1903, mean age 67 ± 7 years) was randomized to raloxifene 60 mg/d (n = 632, RLX60), raloxifene 120 mg/d (n = 648, RLX120) or placebo (n = 623). Study participants received hip dual energy x-ray absorptiometry (DXA) scans at baseline and 1, 2 and 3 year time points. The DXA scans were analyzed with a Hip Structure Analysis (HSA) program that measured BMD and geometric properties of the proximal femur traversing regions of the femoral neck, intertrochanter and proximal femoral shaft. Mean response to treatment (area under curve of repeated measurements) was calculated as a summary measure and used a dependent variable in analysis of covariance with the baseline value as a covariate. Mean differences following three-year treatment with raloxifene, compared to placebo, are presented in the table below. Following treatment with raloxifene, BMD increased and the buckling ratio (an index of structural instability) decreased at all regions, compared to placebo. Bone cross sectional area and section modulus (axial and bending strengths, respectively) increased at the narrow-neck and intertrochanter for both RLX60 and RLX120, but femoral shaft strength showed significant improvement only in the RLX120 group. There were no differences in bone outer diameter between groups, suggesting the rate of periosteal apposition following raloxifene treatment cannot be detected by the HSA technique. Raloxifene treatment improves indices of mechanical strength and stability at all regions of the proximal femur with no apparent dose effect except at the femoral shaft where osteoporotic fragility fractures are rare.

Mean Percent Change from Placebo

*P < 0.002	Femoral Neck RLX60	RLX 120	Intertrochanter RLX60	RLX 120	Femoral Shaft RLX60	RLX120
BMD	2.2%*	2.2%*	1.7%*	1.9%*	1.1%*	1.4%*
Cross Sectional Area	2.2%*	2.3%*	1.7%*	1.8%*	0.7%	1.5%*
Outer Diameter	0.0%	0.1%	0.0%	0.0%	−0.5%	0.0%
Section modulus	2.1%*	2.2%*	1.3%*	1.7%*	−0.1%	1.1%*
Buckling ratio	−2.5%*	−2.4%*	−2.3%*	−2.1%*	−1.6%*	−1.4%*

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RALOXIFENE SKELETAL EFFECTS ARE IMPROVED WITH WEEKLY TERIPARATIDE [RHPTH (1–34)] IN OSTEOPENIC OVARIACTOMIZED RATS

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We evaluated whether combining daily raloxifene (RLX) with weekly teriparatide (TPTD) treatment provided additional skeletal benefits beyond either treatment alone. 6-month-old Sprague Dawley rats were ovariectomized, except for sham-ovariectomy controls (Sham), and permitted to lose bone for 1 month before treatment with agents for the following 3 months. Groups included Sham, ovariectomized controls (Ovx), and ovariectomized animals treated with RLX 1 mg/kg/d, TPTD 10 or 30 ug/kg once a week or RLX + TPTD combinations. Micro-CT, biomechanical failure testing, and histomorphometry were used to analyze the skeletal effects. In lumber vertebrae, bone mineral content (BMC, −16%), bone mineral density (BMD, −15%), peak load (−25%), and energy to break (−36%) were significantly reduced in Ovx relative to age matched Sham controls. RLX vertebrae had BMD, peak load, and energy to break that

were intermediate between Sham and Ovx, while weekly TPTD had no effect relative to Ovx. Daily RLX plus weekly TPTD improved vertebral BMD over RLX alone to Sham levels. Vertebral BMC, peak load, and energy to break for the combination tended to be higher than RLX alone, with RLX + TPTD30 tending to be more efficacious than RLX + TPTD10. Analysis of cortical bone showed no differences between groups, except for the femoral midshaft of RLX + TPTD10, which was significantly stronger than Ovx. Dynamic histomorphometry of the proximal tibial metaphysis showed reduced eroded surface for RLX + TPTD compared to Ovx, similar to RLX alone, but with enhanced mineral apposition rate for RLX + TPTD30 relative to RLX alone. Therefore, the skeletal efficacy of daily RLX was improved by combining RLX with weekly TPTD in osteopenic ovariactomized rats.

P151

STRONTIUM RANELATE REDUCES THE RISK OF VERTEBRAL FRACTURES IN OSTEOPOROTIC POSTMENOPAUSAL WOMEN WITHOUT PREVALENT VERTEBRAL FRACTURE

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Strontium ranelate is a new anti-osteoporotic agent having demonstrated its efficacy on both vertebral and non vertebral fractures: Two large phase III randomized, double blind, placebo controlled clinical trials, SOTI (1649 patients with prevalent vertebral fracture and low lumbar BMD) and TROPOS (5091 patients aged above 70 years and low femoral neck BMD) were conducted to assess the efficacy of strontium ranelate in reducing the risk of osteoporotic fractures in postmenopausal osteoporotic women. It has been shown that strontium ranelate significantly reduces the risk of vertebral fracture (SOTI study) and non vertebral fractures including hip fractures (TROPOS study).

A pre-planned meta analysis was performed on the pooled data from SOTI and TROPOS trials. Among the whole population of these studies, 2605 osteoporotic postmenopausal women without prevalent vertebral fracture (VF) were included and received strontium ranelate 2 g/day orally (n = 1285) or placebo (n = 1320) plus a Calcium/Vitamin D supplementation in both groups during 3 years. Vertebral X-rays were performed yearly (semi-quantitative assessment).

No statistical differences between groups were detected for main baseline characteristics: mean age (SD): 75(5) years; time since menopause: 26(7) years; mean(SD) Lumbar T-score: −2.70(1.53); mean(SD), Femoral Neck T-score: −2.97(0.56).

A significant reduction in the incidence of patients experiencing a VF was demonstrated in the intent-to-treat population over 3 years with a reduction of the relative risk by 48% (95%CI [0.40 ; 0.67], P < 0.001). 87 patients in strontium ranelate group and 161 patients in placebo experienced a vertebral fracture during the study.

Strontium ranelate has already demonstrated its efficacy in reducing the risk of VF (41%) in patients with prevalent VF in SOTI study. The present analysis confirms its antifracture efficacy (reduction of the risk of 48%) in postmenopausal osteoporotic women without prevalent VF. Strontium ranelate is well-tolerated.

Strontium ranelate is a new anti-osteoporotic agent effective in reducing the risk of vertebral fracture in post-menopausal women with or without prevalent vertebral fracture.

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PATIENTS AT HIGH RISK OF HIP FRACTURE BENEFIT FROM TREATMENT WITH STRONTIUM RANELATE

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The lifetime risk of a hip fracture from age 50 years has been estimated at 17% for Caucasian women and the incidence rises exponentially in women over 74 years old.

Strontium ranelate has been shown to significantly reduce the risk of vertebral fracture in women with established post-menopausal osteoporosis by 41% (SOTI study) and by 45% in those patients without prevalent vertebral fracture (TROPOS study) over 3 years.

The international TROPOS study was designed to evaluate the efficacy of strontium ranelate in reducing the risk of non-vertebral fractures. A total of 5091 patients aged above 70 years, with a low femoral neck BMD (T-score < -2.5 SD) were randomized to receive strontium ranelate 2 g daily orally over 3 years. A significant reduction of 16% ($P = 0.04$) and 19% ($P = 0.031$) respectively in the relative risk of non-vertebral osteoporotic fracture and major osteoporosis-related fracture was demonstrated in the intention to treat population. The efficacy of strontium ranelate in reducing the risk of hip fracture was investigated in a subset of particular medical interest, namely women of 74 years and above and with a baseline femoral neck BMD T-score lower or equal to −3 (calculated according to the centralized normative data). A total of 1977 patients are represented in this subset: 982 patients in the strontium ranelate group and 995 patients in the placebo group. The main baseline characteristics of this subset were similar between treatment and control groups and were as follows: mean (SD) age of 79.6 (4.5) years; menopause duration of 31.5 (7.0) years; femoral neck BMD T-score of −3.6 (0.5).

In ITT, over 3 years, a significant reduction of 36% in the relative risk of hip fracture risk was observed (RR = 0.64, 95%CI [0.412;0.997]; $P = 0.046$).

These results demonstrate that strontium ranelate is a new and innovative anti-osteoporotic treatment which is effective in reducing hip fracture in high risk, osteoporotic postmenopausal women.

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STRONTIUM RANELATE REDUCES THE RISK OF VERTEBRAL FRACTURES IN POSTMENOPAUSAL WOMEN WITH OSTEOPENIA.

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Strontium ranelate 2 g/day is an orally active anti-osteoporotic agent which reduces over 3 years the risk of vertebral fractures by 41%, non-vertebral fractures by 16% and hip fractures by 41% in postmenopausal women with osteoporosis based on results of a phase III program including 2 international randomised, double blind, placebo controlled clinical studies: SOTI (1649 patients with low lumbar BMD and having at least one prevalent vertebral fracture) and TROPOS (5091 patients with low femoral neck BMD).

An analysis was performed on the pooled data from SOTI and TROPOS studies. Amongst the whole population, 409 patients with lumbar and/or femoral neck T-score between −1 and −2.5 and both T-scores > −2.5, with or without prevalent fractures were included and received strontium ranelate 2 g/d orally or placebo for 3 years, associated to calcium and vitamin D supplementation according to the patient's status. Vertebral X-rays were performed yearly.

No relevant differences between groups were detected for the main baseline characteristics: mean(SD) age: 73(6) years; time since menopause: 25(8) years; mean(SD) Lumbar T-score: −1.20(1.15); mean(SD) Femoral Neck T-score: −2.06(0.44).

In the intent-to-treat population, strontium ranelate was associated with a 62% reduction in the relative risk of vertebral fracture over 3 years (as assessed through semi-quantitative method by a central reading centre) (RR = 0.38, 95%CI[0.21;0.70], $P = 0.001$).

Amongst the 409 described patients, 43% of patients presented an osteopenia according to their BMD values (described above) and had no prevalent fracture. In this subgroup, strontium ranelate reduced the risk of vertebral fracture by 72% over 3 years (RR = 0.28; 95% CI [0.07; 0.99]) ($P = 0.045$).

We infer that strontium ranelate, a new anti-osteoporotic agent, reduces the risk of vertebral fractures in women with osteopenic range of BMD by 62% and in women with osteopenia without any prevalent fracture by 72%.

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SYSTEMATIC EDUCATION OF PATIENTS INCREASES KNOWLEDE ON OSTEOPOROSIS—INTERIM ANALYSIS FROM A RANDOMIZED PROSPECTIVE TRIAL

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Pharmacological treatment of osteoporosis with HRT, bisphosphonates, raloxifen, and PTH reduce the incidence of fractures significantly. Also, lifestyle modification and fall prevention programs have significant positive effects. Unfortunately, compliance with pharmacological therapy is low in clinical practice and seems inversely related with educational level.

Aim: We hypothesized that patients knowledge on osteoporosis may be increased by systematic education.

Participants and design: One-hundred-and-nineteen patients aged 48 to 81 years who were recently diagnosed with osteoporosis and started on specific treatment were randomized to either the ''school'' or ''control'' group. In the school-group, patients attended four classes with 6–10 participants during four weeks. Teaching was performed by nurses, physiotherapist, dieticians, and doctors and the classes covered ''facts on osteoporosis'', ''fractures and pain'', ''diet'', ''preventive measures'', ''balance and exercise'', and ''medical treatment''. Teaching was designed to increase the patient's empowerment. In this interim analysis, the patient's knowledge on osteoporosis was tested at study entry and at 3 months using a recently developed and validated questionnaire yielding a score between 0 and 28 points (ref).

Results: At study entry, no differences in age or score (23[3–27] versus 22[8–28] points) were seen between the school and control groups. The change in knowledge during the study, however, differed significantly between the two groups ($P < 0.05$). In the school group the score increased (2 [−8 to 14] points, $P < 0.001$) while no change (0 [−7 to 11] points, n.s.) were seen in the control group.

Conclusion: Systematic education of patients with osteoporosis significantly increases their knowledge on the disease. It remains to be demonstrated whether increased knowledge translates into increased compliance with treatment or appropriate lifestyle modifications.

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IMPLANT INTEGRATION IS IMPROVED BY IBANDRONATE IN OSTEOPENIC RATS

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Aims: Use of cementless and hybrid total joint replacement is increasing. Effective osseointegration of these implants is required for early secondary stabilisation. There is a need for agents that can enhance this process, especially in patients with elevated bone turnover in whom osseointegration may be impaired. Bisphosphonates normalise the high rate of bone resorption commonly associated with osteoporosis, while inhibiting bone formation to a lesser extent, leading to a net gain in bone mass. We investigated the effects of ibandronate, a potent, nitrogen-containing bisphosphonate, on the osseointegration of titanium-only and hydroxyapatite (HA)-coated implants in ovariectomised (OVX) rats. HA-coated implants are osseoconductive, supporting the ingrowth of capillaries, perivascular tissues and osteoprogenitor cells into the implant structure.

Methods: Three months after ovariectomy or sham operation, 84 rats received titanium-only or hydroxyapatite (HA)-coated titanium femoral implants. OVX animals were then randomly assigned to 4 weeks treatment with daily subcutaneous (s.c.) saline (OVX control) or ibandronate (1 mcg/kg or 25 mcg/kg) injections. These ibandronate doses were calculated to be analogous to those used to treat osteoporosis or metastatic bone disease, respectively, in humans. The sham-operated group received saline (sham control).

Results: S.c. ibandronate injections increased lumbar spine BMD relative to the OVX control animals and to a level similar to that observed in the sham-

operated group. In the animals that received titanium-only implants, osseointegration (determined by histomorphometric analysis and expressed as a percentage of osseointegration surface [OIS]) did not differ between treatment groups. However, in the OVX animals that received HA-coated implants, 1 mcg/kg and 25 mcg/kg ibandronate increased OIS by 113.5% and 185%, respectively, relative to OVX control animals. Moreover, OIS was 56.5% lower in the OVX control group than in the sham control group.

Conclusions: These findings indicate that OVX-induced bone loss impairs osseointegration of HA-coated titanium implants. Ibandronate is able to reverse these effects and increase osseointegration to a level similar to that observed in non-OVX animals. These results suggest a role for ibandronate in enhancing the osseointegration of HA-coated implants in patients with elevated bone turnover undergoing joint replacement surgery.

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ONCE-MONTHLY ORAL IBANDRONATE A NEW BISPHOSPHONATE DOSING CONCEPT

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Oral daily or weekly bisphosphonates may be inconvenient for some patients, potentially impairing adherence to therapy and therapeutic outcomes. Less frequent oral regimens are likely to enhance patient acceptability. Ibandronate is a potent, nitrogen-containing bisphosphonate with proven fracture efficacy when given daily or intermittently (between-dose interval >2 months). A randomised, double-blind, placebo-controlled, phase I, dose-finding study (Monthly Oral Pilot Study: MOPS) explored the safety, pharmacodynamics and pharmacokinetics of once-monthly oral ibandronate. A total of 144 postmenopausal women received 3 cycles of oral monthly placebo, 50 mg, 50 mg (first cycle) then 100 mg (remaining cycles), 100 mg or 150 mg ibandronate. Additional calcium and vitamin D supplements were not administered. Oral monthly ibandronate was well tolerated, with a safety profile similar to placebo. Once-monthly ibandronate was highly effective in decreasing bone resorption. Versus baseline, dose-dependent and substantial decreases in sCTX and uCTX were observed after 3 months (30 days after final dose): −12.3% and −5.5% for placebo and −56.7% and −54.1% for 150 mg ibandronate, respectively. Dose-related increases in exposure (AUC and Cmax) to ibandronate were also reported. A randomised, double-blind, phase III, non-inferiority study (Monthly Oral iBandronate In LadiEs: MOBILE) is ongoing to establish the efficacy and safety of oral monthly ibandronate in 1,600 women (aged 55–80 years; menopausal for at least 5 years) with osteoporosis (spinal BMD T-score <−2.5 and >−5.0). Participants are receiving 2.5 mg oral daily ibandronate, 100 mg oral monthly ibandronate (as 50 mg doses on two consecutive days), 100 mg or 150 mg oral monthly ibandronate (on a single day) for 2 years. All patients are taking daily calcium (500–1500 mg) and vitamin D (400IU) supplements. Adverse events, including clinical vertebral and non-vertebral fractures, are being continuously monitored. The primary endpoint is lumbar spine BMD change at 1 year. Fracture efficacy will be concluded if the monthly regimens show non-inferiority to the proven daily regimen for this endpoint. Oral monthly ibandronate is expected to provide an optimal combination of efficacy, tolerability and patient convenience, leading to improved patient outcomes in postmenopausal osteoporosis.

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KUOPIO OSTEOPOROSIS STUDY - FRACTURE PREVENTION STUDY (OSTPRE-FPS)

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Kuopio Osteoporosis Study - Fracture Prevention study (OSTPRE-FPS)
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A new population based study of Kuopio University, Bone and Cartilage Research Unit (BCRU), investigates in a 3-year randomized study, if Calcium and Vitamin D supplementation decrease fractures and falls in elderly women. The study population is based on the population of Kuopio Osteoporosis Study, which included all the women born in 1932–41 (n = 14 220) in Kuopio Province. In autumn 2002, a postal enquiry was sent to 5407 women (>65 years), to ask willingness to participate in a prospective fracture prevention study. In all, 4189 questionnaires came back and 77.2% were willing. Three thousand voluntary women were randomised into two groups of equal size (n = 1500 + 1500). During February-April 2003 the women in the supplementation group (n = 1500) were given Vitamin D 800 IU + Calcium 1000 mg (Calcichew D3 Forte® 1 tablet twice a day, Leiras–Nycomed, Finland), and the women in the control group (n = 1500) were not supplemented. All women were asked to continue their previous lifestyle and other medication (including osteoporosis therapy).

The supplementation has been delivered and accounted by local pharmacies. A subgroup of 600 women will be intensively followed up at BCRU, where the women will be examined twice, at the beginning and at the end of the 3-year trial. By now (December 2003), 468 women have visited BCRU and several measurements have been performed: BMD of total body, lumbar spine and femoral neck (DXA) and calcaneus (pDXA and QUS), balance tests, muscle strength, quality of life (WHQ, Qualeffo, EUROQUOL), food and physical activity diaries, laboratory tests (vitamin D and calcium-levels, bone biochemical markers, PTH, DNA). Phone interviews and postal enquiries will be performed to get information about falls and fractures during the study. The results will be used to make recommendations how to prevent falls and fractures in elderly people and the acceptance and benefits of Calcium and Vitamin D supplementation as a population scale intervention will be studied.

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ANTIFRACTURE EFFICACY OF ORAL DAILY AND INTERMITTENT IBANDRONATE IS SUPPORTED BY THE RIGOROUS METHODOLOGY USED TO DIAGNOSE FRACTURE

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Aims: A significant reduction in the risk of new vertebral fractures was reported with oral daily (62%) and intermittent ibandronate (50%) in the BONE study (oral iBandronate Osteoporosis vertebral fracture trial in North America and Europe). As the study was performed in 73 centres in Europe and North America, standardisation of X-ray technique and reproducibility of fracture diagnosis was considered essential. To enhance the already high-quality protocol, a system for cross-validating fracture diagnoses was implemented.

Methods: X-rays were read and diagnoses made at single centres in Europe and North America. Although morphometric criteria were used to establish prevalent and new incident fractures, qualitative confirmation, including differential diagnosis of deformation, by an expert radiologist was also required. To ensure between-centre consistency of diagnosis, the European centre reviewed all North American films (qualitative assessment only), blind to the original North American diagnoses. The North American centre then re-examined the films and submitted its final diagnoses.

Results: Agreement between morphometric and qualitative diagnoses for incident vertebral fractures was excellent: of the 995 film sets reviewed by both centres, only two discrepancies were found in final diagnosis (kappa coefficient: 0.97; 95% CI, 0.91, 1.0). Final morphometric diagnoses were also consistent between the centres: there were discrepancies in only four patients (kappa coefficient: 0.94; 95% CI, 0.88, 1.0). This finding corresponds to a potential difference of two fractures in the trial results. The use of single baseline X-rays for assessments of prevalent fractures, rather than the series used for incident fractures, made diagnosis more complex. For this reason, and also differences in deformation aetiology (e.g. degenerative, traumatic, etc), 21% (359/1,715) of the prevalent fractures identified by morphometric means did not fulfil the criteria for osteoporotic fracture (kappa coefficient: 0.87; 95% CI 0.85, 0.88). Despite this finding, agreement between centres on final diagnoses of prevalent osteoporotic fractures was achieved in all but six patients (8%).

Conclusions: Successful between-centre standardisation of fracture diagnosis provides strong support for the methodology used in BONE and associated fracture outcomes.

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EFFECT OF BISPHOSPHONATE THERAPY ON SERUM OSTEOPROTEGERIN LEVELS IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS

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Bisphophonates are analogs of pyrophosphate with a potent inhibitory effect on bone resorption. They are taken up by osteoclasts and inhibit farnesyl diphosphate synthase, which is essential for osteoclast activity and survival. In addition to the direct effects of bisphosphonates on osteoclasts, there are evidences that these compounds also act on the osteoclasts indirectly through the osteoblasts. In bone remodeling process, RANKL is essential for osteoclasts formation and activation, whereas osteoprotegerin (OPG) neutralizes RANKL. Although OPG is capable of inhibiting bone resorption *in vivo*, whether bisphosphonates regulate the production of OPG has remained unclear. In this study, we assessed the effects of the risedronate therapy on serum OPG levels in randomized prospective trial. We also investigated the associations of serum OPG levels with various indices of bone metabolism. 111 women (65.8 ± 3.7 years) with postmenopausal osteoporosis were treated with placebo or risedronate (5 mg daily) for 6 months and all received 1000 mg of calcium and 250 IU of vitamin D. Bone mineral density of lumbar spine were measured before, and 6 months after the treatment. The biochemical markers of bone turnover (sCTx, NTx/Cr and sALP) were measured before the treatment and 1, 3 and 6 months after the treatment. The serum OPG levels were measured before, 1 and 6 months after the treatment. There were negative correlations between the baseline OPG levels and the baseline sALP or sCTx levels($r = -0.22$, $P < 0.05$; $r = -0.21$, $P < 0.05$). A significant correlation was also found between the OPG and lumbar spine bone mineral density at baseline($r = 0.26$, $P < 0.05$). The women treated risedronate had significant increases in bone mineral density at lumbar spine. Risedronate reduced biochemical markers of bone resorption and bone formation by approximately 50%. Serum OPG levels were not changed during the treatment period in both risedronate and placebo group. However, in subgroup who showed higher OPG levels initially, OPG levels significantly decreased after risedronate therapy. In conclusion, bisphophonate therapy cause low bone turnover state and this may lead to downregulation of OPG.

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DRUG–DRUG INTERACTIONS WITH IBANDRONATE ARE UNLIKELY

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Aims: Patients receiving ibandronate for osteoporosis or metastatic bone disease often take concomitant medications. Thus, the potential for drug-drug interactions is an important consideration.

Methods: The pharmacology and pharmacokinetics (PK) of ibandronate have been extensively studied in animals and human subjects. Protein binding, renal excretion and effects on cytochrome P450 (CYP) activity have been examined *in vitro* and *in vivo* in preclinical studies. Clinical PK studies in human volunteers and patients have also explored the potential for drug-drug interactions with common concomitant medications.

Results and discussion: The level of protein binding, a factor known to influence drug-drug interactions, of ibandronate, at the range of concentrations observed in human serum, is relatively low (86%). In rats, renal clearance of ibandronate and glomerular filtration rate was not affected by high doses of classical anionic and cationic inhibitors of renal transport systems. These findings suggest that these systems have minimal, if any, involvement in the secretion of ibandronate and that the potential for interactions with drugs excreted by these systems is low. In rats, no induction of CYP activity in the liver was observed. In addition, no affinity for human liver CYP isoenzymes (1A2, 2A6, 2C9, 2C19, 2D6, 2D1 or 3A4) was reported for ibandronate at concentrations of up to 100 μM (360 μg/ml) in human liver microsomes. Thus, metabolic drug-drug interactions through inhibition of CYP activity are unlikely. When administered concomitantly with drugs commonly taken by postmenopausal women, women with breast cancer and women with multiple myeloma (hormone replacement therapy, tamoxifen, and melphalan or prednisolone, respectively), no, or only minor, changes in PK parameters were observed. All changes were considered clinically irrelevant. When taken with ranitidine, a slight increase in the AUC (20%) of ibandronate was noted. This change was attributable to an increase in gastric pH and was not considered clinically relevant. As with other bisphosphonates, multivalent cations are likely to produce drug-drug interactions at the absorption level, hence the rigorous fasting requirements with bisphosphonate therapy.

Conclusions: These data demonstrate that drug-drug interactions with ibandronate are unlikely, suggesting that ibandronate is suitable for use in patients receiving multiple concomitant medications.

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ALENDRONATE REDUCES FRACTURE RISK IN LOWEST BONE TURNOVER GROUP

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Alendronate reduces the rate of bone turnover to within the premenopausal range, increases BMD, and reduces the risk of both vertebral and nonvertebral fractures. We compared the incidence of fractures among alendronate (ALN) treated women with the lowest bone turnover to other women who received alendronate in the Fracture Intervention Trial. Women with femoral neck T-scores £−1.6 were randomized to alendronate or placebo for 3 years (women with prior vertebral fracture) or 4–4.5 years (no prior vertebral fracture). We used a per-protocol analysis of the turnover marker, bone-specific alkaline phosphatase (BSAP), at 12 months, because the full effect of ALN on turnover markers occurs within a few months and is subsequently maintained. The number and % incidence (95% CI) of women with new nonspine fractures after month 12 (and new vertebral fractures at any time) was calculated separately for women in the lowest 10% of BSAP values in the alendronate group (BSAP < 5.5 ng/mL) and in the placebo group (BSAP < 7.9), and compared to all other women in each treatment arm (Table). The sample size in the low turnover group is small; hence, statistical tests were not performed. The fracture incidence in the 10% of ALN-women with the lowest BSAP was similar or lower than that in other women. Thus, there is no evidence of an increase in risk among women with the lowest BSAP levels, suggesting that bone quality is not impaired during ALN treatment, including women with the lowest bone turnover.

Table: Fracture Incidence by Marker Level and Treatment Group

Group	Nonspine Frx Lowest 10%	Nonspine Frx Upper 90%	Vertebral Frx Lowest 10%	Vertebral Frx Upper 90%
Placebo	(N = 31)	(N = 273)	(N = 20)	(N = 173)
N = 2711	11.4%	11.2%	7.4%	7.1%
95% CI	(8.1, 15.9)	(10.0, 12.5)	(4.8, 11.2)	(6.1, 8.2)
Alendronate	(N = 16)	(N = 230)	(N = 10)	(N = 89)
N = 2769	5.8%	9.2%	3.6%	3.6%
95% CI	(3.5, 9.3)	(8.2, 10.4)	(1.9, 6.7)	(2.9, 4.4)

Frx: fracture; CI: confidence interval

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TERIPARATIDE EFFECTS ON BONES GEOMETRY ARE INDEPENDENT OF MUSCLE AREA AND RADIUS LENGTH

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In a previous cross-sectional study, we showed that compared to placebo, teriparatide-treated patients had significantly higher axial (Ix) and polar (Ip) moments of inertia, determined by pQCT at the mid-distal radius, suggesting an increase in bone strength and improved resistance to fractures. These improvements in bone geometry were not associated with differences in age, height or weight, since values for each patient were adjusted for these variables. However, several other factors may affect bone geometry.

We assess here the influence of muscle cross-sectional area (CSA) and forearm length (L) in the effects of teriparatide on cortical bone architecture.

pQCT scans were performed in 72 postmenopausal osteoporotic women after a median 18 months of treatment with teriparatide at doses of either 20 (n = 29) or 40 (n = 21) ug or placebo (n = 22); and in a control group of 28 healthy men (n = 8) and premenopausal women (n = 20), at a site corresponding to 15% the length of the ulna from the distal radius end. Ix and CSMA were calculated from the scan images. L was measured between the ulnar styloid and the olecranon process.

Linear regression analysis showed a strong relationship between CSMA and Ix in the control group ($r = 0.85$, $P < 0.001$), but not in postmenopausal patients ($r = 0.18$, $P = $ ns). Comparison of regression lines for the placebo and teriparatide groups showed no differences in slope, but elevation was significantly higher in the treatment group ($F = 5.1$, $P = 0.027$). CSMA was not significantly different between the placebo and treatment groups, but both showed significantly lower CSMA than the control group. Despite differences in CSMA, and in agreement with the results of the regression analysis, Ix values

were not significantly different between controls and teriparatide patients, and both showed significantly higher Ix values than placebo. Similarly, a significantly relationship between L and Ix was found for the control group (r = 0.65. $P < 0.01$), but not in patients (r = 0.19, P = ns). Regression lines for the placebo and teriparatide patients showed no differences in slope, but elevation was significantly higher in the treatment group (F = 6.8, P = 0.011). L was not different between treatment groups but both showed significantly lower L values than the control group.

These results suggest that the improvements in bone geometry associated with teriparatide treatment are independent of cross-sectional muscle area and bone length.

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COMPARISON OF RISEDRONATE AND ALENDRONATE IN AN OSTEOPOROSIS CLINC

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INTRODUCTION: Oral bisphosphonates are the treatment of choice for the management of post menopausal osteoporosis. The nitrogen containing bisphosphonates, risedronate and alendronate produce similar reductions in fracture risk. However, the relative effects of these treatments on biochemical bone turnover and bone mineral density (BMD) are not clear.

METHODS: We monitored 57 patients (mean age 70.2 years) with postmenopausal osteoporosis treated with risedronate 5 mg daily over 2 years. This group was compared with data from 29 postmenopausal osteoporotic patients (mean age 73.7 years) treated with alendronate 10 mg daily who were also followed up over 2 years (previously described). Both groups were monitored in the hospital based osteoporosis clinic using BMD (Lunar DXA) and the bone resorption marker, urine N telopeptide crosslinks (NTX, Osteomark) RESULTS (see Tables)

CONCLUSION: Risedronate produced smaller increases in BMD at the LS and FN than alendronate over a 2 year period. In addition, this agent caused a modest suppression in NTX levels when compared with alendronate and levels were not appropriately suppressed (least significant change = 40% reduction from baseline value) until patients had 6 months of risedronate therapy. Whereas NTX levels of patients treated with alendronate were markedly suppressed at 3 months. These modest effects of risedronate suggest it may reduce fracture risk partly through mechanisms other than altering BMD and bone turnover.

Table: BMD (g/cm^2) and NTX (BCE/mmol/creatinine)

	0	24 months	
RISEDRONATE	(n = 57)	(n = 13)	
LS	0.854	0.884, 3.5%, p.001	
FN	0.72	0.735, 2%, NS	
ALENDRONATE	(n = 29)	(n = 29)	
LS	0.76	0.81, 7.5%, p0.001	
FN	0.63	0.65, 3.6%, p0.01	
NTX	0	3 months	6 months
RISEDRONATE	50.7	36.8, −27.5%, p.02	29.7, 41%, p0.01
	(n = 43)		
ALENDRONATE	51.6	25.5, −50%, p.001	25, −49%, p.0001
	(n = 30)		

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SPATIAL DISTRIBUTION OF CORTICAL BMD DID NOT COMPROMISE THE OBSERVED BENEFICIAL EFFECTS OF TERIPARATIDE ON RADIUS GEOMETRY IN OSTEOPOROTIC WOMEN.

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We had previously reported that, relative to placebo, teriparatide-treated patients had significantly higher axial (Ix) and polar (Ip) moments of inertia at distal radius, suggesting an increase in bone strength and improved resistance to fractures. However, bone strength is not only determined by

geometry, but also by bone material properties. Using bone mineral content (BMC) and density (BMD) as surrogates for bone material quality we had attempted to show that for any level of BMC or BMD, teriparatide-treated patients had better distribution of bone, as represented by Ix and Ip. However, this analysis assumed a homogenous distribution of cortical BMD in the bone cross-section. This is likely not the case in teriparatide-treated patients because teriparatide may increase bone porosity and newly formed bone may include incompletely mineralized regions with lower BMD.

We assess here the influence of BMD distribution at the cross-sectional area on the estimation of cortical bone architecture at distal radius.

pQCT scans were performed in 72 postmenopausal osteoporotic women after a median 18 months of treatment with teriparatide at doses of either 20 (n = 29) or 40 (n = 21) ug or placebo (n = 22), using a Stratec 960 pQCT machine at a site corresponding to 15% the length of the ulna from the distal radius end. The density-weighted cortical area axial (Ixw) and polar (Ipw) moments of inertia were calculated by correcting the area of each pixel by the ratio of the individual pixel density and the average density of the cortical area. Using this approach, incompletely mineralized or high-porosity bone areas contribute less to the moments of inertia values.

Volumetric cortical BMD was lower in the teriparatide-treated patients as compared with placebo (886.9 ± 103.5 vs. 904.5 ± 118.4 mg/ccm), but the difference was not statistically significant. Ix and Ip values were significantly higher in the teriparatide-treated patients as compared with the placebo group (Ix 789.4 ± 238.9 vs. 654.7 ± 145.8 mm4, P = 0.008; Ip 2986.4 ± 800.7 vs. 2716.1 ± 728.7 mm 4, P = 0.016). After corrected for BMD, Ixw and Ipw were still significantly higher in the treatment group as compared with placebo (Ixw 1071.4 ± 346.8 vs. 892.5 ± 215.9, P = 0.010; Ipw 3265.9 ± 1050.7 vs. 2850.6 ± 756.2 mm4, P = 0.005)

These results suggest that the putative effects of teriparatide on cortical BMD do not prevent the improvements in cortical bone architecture associated with teriparatide treatment.

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MULTIPARITY REDUCES THE FRACTURE INCIDENCE IN POST MENOPAUSAL WOMEN

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Reproductive history may affect bone mass in post menopausal women. Several authors have correlated the nulliparity with a lower bone mineral density in post menopausal women.

The aim of this study was to evaluate the parity effect on fracture incidence in post menopausal women.

We included 4873 nulliparous women (61 ± 8 year of age) and 4821 multiparous women, who had given birth to ≥3 children, (60 ± 7 years of age). Lumbar spine and femoral bone mineral density were measured by DEXA (GE-Lunar). Fracture history, age of menarche, age of menopause, physical activity, smoking status and calcium intake were evaluated by a questionnaire.

T-test was used to compare means between nulliparous and multiparous group. A multiple regression test was used to determine correlations between both groups.

All results showed statistically differences between groups. Nulliparous women had a lower BMD in lumbar spine (0.941 g/cm^2 vs 0.971 g/cm^2; $P < 0.001$), femoral neck (0.783 g/cm^2 vs 0.818 g/cm^2; $P < 0.001$), trochanter (0.655 g/cm^2 vs 0.692 g/cm^2 $P < 0.001$), and total femur (0.840 g/cm^2 vs 0.886 g/cm^2; $P < 0.001$). Moreover, nuliparous women had a higher fracture incidence (1445 vs 1242 cases). Hip fracture had a double incidence in nuliparous group (80 vs 41 cases). There was a statistical significant difference in weight between both groups, with nulliparous women weighing less, and a higher fracture incidence and a lower BMD.

In conclusion, the higher weight in multiparous women may have a positive effect on bone mass, with an increase in BMD and a decrease in fracture incidence, especially on weight-bearing sites.

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COMBINED TREATMENT OF ALENDRONATE PLUS ALPHACALCIDOL (ONE-ALPHA® LEO PHARMA©) ON BONE MASS IN POSTMENOPAUSAL OSTEOPOROSIS

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In many studies, alphacalcidol (Alfa) has been shown to be effective in Postmenopausal Osteoporosis (PMO) in reducing the loss of bone mass (BMD) and in others reducing the fractures 1. Alendronate (ALN) has been documented to increase BMD and demonstrated a 50% reduction on vertebral and femoral fractures 2. Combination studies of Alfa with the classical drugs for PMO are very rare and especially with ALN not existed in the literature. The main objective of this study was to determine the efficacy of the combination of ALN +

Alfa on BMD and compare it with the ALN + Calcium (Ca) classical combination.

226 postmenopausal women (MA = 54.2 ± 7.5 years) having PMO (T-score < −2,5 on Lumbar Spine (LS) and/or Femoral Neck (FN)), were enrolled in this randomized open study. Patients taken drugs or having diseases that could alter the bone metabolism were excluded. Standard biochemical measurements (e.g. Ca, P in blood and urine, Alp, Cr Alb, SGOT, SGPT were performed every 3 months) and BMD (LS + FN measured with Hologic Delphi) was measured at the beginning and after 12 months of treatment. The patients were divided in two groups. Group A of 114 patients has received ALN 10 mg plus 500 mg of Ca per day and Group B of 112 patients has received ALN 10 mg plus Alfa 0,5 μg per day (One-Alpha® LEO Pharma©) for 12 months. There is no demographic difference between groups. At the end of the study 102 of the Group A and 98 from the Group B patients were evaluated and analyzed with the paired T-test of the percentage change in BMD with basal values. 26 patients discontinued the study. 8 of them because of adverse gastrointestinal events related to ALN and 3 because of transient hypercalcaemia, 1 from the group A and 2 from the group B.

Our results showed that after 12 months of treatment the BMD of Group A has been augmented by 3.5% (± 2.9) at LS and 2.5% (± 2.61) at FN and of Group B by 4.3% (± 2.5) at LS and 3.3% (± 2.03) at FN. The statistical significance between the groups was P = 0.03 at LS and P = 0.004 at FN.

Out data shows clearly that the combination of 0.5 μg of alphacalcidol (One-Alpha®) with alendronate (Fosamax®) 10 mg/day is more effective on BMD of PMO than ALN 10 mg with 500 mg Ca/day.

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RISEDRONATE PROTECTS SKELETAL MASS DURING INTERCURRENT ILLNESS

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Background: Serious illness, often requiring hospitalization and surgery, is common in the elderly and has been hypothesized to contribute to so-called "age-related" bone loss. Antiresorptive agents have been predicted to protect against bone loss occurring as a consequence of such illness episodes.

Setting: Retrospective review of adverse event and BMD data in the 9578 subjects involved in the risedronate phase III trials.

Methods: 243 study participants underwent hospitalization while under study (for various non-skin cancers, myocardial infarction, pneumonia, CVA, gallbladder disease, and pancreatitis, as well as skeletal fractures) and had paired measurement of BMD prior to and following hospitalization. 104 subjects received risedronate 5 mg and 139 received placebo. Median inter-measurement interval was 547 days, and median onset of hospitalization was at day 120 within that interval. Additionally, 286 non-hospitalized controls were selected for both the placebo and the risedronate-treated subjects. Matching of these controls was primarily by visit interval.

Results: Mean annualized percent change in BMD ± SEM from the pre-hospitalization visit was +2.3% ± 0.56 at the lumbar spine (LS), +1.4% ± 0.68 at femoral neck (FN), and +0.6% ± 0.48 at femoral trochanter (FT) for subjects receiving risedronate. The corresponding values for the placebo-treated hospitalized subjects were −0.7% ± 0.40 at LS, −1.1% ± 0.56 at FN and −2.7% ± 0.58 at FT. The difference between risedronate and placebo at all three sites was statistically significant (P < 0.001, 0.004, and < 0.001, respectively). There was no difference in rate of change in the risedronate-treated subjects between those hospitalized and those non-hospitalized. By contrast, rates of change at all three sites were more negative in the hospitalized, placebo-treated subjects than in the non-hospitalized controls, with the difference statistically significant at LS (P = 0.019) and FT (P = 0.002).

Conclusion: As expected, there is a tendency to lose bone across a period of illness serious enough to require hospitalization. This bone loss is prevented in patients receiving risedronate.

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RISK-BENEFIT ASSESSMENT OF RALOXIFENE INFLUENCE OF BASELINE CARDIOVASCULAR RISK

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Raloxifene is a selective estrogen receptor modulator (SERM) indicated for the prevention and treatment of osteoporosis in postmenopausal women. We used the global risk index defined in the Women's Health Initiative (WHI) estrogen-progestin trial to assess the overall risk-benefit profile of raloxifene (RLX) and assessed whether this profile is influenced by cardiovascular (CV) risk. The Multiple Outcomes of Raloxifene Evaluation (MORE) 4-year osteoporosis treatment trial randomized 5133 postmenopausal women (mean age, 67 yrs) to placebo (N = 2576) or RLX 60 mg/d (N = 2557). Global index events included coronary heart disease (non-fatal MI, coronary death, or silent MI determined by ECG), stroke, pulmonary embolism, invasive breast cancer, endometrial cancer, colorectal cancer, hip fracture, and total mortality. Events are adjudicated by physicians blind to treatment assignment. Cox proportional hazard models were used to analyze the first occurrence of any global index event. Baseline CV risk was assessed by a quantitative risk score based on prior CV event history or CV risk factors. Women treated with RLX 60 mg/d compared with placebo had a significantly lower risk of experiencing a global index event (HR, 0.75; 95% CI, 0.60–0.96); the risk tended to decrease further as CV risk increased (Table). Contributing to this effect was a lower risk of major CV events (MI, stroke, and coronary death) among women treated with RLX 60 mg/d that also tended to decrease further with increasing CV risk (Table). The significant reduction in global index with RLX suggests a favorable risk-benefit profile for prevention and treatment of osteoporosis in postmenopausal women overall and in those at increased CV risk.

Table:

CV Risk Points	N*	Global Index HR (95% CI)	CV Events† HR (95% CI)
Total Cohort	5133	0.75 (0.60–0.96)	0.71 (0.48–1.06)
> = 2	3106	0.76 (0.58–1.00)	0.67 (0.44–1.02)
> = 3	1726	0.66 (0.47–0.92)	0.62 (0.38–1.01)
> = 4	676	0.50 (0.30–0.81)	0.41 (0.21–0.82)
> = 5	252	0.32 (0.15–0.69)	0.16 (0.05–0.53)
> = 6	186	0.39 (0.16–0.95)	0.17 (0.04–0.74)

*Data represent hazard ratio (95% CI) for RLX 60 mg/d vs placebo †MI, coronary death, and stroke

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THE UPPER GASTROINTESTINAL TOLERANCE OF HIGH-DOSE DAILY ORAL RISEDRONATE IN AN NSAID-USING POPULATION

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The favorable gastrointestinal (GI) tolerance of risedronate 5 mg daily in the treatment of osteoporosis has been demonstrated in a clinical database involving over 10,000 patients (Taggart 2002). This database included over two-thirds of patients with either a history of GI tract disease, or who prospectively received non-steroidal anti-inflammatory drugs (NSAIDs). We report here additional experience on the GI tolerance of risedronate from an osteoarthritis patient population receiving up to 3 times the dose used in the treatment of osteoporosis.

Two, Phase III placebo-controlled studies were conducted in 2483 patients with a diagnosis of medial compartment knee osteoarthritis. Subjects received orally either placebo, 5 or 15 mg of risedronate daily, or weekly risedronate at doses of either 35 mg (in Europe) or 50 mg (in North America) for 24 months. This report will focus on adverse event profile of placebo, 5 mg and 15 mg doses, since these were the common doses across both studies.

There were 2154 patients (87%) who completed the month 24 visit. The average age was 62 years with the majority (60%) being postmenopausal women. Forty-one percent of patients had a history of GI disease. The incidence of

Table:

Treatment (n)	Placebo (622)	Ris. 5 mg/d (628)	Ris. 15 mg/d (609)
Withdrawals due to AEs	12%	9%	11%
Upper GI AEs	18%	20%	17%
Mod to severe upper GI AEs	8%	10%	7%

NSAID use during the study period was 87%. Adverse event (AE) data are shown in the table for withdrawals due to AEs, as well as overall incidence and severity of upper GI AEs.

Overall, withdrawals due to AEs were low and comparable among treatment groups. The GI tolerability of risedronate was comparable to placebo with regard to incidence of withdrawals due to AEs and upper GI incidence and severity of AEs. Weekly doses of 35 and 50 mg were also similar when compared to their respective placebo groups.

In summary, risedronate is well-tolerated even when given at high doses in a population at risk for increased GI side effects. This study also confirms previous observations of the favorable tolerability of 5 mg daily and 35 mg weekly used in the treatment of osteoporosis.

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PROPORTION OF BMD RESPONDERS IS SIMILAR WITH RALOXIFENE AND ALENDRONATE

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Aim of the Study: ISCD guidelines suggest that the main objective of monitoring BMD in patients undergoing antiresorptive therapy is to identify patients with significant BMD loss. No change in therapy is indicated if theBMD increases or remains the same when compared to the least significant change (LSC) [J Clin Densitom 2002;5:S29–S38]. This analysis compares the proportion of BMD responders for raloxifene and alendronate.

Methods: A randomized, double-blind, placebo controlled trial compared changes from baseline in lumbar spine and femoral neck BMD at 1 year in postmenopausal women with low bone mass (femoral neck T-score <-2.0) treated with raloxifene 60 mg/d (n = 82) or alendronate 10 mg/d (n = 83). All women received calcium (500 mg/d) and vitamin D (400–600-IU/d). LSC was calculated as DXA precision multiplied by 2.77 (95% confidence interval).

Results: Assuming 1% precision, the proportion of women who gained BMD or had a BMD loss no more than the LSC (3%) at the lumbar spine was 97% and 98% in the raloxifene and alendronate groups, respectively. Also, 95% of women in both the raloxifene and alendronate groups had gained BMD or lost no more than 3% of BMD at the femoral neck. For lumbar spine or femoral neck BMD, the proportions of BMD responders were 92% with raloxifene and 93% with alendronate.

Conclusion: The proportion of women who responded to therapy, as measured by changes in lumbar spine or femoral neck BMD, was similar for raloxifene and alendronate, when precision error was taken into account.

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ANALYSIS OF AGE SUBGROUPS AMONG PATIENTS TREATED WITH ALENDRONATE OR RALOXIFENE IN THE EFFECT STUDY

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Aim: To evaluate the consistency of effect of alendronate (ALN) and raloxifene (RLX) across two age subgroups (<65 vs. greater than or equal to 65) in a randomized, placebo-controlled trial.

Methods: Patients were treated with ALN 70 mg once-weekly or RLX 60 mg daily for 1 year. Prespecified age subgroup (<65 and greater than or equal to 65) analyses were performed on endpoints of % change from baseline at 12 months in lumbar spine (LS) and total hip BMD, and urinary N-telopeptide (NTx) to assess consistency of treatment. Post-hoc analysis of % of patients with greater than or equal to 0% increase in LS BMD was included. Interaction between treatment difference and age subgroup was tested.

Results: Of 451 patients, 243 (53.9%) were <65 years [123 ALN, 120 RLX] and 208 (46.1%) were greater than or equal to 65 years [98 ALN, 110 RLX].

Treatment differences were consistent across age groups: $P = 0.119$, 0.974 and 0.470 for interaction between treatment difference and age subgroups for % change in LS BMD, % change in greater than or equal to 0% LS BMD, and % change in NTX respectively. The same was true for increases in total hip BMD.

Conclusions: ALN 70 mg once-weekly provided greater increases in BMD, greater reduction in resorption markers, and a greater percentage of responders than RLX, regardless of age.

Table:

Age Subgroup	ALN	RLX	Difference
<65 LS BMD % Change	4.1	1.3	2.8
% = or	91.7	69.5	22.2
Above 0 % Change in NTX	−59.0	−21.5	−47.8
At Least 65 LS BMD % Change	4.5	2.6	2.0
% = or	95.6	81.2	14.4
Above 0 % Change in NTX	−60.9	−34.4	−40.5

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REDUCTION IN BACK PAIN FOLLOWING TERIPARATIDE COMPARED WITH ALENDRONATE TREATMENT OF POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Postmenopausal women with osteoporosis treated with teriparatide 20 and 40 mcg/d (TPTD20 and TPTD40) had significantly reduced risk of back pain, moderate or severe back pain, compared to placebo (Neer NEJM 2001, Genant ASBMR 2003). The purpose of this analysis was to compare the incidence of back pain occurring in postmenopausal women treated with teriparatide or with alendronate. Back pain data were analyzed from two double-blind trials comparing oral alendronate 10 mg/d (ALN10) plus placebo injection with oral placebo plus teriparatide injection in postmenopausal women with osteoporosis. In study A, women were randomized to TPTD20 (N = 102) or ALN10 (N = 101) for 18 months. In study B, women were randomized to TPTD40 (N = 73) or ALN10 (N = 73) for a median 14 months and 52 previous TPTD40 and 53 previous ALN10 women completing this trial were enrolled in a follow-up study. Compared with women treated with ALN10, women treated with TPTD20 had reduced risk of back pain ($P = 0.051$), moderate or severe back pain ($P = 0.003$), and severe back pain ($P = 0.04$), respectively (Table, I). Women treated with TPTD40 had reduced risk of back pain ($P = 0.012$) and moderate or severe back pain ($P = 0.016$) versus women treated with ALN10 (Table, II). During the trial plus 18 months of follow-up, the TPTD40 group had reduced risk of back pain ($P = 0.015$), and moderate or severe back pain ($P = 0.016$) versus the ALN10 group (Table, III). In conclusion, women randomized to teriparatide had reduced risk of back pain compared to women randomized to alendronate.

Table: Back Pain after ALN10 and TPTD20 or TPTD40 treatment

	ALN10 % (n)	TPTD % (n)	Relative Risk (P-value)
I. Study A† Back Pain	38.6 (39)	25.5 (26)	.73 (0.051)
Moderate or Severe Back Pain	32.6 (33)	14.7 (15)	.56 (0.003)
Severe Back Pain	11.9 (12)	3.9 (4)	.48 (0.04)
II. Study B* Back Pain	19.2 (14)	5.5 (4)	.29 (0.012)
Moderate or Severe Back Pain	13.7 (10)	2.7 (2)	.20 (0.016)
Severe Back Pain	2.7 (2)	1.4 (1)	.52 (NS)
III. Study B + 18 Mo* Back Pain	28.3 (15)	9.6 (5)	.34 (0.015)
Moderate or Severe Back Pain	18.9 (10)	3.9 (2)	.20 (0.016)
Severe Back Pain	5.7 (3)	1.9 (1)	.33 (NS)

† = TPTD20 versus ALN10, * = TPTD40 versus ALN10

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COMPARATIVE EFFICACY OF THERAPIES FOR POSTMENOPAUSAL OSTEOPOROSIS: RESULTS OF THE YAMAGUCHI OSTEOPOROSIS PREVENTION STUDY (YOPS)

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This randomized controlled trial was conducted at 3 hospitals to assess the comparative effectiveness of several medications on bone mineral density (BMD), biochemical bone markers, and a new vertebral fracture incidence in

postmenopausal osteoporosis. A total of 513 postmenopausal women aged 50–75 with osteoporosis were randomly allocated into seven groups: hormone replacement therapy (HRT, conjugated estrogen 0.625 mg/day plus medroxyprogesterone 2.5 mg/day); alendronate (5 mg/day); etidronate (200 mg/day, 14 days per 3 months); eel calcitonin (CT, 20 IU/week); active vitamin D (alfacalcidol 1 micro g/day); vitamin K2 (45 mg/day); and control (no treatment). Thoracic and lumbar spine radiographs and BMD at distal 1/3 radius were assessed at baseline and at every 3 months, along with markers of bone turnover [serum bone specific ALP, serum osteocalcin, urinary NTX, and urinary deoxypyridinoline (DPD)]. Mean changes in BMD relative to baseline after the 2-year treatment in HRT, alendronate, etidronate, CT, active vitamin D, vitamin K and control was 2.0%, 2.3%, −0.8%, 1.4%, −3.4%, −1.9% and −3.3%, respectively. In control, the incidence of new vertebral fractures during the 2-year treatment was 25.8%. By intention-to-treat analysis, vertebral fracture risk was significantly reduced with HRT (RR = 0.35, 95% CI = 0.14–0.83), alendronate (0.35, 0.14–0.83), etidronate (0.40, 0.17–0.92), CT (0.40, 0.17–0.93), and vitamin K (0.44, 0.20–0.99). Although the number of vertebral fractures was nominally lower in active vitamin D group than in control group, the study did not have statistical power to detect differences in fracture rates (0.56, 0.26–1.12). Logistic regression analysis revealed that changes in BMD relative to baseline at month 3 significantly predicted the new vertebral fracture risk (OR: 2.17 in HRT; 2.64 in alendronate; 2.54 in etidronate; and 3.53 in CT; $P < 0.05$). Changes in NTX and DPD relative to baseline after 3 months were also significant predictors of the incidence of new vertebral fractures (OR: 1.83 and 2.02 in HRT, respectively; 3.04 and 3.87 in alendronate; 2.04 and 1.87 in etidronate; 3.07 and 2.07 in CT; $P < 0.05$). In conclusion, our results demonstrate the importance of measurements of BMD and markers of bone resorption at month 3 in identifying women for whom drug therapy to prevent vertebral fracture is appropriate. Clinicians should consider these results when selecting antiosteoporosis therapies for postmenopausal women.

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BONE TURNOVER MARKERS AND IGF-I LEVELS IN BLOOD ARE NOT MODULATED BY ERYTHROPOIETIN IN RECREATIONAL ATHLETES

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Erythropoietin (EPO) treatment increases bone turnover and IGF axis markers in patients on dialysis (Malyszko et al., 2002 Nephron 90: 282–289). It is not known whether EPO exerts similar effects in normal healthy adults, which is of importance if these GH-responsive biochemical markers are used for a GH doping test in sport. The aim was to determine the effects of EPO treatment in young recreational athletes on markers of bone turnover (PINP and ICTP), connective tissue turnover (PIIINP) and on IGF axis markers (IGF-I, IGFBP-3, ALS).

Fifteen male Caucasian recreational athletes, aged 27 ± 4 years were administered 50 U/kg recombinant hEPO with oral iron, 3 times/week for 25 days. Bone turnover and IGF axis markers were measured by radioimmunoassay in samples collected at baseline, after 10, 22 and 24 days of treatment, and after a 4-week washout period. Results were analysed by a mixed-effects ANOVA model.

EPO treatment resulted in approximately three-fold elevation of serum EPO and marked elevation of measures of erythropoiesis including haematocrit ratio (by approximately 0.06) and doubling of reticulocyte haematocrit. Mean levels of PINP, ICTP, or PIIINP did not change significantly in response to EPO treatment (Treatment effect mean $\pm$ SE: PINP 0.6 ± 3.9 ug/L, ICTP 0.07 ± 0.17 ug/L, PIIINP -0.32 ± 0.25 ug/L). Similarly, no significant changes in IGF-I, IGFBP-3 and ALS were observed during or after treatment.

In summary, EPO administration in young healthy adults did not change the bone and connective tissue turnover markers ICTP, PINP and PIIINP or the IGF axis markers IGF-I, IGFBP-3 and ALS. This indicates that concurrent use of EPO in athletes should not affect the validity of a GH doping test using these indirect markers. (Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program).

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EVALUATION OF BONE DENSITY FOLLOWING PERIODONTAL SURGICAL THERAPIES

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The aim of present study is to evaluate new bone density by densitometric measurement following periodontal surgical therapies, and to analyze significant factors associated with clinical outcome. The material comprised 40 female pa-

tients with chronic periodontitis separated into two groups. Group A consisted of 20 patients aged from 25–40 years without any systemic disease and the B group patients were aged from 45–65 years with osteoporosis diagnosis with systemic therapy. Patients were given the initial phase of periodontal therapy – oral hygiene instructions, full mouth scaling and root planing. Before surgical therapy periodontal indexes (deep of infrabony defects and/or furcation defects [> 6 mm] were measured); radiography and radiodensitometric measurements were also performed. Bone density was measured by means of computer program "THROPHY-RWG-UI" digital x-ray system, and expressed as a part of grayscale, ranked from 0–256, with always the same exposure time (5). Following reevaluation, each patient was treated with surgical therapy. During surgery mucoperiostal full thickness flap including two vertical releasing incisions were applied. Infrabony defects were filled with Bio-oss spongiosa and covered with Bio-gide (Geistlich-Biometrials). Control measurements were done after 6 months and after 1 year. After 6 months post surgical measurements of bone density in both groups showed bigger density values (Group A, $+84 \pm 50$, $P < 0.0001$; Group B, $+12 \pm 14$, $P < 0.002$), and difference between group A and group B was in favor of group A ($P < 0.0001$). After 1 year post surgical measurements of bone density in both groups showed minimal increase in value compared to the measurements performed after 6 months (Group A, $+14 \pm 30$, $P = 0.0002$; Group B, $+1 \pm 1$, $P < 0.0001$). However, even this minimal increase was in favor of group A ($P = 0.005$).

The results show the usefulness of periodontal surgical therapy, and they also draw attention to the fact that the regeneration of new bone is rather inferior with female patients suffering from osteoporosis, but without any systemic therapy administrated.

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THE OSTEOPOROSIS CARE GAP IN A COMMUNITY FAMILY MEDICINE GROUP IN MONTREAL CANADA: A PILOT STUDY

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There is increasing evidence to suggest that a large proportion of individuals at increased risk to fracture are not identified and that many of those identified are not adequately or appropriately treated. The purpose of this study was to determine whether this "care gap" exists in community family practice clinics in a large urban centre in Canada. In a large family practice clinic in central Montreal 500 consecutive patients > 50 years were asked to complete 8 questions related to fracture history after age 40 years, glucocorticoid use, past bone density test, spine x-ray, smoking history, and hospitalizations since their last clinic visit. Their clinic charts were reviewed to determine record of fracture history, DXA test results, diagnosis and treatment of osteoporosis. 359 women and 141 men were included. Approximately 90% of the cohort was equally distributed between ages 50–79 years with 10% > 80 years. Approximately 75% of the women and only 8% of the men were sent for bone density test, however there was no discernable rationale for how people were selected for DXA testing. Of those tested 25% of women and 55% of the men had osteoporosis by WHO criteria. Of the women diagnosed with osteoporosis, 47% were treated with a Bisphosphonate, Alendronate or Residronate, and 16% received only Calcium and Vitamin D supplementation. It is concluded that in a large family practice clinic there is an important "osteoporosis care gap". Furthermore, there appears to be no clear rationale for the use of DXA to diagnose osteoporosis and there is no evidence that fracture history plays any role in the assessment of fracture risk. Fewer than 50% of those diagnosed with osteoporosis receive the currently recommended therapy. A follow-up study in a number of family practice clinics is planned.

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BONE MINERAL DENSITY EVALUATION IN TYPE 2 DIABETIC WOMEN

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AIMS: Measure Bone Mineral Density (BMD) in women with Type 2 Diabetes Mellitus and compare with one control group. Establish prevalence of Osteoporosis and/or Osteopenia. Correlate BMD with Body Mass Index (BMI), period of time of menopause (PTM), and period time of diabetes (PTD).

METHODS: We measured BMD with a DEXA equipment in Lumbar Spine (LS) and Femoral Neck (FN) in 45 women with Type 2 Diabetes Mellitus and 53 control patients to match by age and sex. Values of BMD are expressed as T Score units. We performed a t Student test for comparing independent averages, an analysis for lineal correlation and calculate prevalence of osteoporsis and osteopenia using WHO criterias.

RESULTS: For women with Type 2 Diabetes Mellitus: Age 61.7 ± 12 years. PTM: 16.7 ± 10 years. PTD: 8.6 ± 7 years. BMI 26.7 ± 7. BMD in LS: −2.2 ± 1.3 and FN: −2.4 ± 4.5. For Control Group: Age 59.3 ± 10 years. PTM: 16.5 ± 8 years. BMI 26.5 ± 8. BMD in LS: 0.04 ± 0.78 and FN: −0.22 ± 0.88. BMD in type 2 Diabetes Mellitus Group vs Control Group $P < 0.001$ and $P < 0.001$ in LS and FN respectively. We found the following correlations in type 2 Diabetes Mellitus Group: LS vs Age = −0.27. FN vs Age = −0.25. BMI vs LS = 0.35. BMI vs FN = 0.29. LS vs PTD = 0.31. FN vs PTD = −0.55. LS vs PTM = −0.21. FN vs PTM = −0.33. The prevalence of osteoporosis and osteopenia in LS = 80%, and in FN = 76%. At the table we showed the prevalence of osteoporosis and osteopenia according to analyzed region.

CONCLUSIONS: BMD in women with Type 2 Diabetes Mellitus are low when it is compared in front of controls. Lost of bone mass is similar in trabecular and cortical bone in type 2 diabetic patients. There is an inverse correlation between Period Time of Diabetes and BMD in Femoral Neck. The ratio between diabetic patients with osteoporosis and/or osteopenia is higher to 76% in this study.

Table: Prevalence of Osteoporosis and Osteopenia in Type 2 DM

Skeletal Site	n	osteoporosis %	osteopenia (%)	normal (%)
Lumbar Spine	45	25 (56)	11 (24)	9 (20)
Femoral Neck	45	16 (36)	18 (40)	11 (24)

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ONCE-MONTLY NERIDRONATE IN POSTMENOPAUSAL OSTEOPOROTIC WOMEN: A CLINICAL EXPERIENCE

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The purpose of this study was to evaluate the effect of neridronate on women with postmenopausal osteoporosis and patient's adherence to this therapy.

From July 2002 we recruited 21 patients seen at our Department for Diagnosis and Care of Osteomethabolic Diseases in Venaria Reale (Turin, Italy). The group was of females, 59–68 years old, with postmenopausal osteoporosis. The patients were no-responders: they didn't have increase in BM measurements by the lumbar spine DXA after 18 months anti-resorptive therapy, or had severe controindications to oral treatment.

We identified the individual risk-score (by familiar, anthropometric, medications, illnesses data) with particularly care for the nutritional and activity status, and measured Ca, Ca regulating hormones and bone biomarkers. The patients had a DXA an a QUS (Quantitative UltraSound with Lunar Achilles) at baseline. For each one was determined the ICF (International Classification of Function, Disability and Health) and the Spitzer Index (scale for Life Quality). They were informed about the chance of undergo a therapy with an injection once-montly, with all indications and controindications. All the women accepted the new treatment and signed an Informed Consensus.

They received a therapy with neridronate 1 fl 25 mg intramuscolar, once-monthly. The follow up was every 6 months, including clinical visit, control of scales, lab evaluation, measurement of the Stiffness by QUS.

As measured by urinary deoxypyridinoline (uPDP) that decreases for 50 percent of the overflowing, and by others markers, the activity of neridronate on bone methabolism is important like in therapy with others anti-resorptive drugs. Stiffness is increase of 5–6 percent at control with QUS at 12 months. We haven't yet the BMD control because no-one of the women reached the 18 months, when is fixed the DXA. The Spitzer Index and ICF data are in course of evaluation.

The compliance for this therapy is very good. We had only one drop out, a patient who had a RAF with fever and cutaneous rashes at the first injection. The others women of the group are keeping on the protocol. The increase of Stiffness and the reduction of turn-over demonstrate a positive action of this bisphosphonate. The injective way and the monthly rhytm assure the best compliance.

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TAE DO EXERCISE USED FOR MAINTENANCE AND BUILDING OF BONE MINERAL DENSITY IMPROVES DAILY LIFE

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Many studies have documented that exercise can be effective in the maintenance and building of bone mineral density in postmenopausal women. 43 women suffering from osteopaenia and osteoporosis were included in the so called Tae do basic exercise program. Tae do exercise program is originally developed by Dr. Dalibor Krpan. It is founded on Korean traditional marshal art Taekwondo. The basic idea of this type of exercise is to connect the intermittent muscular contraction with mind focused energy. The type of the movement is performed by focused force stimulating the bone on the specific way with preventive and curable effect on osteopaenia and osteoporosis. Tae do exercise are divided in basic program that is suitable for almost all osteoporosis patient and more demanding advanced exercises. Medium age of the investigated sample was 65.6 years, medium age of menopausis (or quitting hormonal substitution) was 48.2 years. Medium BMD L1–L4 vertebrae was 0.755, T score −2.6, medium BMD of the hip was 0.664, T score −1.8. Duration of performing Tae do was in average was 5 months. All the participants exercised 4 times a week, 2 times guided by trained person, 2× times a week at home using literature and repeating previously learned motions. Patients were asked to score quality of life, severity of pain in the bones and mobility with grade from 1 to 10. As expected, we found negative correlation among the quality of life and severity of pain in the bones (correlation factor −0.3) and significant positive correlation among the quality of life and mobility (correlation factor 0.5). When analysing all women there was no correlation among their mobility and duration of exercise. While analysing women that exercised Tae do more than 6 months slightly positive correlation was found (correlation factor 0,3), and when we included women who were exercising more than 12 months a positive correlation was stronger (correlation factor 0.4). Cochrane Database review showed some evidence that exercise is effective at one year or longer on slowing bone loss, and our data fit into. The benefit of Tae do on mobility resembles previous reports that show a positive influence of exercise training for elderly in increasing physical activity in daily life. The results showed above are as the part of the bigger study only preliminary ones. Influence of Tae do on quality of life, osteoporosis and frequency of bone fractures is going to be described in follow up reports.

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BONE FRAGILITY IN PATIENTS AFFECTED BY SYSTEMIC SCLEROSIS

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Systemic Sclerosis (SSc), a chronic inflammation of connettive tissue with unknown aetiology, is characterized by microcirculatory alteration, immune system derangement and abnormal deposition of collagen. Life-span of these patients is significantly increased in recent years with enhanced risk of age-related disease onset such as osteoporosis. Poor and controversial reports are available on bone modifications in patients with SSc. Bone Mineral Density (BDM) effectively distinguishes patients with osteoporosis from normal subjects. Bone ultrasonography validly provides information on bone mass including structural characteristics. Aim of this study was to assess the prevalence of osteopenia/osteoporosis in a cohort of 38 female SSc patients [mean age 52 (26–72) years, mean disease duration 6 years (2 months–19 years)]. Bone density was measured at heel by ultrasonography (Achilles Express, Lunar Corp. USA) and at thigh neck and lumbar spine by DEXA Hologic 4500. Rachis morphometry was evaluated by spine Analyzer software (CAM Diagnostic).

Nineteen out of 37 patients (51.4%) showed a T-score < -2.5 at heel ultrasonography, while 7/35 (21.2%) patients showed a T-score < -2.5 at thigh level and 13/33 (39.3%) patients at lumbar spine level. Rachis morphometry revealed at least one vertebral fracture in 26 on 29 (89.7%) patients.

In conclusion, our results indicate an increased bone weakness in patients with SSc suggesting that bone modifications with osteoporosis is frequent as other tissue alterations in SSc patients. In this study ultrasonography was superior than DEXA in predicting morphometric alterations.

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EFFECT OF 2β-(3-HYDROXYPROPOXY)-CALCITRIOL ON BONE MASS AND STRENGTH AND BONE METABOLISM IN OVARIECTOMIZED MICE

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EFFECT OF 2β-(3-HYDROXYPROPOXY)-CALCITRIOL ON BONE MASS AND STRENGTH AND BONE METABOLISM IN OVARIECTOMIZED MICE.

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[Abstract] Objective This study is to compare the effects of a synthtic (3-Hydroxypropoxy)-Calcitriol (ED-71)with 17β-Estrodiol (E2) on bone mass bone

strength and bone metabolism. Methods Bone mineral density (BMD), bone mineral content (BMC), bone strength and bone histomorphometric parameters were measured in 36 female Kunming mice, weighing an average of 35 g, were randomly divided into 4 groups. Results After 6 weeks treatment of ED-71 at 0.4 μg/Kg/week and E2 at 30 μg/Kg/day in ovariectomized (OVX) mice, compared with OVX mice femoral BMD and BMC increased respectively 3.8%, 5.9% and 3.2%, 5.7%; maximun load of femur increased respectively 18.7% and 16%; trabecular bone volume of lumbar vertebra increased respectively 10.6% and 16.1 and serum alkalinephosphatase decreased respectively 58% and 37%. Conclusions ED–71 significantly increased BMD, BMC and bone strength and significantly inhibited bone turnover in OVX mice. Also ED–71 could not induce calcemia and uterus proliferation. However E2 significantly induce uterus proliferation.

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THERAPUTIC EFFICACY OF 1,25DIHYDROXY VITAMIN D3 IN PREDICT OF OSTEOPOROSIS INDUCE GLUCOCORTICOID IN RATS

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Theraputic efficacy of 1,25dihydroxy vitamin D3 in predict of osteoporosis induce Glucocorticoid in rats.

Abstract

Introduction: Glucocorticoid induce osteoporosis was characterized by increased bone resorption and decreased of new bone formation.

The present study was designed to the effects of 1,25dihydroxy vitamin D3 (calcitriol) in prevent of osteoporosis by induced Glucocoticoid with assaied biochemical markers, bone mineral density and hitomorphometry of lumber vertebrae body in rats.

Methods and material: Total duration of the experiment was four weeks. Twenty-four male spargue Dawley rats (7 week old and 180 gr weight) were randomly divided into four groups: Group 1(n = 6), was abase line control. Group 2 (n = 6), get only normal saline (0.9%). Group 3 (n = 6) get Methylprednisolone Acetate (MPA), 0.2 mg/kg subcutaneously 3 times for a week and group 4 (n = 6), get MPA (previously doses) with calcitriol (0.1 microgram/kg/day/oral).

For evaluation of biochemical agent changes in serum Calcium, Acid phosphatas and osteocalcine were measured before and after treatments.

Bone mineral density (BMD) of lumber vertebrae was measured by Dual x-ray absorptiometry (DEXA).

The first lumber vertebrae bone volum evaluated by histomorphometry.

Results: The results showed that, the serum Calcium level un affected in MPA and MPA with calcitriol treatment groups (P < 0.05), but the serum Acid phosphatase level increased, and serum osteocalcine level and BMD of lumber vertebrae decreased in MPA group (P < 0.05). The mean of serum Acid phosphatas level, decreased and the mean of serum osteocalcine level and BMD of lumber vertebrae increased in MPA with calcitriol group. Although, in the end of experimental period these results weren't significient diffrence (p.0.05) with MPA group.

In histomorphometric analysis, MPA decreased trabecucar bone volume and trabecular number per area of bone surface in the first lumber vertebrae (P < 0.05). Combined administration of calcitriol with MPA for 4 weeks prevented decreased trabecular bone volume and trabecular number, also the trabecular separation was decreased (P < 0.05).

Conclusion: Combined administration of calcitriol with Methylprednisplne Acetate showed a preventative effect against bone loss.

Key word: Glucocorticoid, Osteoporosis, Bone metabolism markers, BMD, Rat.

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DXA AND QUS ARE EQUALLY GOOD IN DISCRIMINATING OSTEOPOROTIC HIP FRACTURE PATIENTS FROM MATCHED CONTROLS

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Dual Energy X-ray Absorptiometry (DXA) and Quantitative Ultrasonometry (QUS) of the heel are two techniques used for assessing osteoporotic fracture risk. In this pilot study we evaluated the ability to discriminate patients with hip fracture from healthy controls using DXA and QUS in postmenopausal women.

We included 22 Patients mean age 76.5 ± 5.4 years with an incident osteoporotic hip fracture and 22 age and BMI matched controls. Women in the control group with a history of osteoporosis or with a fracture or diseases or treatments known to affect bone metabolism were excluded from the study. BMD was measured by DXA (Prodigy, GE Lunar) at the spine and hip. QUS

was performed at the os calcis using the Achilles+ device as well as the Achilles Insight device (both from GE Lunar).

DXA results of women with hip fractures at the femoral neck showed statistically significant lower T-score of −2.6 and Z-score of −0.8 compared to T-score of −1.7 and a Z-score of 0.1 in healthy controls ($P \leq 0.008$ and $P \leq 0.01$). In women with hip fracture, DXA results of the Spine (L1–L4) showed a T-score of −2.1 and a Z-score of −0.3, compared to age and BMI matched controls who showed a T-score of −1.4 (L1–L4) and a Z-score of 0.3 (L1–L4) (difference not significant). In accordance to the hip DXA results, measurement at the Os Calcis (Achilles+ and Achilles Insight) also showed significant differences between the groups. The T-scores were −3.3 and −2.6 in women with hip fracture compared to a T-score of −2.3 and −1.6 ($P \leq 0.01$) in controls. The Z-score was −0.9 and −0.3 in women with hip fracture compared to 0.1 and 0.8 ($P \leq 0.01$ and $P \leq 0.005$) in controls.

The results of our pilot study confirm the capability of DXA and QUS devices to discriminate patients with prevalent hip fracture from healthy controls. This significant difference could be observed by DXA at the hip and by both heel ultrasonometers but not for DXA of the spine. Further large scale longitudinal studies are needed to evaluate the diagnostic capabilities of DXA and QUS.

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PRECISION AND ACCURACY OF THE LUNAR BRAVO, A COMPACT DXA SYSTEM

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The Lunar Bravo (GE Medical Systems) is a small footprint spine/hip DXA scanner designed for offices with limited space. The Bravo scanner arm rotates to the side for easy patient access and positioning. It uses established pencil beam technology with several features previously found only in fan-beam systems. These include a spine/bilateral femur mode (OneScan) that eliminates the need to reposition between spine and femur scans, automated software to help identify problem scans, and integrated physician reporting software. We evaluated the precision of the Bravo and its accuracy compared to an existing DXA system.

Twenty-six women with an average age 55 ± 10 years had spine and dual femur measurements on Bravo and on the Lunar Prodigy (GE Medical Systems). Each subject was measured 3 times on Bravo and on Prodigy. Subjects were repositioned between scans. BMD results were obtained using manufacturer-recommended analysis protocols. Precision error was calculated as the RMS standard deviation for the repeat measurements (%CV). Bravo spine and hip BMD values were compared to Prodigy values using a two-tailed, paired t-test based on the first measurement obtained from each densitometer.

Bravo precision error was slightly higher than with the Prodigy but consistent with published values for other fan-beam DXA systems. Bravo and Prodigy spine BMD values and femur neck BMD values were highly correlated (r = 0.98 and 0.99 respectively) and not significantly different. There were small but significant differences (∼2%) in Bravo and Prodigy trochanter and total femur BMD values. We conclude that the Bravo provides accurate and precise spine and hip DXA measurements, consistent with results from other bone densitometers, making it a valuable alternative for practices with space limitations.

Table:

	L1–L4 Spine	Femur Neck	Femur Troch.	Total Femur	Dual Tot. Femur
Bravo Precision	1.1%	1.7%	1.4%	0.9%	0.6%
Prodigy Precision	1.1%	1.1%	1.1%	0.8%	0.5%
Bravo BMD	1.091	0.868	0.740	0.925	0.926
Prodigy BMD	1.096	0.865	0.753	0.943	0.943

Precision is given in %CV; BMD is given in g/cm^2

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PERCEPTION OF OSTEOPOROSIS IN THE GERMAN POPULATION

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Awareness for and knowledge about diseases, their risk factors, outcome and treatment possibilities are strong factors for the patients support for diagnostic interventions, treatment adherence and long term compliance.

Objective of this research executed by an independent market research institute, EMNID, was to assess awareness, knowledge, attitudes and medication habits regarding osteoporosis in the German population and compare it with the awareness of some other common diseases.

In January 2003 a telephone survey was performed in a representative sample of 2309 Germans between 20 and 80 years. 53% of participants were female, 33% aged 50+, 6% had been previously diagnosed with osteoporosis.

Knowledge of osteoporosis as being a chronic disease was only 3%, significantly lower than for asthma (27%) and diabetes (20%). Even the population at highest risk had rather low information about osteoporosis: 55% of the men aged 50+ and 63% of the women aged 50+ perceived the progression of osteoporosis after diagnosis as slow. Only 17% of the men aged 50+ and 12% of women aged 50+ associated an increased risk to die with osteoporosis. Awareness about illness, progression and mortality risk worsens with increasing age. Only 12% of the population aged 50+ feared to suffer osteoporosis in the future, other diseases like cancer or cardiac infarction were perceived to be of greater threat. Consistent with this, 71% of the total population assessed themselves not being at risk for osteoporosis. Regarding the most convenient dosing frequency treating a chronic disease, 55% of the total population preferred daily intake vs. 35% weekly. However for a medication with intake instructions like bisphosphonates, 60% preferred weekly medication vs. 32% for daily intake.

We conclude that ignorance about osteoporosis is still common in the total population and the elderly aged 50+. An effective communication framework between physicians, patients and their organisations is required to change this situation. Preference for intake of medications depends on the mode of administration and the habits of the patients, with preferences for either daily or weekly intake.

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ASSESSMENT OF FRACTURE RISK AND OSTEOPOROSIS IN PATIENTS OF 50 YEARS AND OLDER WITH A RECENT FRACTURE

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The Dutch Guidelines for Osteoporosis (April 2002) recommend a case-finding strategy based on risk factors. A fracture in a woman above the age of 50 years is one of the risk factors, which should lead to bone mineral density assessment. The aim of this project is to implement case-finding for osteoporosis in patients with a recent fracture diagnosed in the Emergency Department according to the Dutch Guidelines. All patients of 50 years and older who came to the Emergency of two hospitals (VU University Medical Centre and Slotervaart Hospital) with a fracture were invited to participate. Polytrauma patients and patients with scull fractures were excluded. From January 2003, till December 2003, 702 patients were included in the study. All patients received information about osteoporosis and a questionnaire on the following risk factors: previous fractures, vertebral fracture; mother with hip fracture; low body weight; serious immobility; use of corticosteroids. The risk score was calculated according to the Dutch Guidelines in 235 patients who completed the questionnaires and in whom DXA of the spine and hip were performed. The results of the case-finding were used to evaluate the incidence of osteoporosis in patients with fractures and in patients with high risk scores. An advice was prepared for the general practitioner based on risk score and BMD. The advice included the diagnosis, and the proposed treatment (no treatment, calcium and vitamin D, bisphosphonates or raloxifene, referral to a specialist).

From the 702 included patients, 287 (40.9%) completed the questionnaires and 235 (33.5%) underwent DXA. The first 235 patients were diagnosed as follows: normal BMD 64 (27.2%), osteopenia 110 (46.8%), osteoporosis 61 (26.0%). The advice in the first 209 evaluated patients were: no treatment or extra calcium and vitamin D in 122 patients (58.4%), and treatment with bisphosphonates or raloxifene in 87 patients (41.6%), including all patients with osteoporosis and some patients with osteopenia and several risk factors. Some of the patients with severe osteoporosis were referred to the specialist according to the Guidelines. In conclusion, the acceptance of this case-finding program by the patients is moderate. The majority of patients who complete the questionnaire, also comes for DXA (81.9%).

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WITHDRAWN

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DIGITAL X-RAY RADIOGRAMMETRY (DXR) IDENTIFIES KNEE OSTEOARTHRITIS PATIENTS WITH LOW SPINAL AND HIP BONE MINERAL DENSITY (BMD)

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AIMS: Patients with Osteoarthritis (OA) do usually have a higher BMD than age- and sex matched controls. DXR has been shown to predict low forearm BMD. The aim of this study was to evaluate if DXR was able to identify patients with a low BMD at the lumbar spine and hip in a population with a low pre-test probability of having osteoporosis (OP).

PATIENTS AND METHODS: Patients with painful knee OA were prospectively included in a one-centre, randomised, double blind, placebo controlled clinical trial to evaluate the effect of oral chondroitin sulphate treatment on the knee joint. A selection of these patients were asked to undergo DXR of both hands using the Pronosco X-posure system[TM] (Sectra-Pronosco, Vedbaek, Denmark) and Dual Energy X-Ray Absorptiometry (DEXA) measured on a Hologic QDR 4500 A (Hologic Inc., Waltham, MA). The correlation between both hands DXR values and between the mean hand DXR and vertebral and hip DEXA measurements was assessed using Spearman rank correlation. Mann Whitney test was used to assess differences between patients with high and low BMD T-scores.

RESULTS: DEXA measurements were available in 151 patients (75 M, 76 F; aged 62.6 ± 9.2 yrs, range 40–82 years). DEXA showed a decreased vertebral BMD in 72 patients (45% of all patients; T-value < −1). Nineteen of these 72 patients had spinal osteoporosis (12% of all patients; T-value < −2.5). DXR measurements were available in 154 patients (78 M, 78F). Spearman test showed an excellent correlation between the left and right hand BMD and porosity value ($P = 0.951$ and $P = 0.414$). A significant relationship was found for the mean T-score of hand DXR and spinal BMD T-score ($P < 0.0001$) and the T-score of at least one hip or the mean of both hips ($P < 0.0001$ and $P < 0.0001$). Mann-Whitney test revealed a significant difference of the mean T-scores of DXR of both hands between the group with normal and low T-scores at the vertebral spine ($P = 0.0034$). However, only the hand BMD parameter was able to discriminate patients with low BMD T-score at the spine or hips, but not porosity.

CONCLUSION: DXR is able to reliably discriminate patients with decreased bone mass (T-score < −1) both at the lumbar spine and hip. Therefore, in patients with a low pre-test probability for the presence of osteopenia/osteoporosis as those with OA measured in this study, DXR could effectively identify those patients who should undergo DEXA measurement.

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STONTIUM RANELATE TREATMENT PREVENTS OVARIECTOMY INDUCED BONE LOSS IN RATS BY MAINTAINING THE BONE FORMATION AT A HIGH LEVEL

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Strontium Ranelate is a new active compound in postmenopausal osteoporosis. Static and dynamic bone histomorphometry assessed its effects in prevention of ovariectomy-induced bone loss. Six-month old Sprague-Dawley rats were either ovariectomized (OVX) or received sham (SHAM) surgeries. One day after ovariectomy, 3 OVX groups were treated daily for 52 weeks with 125, 250, or 625 mg/kg of strontium ranelate and one received vehicle. Vehicle-treated OVX and SHAM animals served as controls. Regarding the control groups after 1-year treatment, a 72% reduction in cancellous bone volume (BV/TV) in OVX vs. SHAM ($P < 0.01$) was noted in the proximal tibia. The reduced BV/TV was related to a 70% decrease in trabecular number (Tb.N) and a 394% increase in trabecular spacing (Tb.Sp;both $P < 0.01$). Similar findings were observed in the lumbar vertebra: decreases of 49% and 36% in BV/TV and Tb.N, respectively and a 108% increase in Tb.Sp ($P < 0.01$ for all parameters). Significant increases in bone formation were also observed at these two sites, confirming the presence of high-turnover bone loss in the OVX controls. At the proximal tibia, strontium ranelate treatment showed positive, dose-dependent effects on all parameters compared to OVX: increased BV/TV (116%) and Tb.N (64%) and decreased Tb.Sp (53%) in rats treated with 625 mg/kg/d of strontium ranelate ($P < 0.01$ for all parameters). In this treated group, trabecular thickness (Tb.Th) was increased by 23%. In vertebrae, strontium ranelate at the dose of 625 mg/kg/d increased BV/TV by 40% ($P < 0.05$) and Tb.N by 28% ($P < 0.05$), increased Tb.Th by 12% and decreased Tb.Sp by 31% ($P < 0.01$), respectively, in comparison with OVX. In the proximal tibia as in lumbar vertebrae, bone structure improvements appeared to be a consequence of strontium ranelate's effects on bone formation as the bone formation rates (BFR/BS) in strontium ranelate treated animals were equivalent to those observed in OVX. Finally, the

absence of any mineralization defect under strontium ranelate treatment was confirmed as no modification of the osteoid tissue and of the mineral apposition rate was noted.

These results indicate that strontium ranelate treatment prevents OVX-induced bone loss via a pathway that stimulates bone formation at a high level when bone resorption is decreased.

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STRONTIUM RANELATE DOSE-DEPENDENTLY INCREASES BONE STRENGTH AND INTRINSIC BONE QUALITY IN INTACT FEMALE RATS

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Recent clinical studies have demonstrated that strontium ranelate reduces the risk of vertebral and non-vertebral fracture in postmenopausal osteoporotic women. In the present study, we investigated the long-term effects of strontium ranelate on bone strength in intact female rats at the level of L4 lumbar vertebra. Four groups of 30 rats (seven-week old at treatment initiation) were fed ad libitum a diet containing strontium ranelate at a daily dose of 0 (control), 225, 450 or 900 mg/kg/day, for 104 weeks. From the load deflection curve, obtained by compression of the vertebral body, maximal load, stiffness, yield point, total energy (E), and elastic and plastic energy were measured.

Strontium ranelate treatment dose-dependently increased maximal load (up to +20% at 900 mg/kg/d, $P < 0.05$) and yield point (up to +13%, ns) without affecting stiffness, indicating no mineralization defect, thus suggesting an improvement of the bone quality. This was confirmed by the increase of energy to failure (up to +54.5%, $P < 0.05$) achieved with strontium ranelate treatment at 900 mg/kg/d, which was essentially due to a significant increase in plastic energy (+136%, $P < 0.01$), with an increase in elastic energy (+26%, NS). These results strongly suggest that bone formed under strontium ranelate treatment is able to withstand greater deformation before fracture while possessing similar elastic properties to normal bone. Such modifications observed under strontium ranelate treatment are in agreement with an improvement of intrinsic bone quality leading to greater bone resistance.

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STRONTIUM DIRECTLY STIMULATES OSTEOCLAST APOPTOSIS

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Strontium ranelate reduces the risk of vertebral and non-vertebral fracture in women with post-menopausal osteoporosis by inducing a decrease in bone resorption and an increase in bone formation. As strontium (Sr^{2+}) is a bone-seeking agent, high concentrations of Sr^{2+} are likely to occur in the sub-osteoclastic compartment and in the vicinity of the cells during bone resorption. High concentrations of extra-cellular calcium (Cao^{2+}) are known to down-regulate osteoclastic bone resorption, at least partly by inducing osteoclast (OC) apoptosis. The objective of the present study was to specify the role played by extra-cellular Sr (Sro^{2+}) concentrations on bone resorption and OCs activities. Using

10-days old rabbit purified OCs, the effects of Sro^{2+} alone (1.8–24 mM) or in combination with Cao^{2+} (1.8–20 mM) were assessed on bone resorbing activity by pits area measurement. OCs were seeded on bovine bone slices and adherent cells cultured in the presence of Sro^{2+} for 48 hrs. Apoptosis of isolated OCs was assessed by Hoechst staining, and confirmed by DNA scales electrophoresis.

Sro^{2+} inhibited osteoclastic bone resorption from 12 mM (−25%, $P < 0.05$) to 24 mM (−50%, $P < 0.01$), and Sro^{2+} dose-dependently stimulated OC apoptosis. Independently, Sro^{2+} and Cao^{2+}, around 20 mM, induced a similar rate of OC apoptosis (approx. 50%). Tested together, Sro^{2+} and Cao^{2+} have additional effects on bone resorption as well as on mature OC apoptosis. The use of specific inhibitors of intracellular Ca^{2+} signaling pathway (U73122, Caffeine, 2APB and SKF-96365) indicates that the transduction pathways involved in Sr- and Ca-induced OC apoptosis are different but cumulative.

Our data strongly suggest that exposure of OCs to an increasing amount of Sro^{2+} is responsible for a decrease in the bone resorption process mediated, at least in part, by the induction of OC apoptosis. Although, Sro^{2+} and Cao^{2+} both stimulate a G protein-coupled receptor, which could be the calcium-sensing receptor, they have differential intracellular effects which independently trigger OC apoptosis and could act in a cooperative manner. These results support the mechanism of reduced bone resorption observed in various *in vivo* and *in vitro* experiments with strontium ranelate.

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HEEL ULTRASONOMETRY AS A TOOL FOR IDENTIFYING PATIENTS AT HIGH AND LOW RISK FOR OSTEOPOROSIS

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Quantitative ultrasonometry of the heel is an alternative, low-cost method that can be used to identify women likely to have osteoporosis at the hip or spine as measured by DXA. We determined T-score cutpoints for the Achilles bone ultrasonometer to identify women at high and low risk for osteoporosis. The Lunar Achilles InSight (GE Medical Systems) is an imaging ultrasonometer with 8–10 second measurement time; the complete examination takes 3–5 minutes.

A total of 163 women (mean age 67 ± 12 years) had a spine and dual femur measurement with the Lunar Prodigy (GE Medical Systems) and a heel ultrasound measurement (Achilles InSight). Osteoporosis was diagnosed if the lowest DXA T-score at spine (L1–L4) or left or right femur (neck, trochanter or total) was ≥−2.5. Using the binormal fit to the ROC data, InSight T-score cutoffs for a likelihood ratio for a positive test (LR +) ≥5 and negative test (LR −) ≤ 0.2 were calculated.

We found 86% of the women with InSight T-score >−1.0 did not have osteoporosis at the spine/hip (negative DXA). The 88% sensitivity for a negative test is statistically not different from the 90% level recommended by the ISCD for referring subjects from peripheral ultrasonometry to central DXA. Also, 83% of the women with an InSight T-score £−1.8 had osteoporosis at the spine or hip. Women with InSight

T-Scores £−1.8 are at high risk, and could be considered for treatment and DXA monitoring measurements. Women with InSight T-scores >−1.0 could be considered at low risk and scheduled for retesting in future depending on risk factors. Women with an InSight T-score between −1.8 and −1.0 should be referred for DXA assessment.

We conclude that the Achilles InSight can be used as a valid screening tool to select candidates for axial DXA.

Table:

DXA Test	InSight T-Score	Sensitivity	Specificity	LR +	LR−
Negative	>−1.0	88%	58%	2.1	0.2
Positive	≤ −1.8	58%	89%	5.0	0.5

P193

STRONTIUM IS A FULL AGONIST OF THE EXTRACELLULAR CALCIUM-SENSING RECEPTOR (CAR) TRANSFECTED IN HUMAN EMBRYONIC KIDNEY CELLS

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Strontium ranelate has been shown to be effective in reducing fracture risk in women with postmenopausal osteoporosis but its cellular mechanism of action has not yet been fully elucidated. Extracellular strontium ($Sr^{2+}o$), similarly to extracellular calcium ($Ca^{2+}o$), could exert its actions, in part, via the extracellular calcium-sensing receptor (CaR), as the atomic and ionic structures of strontium and calcium are close. The goal of this study was to evaluate whether $Sr^{2+}o$ directly activates the CaR by assessing changes in intracellular transduction pathways and biological responses namely, elevations in the cytosolic calcium concentration ($Ca^{2+}i$), accumulation of inositol phosphates (IPs), activation of mitogen-activated protein kinase (MAPK) and stimulation of the activity of a non-selective cation channel (NCC). These pathways were tested in CaR-transfected or non-transfected (control) HEK293 cells. Raising the level of $Ca^{2+}o$ (0.1–10 mM) produced a dose-dependent activation of the CaR in CaR-transfected HEK293 cells as assessed by increases in $Ca^{2+}i$, enhanced accumulation of IPs, activation of MAPK, and increased activity of the NCC. $Sr^{2+}o$ (0.1–10 mM) also dose-dependently activates the CaR in CaR-transfected HEK293 cells as assessed by the same four parameters. The efficacy of $Sr^{2+}o$ is similar to that of $Ca^{2+}o$ for activation of the NCC and MAPK, and about 30% lower for stimulating increases in $Ca^{2+}i$ and accumulation of IPs. Neither $Sr^{2+}o$ nor $Ca^{2+}o$ had any effect on these four parameters in non-transfected cells. The results obtained in this study show that $Sr^{2+}o$ is a full agonist of the CaR. Thus $Sr^{2+}o$ could exert some of its actions *in vivo* via the CaR receptor.

P194

TERIPARATIDE REDUCES THE CASCADE OF VERTEBRAL FRACTURE RISK AS MEASURED BY THE SPINAL DEFORMITY INDEX

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Teriparatide [rhPTH (1–34)] was shown to increase bone mineral density and reduce the risk of vertebral and nonvertebral fractures in a double-blind study of 1,637 postmenopausal women with osteoporosis (Neer NEJM 2001). Patients were randomized to receive placebo or teriparatide 20 (TPTD20, commercially available) or 40 mcg/day for a median treatment duration of 19 months. Two critical determinants of future fracture risk in women with osteoporosis are the number and severity of prevalent vertebral fractures as measured by the Spinal Deformity Index (SDI). We assessed TPTD20 vertebral fracture efficacy as measured by increases from baseline in SDI score. Spinal radiographs were assessed using a visual semiquantitative technique (Genant JBMR 1993). For each radiograph, fractured vertebrae were assigned scores of 1, 2, or 3 for mild, moderate, or severe fractures, respectively, and the SDI was calculated by summing these scores. The proportion of patients having increases in baseline-to-endpoint SDI scores of > 1, > 2 and > 3 units were analyzed using a logistic regression model that included therapy and baseline SDI as predictor variables. In placebo patients with increasing baseline SDI score, there was a marked increase in the risk of SDI scores worsening by > 1, > 2 and > 3 units. However, this cascade of vertebral fracture risk was mitigated in TPTD20-treated patients. Results in the placebo group confirm that SDI scores provide important prognostic information. Specifically, with increasing baseline SDI, there was a cascade of risk for new or worsening vertebral fractures, as measured by the increase in SDI. This cascade of risk for future vertebral fracture was mitigated following TPTD20 treatment indicating that TPTD20 alters the natural history of the progression of osteoporosis.

P195

STRONTIUM RANELATE TREATMENT PRESERVES BONE CRYSTAL CHARACTERISTICS AND BONE MINERAL REACTIVITY

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In this work, the effects of strontium ranelate on the characteristics of bone mineral crystals, ionic exchanges at the mineral level and dissolution properties of bone apatite were determined. Female Cynomolgus monkeys were treated with 0, 200, 500, 1250 mg/kg/d of strontium ranelate for 52 weeks, 4 were sacrificed at the end of treatment, and 2 after a 10-week reversibility period. On powdered samples of the humeral diaphysis, metaphysis and epiphysis, the Ca, Sr, P, Mg, and CO3 ions contents (chemical measurements), crystals size (X-ray diffraction) and fine structural characteristics (FTIR spectroscopy) were determined. Diaphysis powdered samples underwent an exchange test (in a solution of Ca(NO3)2 for 30 min) or a dissolution test (in acetate, 0.1 M, pH 5, for 60 min at 37 °C). Powders were analysed before and after these tests by the techniques previously described. Release of ions in the acidic buffer during dissolution, was determined by chemical measurements and Ca release with a Ca-selective electrode.

Strontium ranelate treatment induced a dose-related increase in the bone Sr content with no modification of the stoichiometry, crystal size and nonapatitic environments. Sr uptake as well as its release was observed preferentially in the epiphysis and metaphysis. This can be due to higher surface contact with bone fluids for the epiphysis and metaphysis than for the diaphysis and/or to differences in turnover rate and crystals characteristics in these locations. The exchange test demonstrated a slight constant exchange rate of 9–12% Sr in diaphysis crystals, independent of the treatment dose and bone Sr content. This indicated that easily exchangeable Sr from bone was located in labile hydrated nonapatitic environments on crystal surface. Strontium ranelate treatment had no effect on the bone dissolution rate, or on the amounts of Ca, Mg and P ions released in the buffer. The amount of Sr ions released was dependent on the bone Sr content.

These data demonstrate the strontium ranelate safety at bone apatite crystal level and the preservation of the apatite reactivity, based on the absence of changes on exchange and dissolution properties after long-term treatment using a dose up to 40 times the human therapeutic dose of 2 g/day.

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CORRELATION OF THE HOLOGIC QDR EXPLORER AND DISCOVERY WHOLE BODY DENSITOMETERS

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The QDR Explorer and Discovery are linear scanning fan beam densitometers that support Whole Body applications. BMD and body composition correlation studies were performed on the two densitometers.

Twenty-three subjects ages 25 to 85 years old were scanned at the spine, hip, forearm and whole body on both the Explorer and Discovery. Linear correlation analysis with the intercepts unrestricted revealed high BMD correlation (r > 0.99) at all sites. Since the intercepts were not statistically significant, the slopes and RMSE were calculated with the intercept restricted to zero. None of the slopes were statistically different from unity, and all of the RMSE's were less than 0.020 g/cm^2.

In addition to BMD, correlation of whole body composition results were compared. The agreement of total mass was exceptional, with an r = 0.9998, no intercept, a slope of 1.0, and an RMSE of only 164 g. The percent fat of the two instruments was also very highly correlated at r = 0.995, and had a small offset of $1.2 \pm 0.6\%$. However, with the intercept restricted to zero, the slope was 1.000 ± 0.005 and the RMSE was only 0.7%. The lean mass did not exhibit a significant offset, had a slope of 1.000 ± 0.002, and an RMSE of 470 g. The precison of the whole body composition on the Explorer was measured on fourteen adults measured in triplicate with repositioning. The fat mass precision, expressed as a standard deviation, was 294 g and the lean mass precision was similar at 308 g.

The two fan beam densitometers exhibited a close correspondence for both BMD and body composition, with results similar to comparisons between pencil beam densitometers of the same manufacturer.

Table: BMD correlation results

	r	Slope $\pm$ SEE	Intercept	RMSE g/cm^2
AP Spine	0.993	0.999 ± 0.004	N.S.	0.019
Total Hip	0.996	1.004 ± 0.003	N.S.	0.016
Femoral Neck	0.995	0.993 ± 0.004	N.S.	0.016
Radius + Ulna	0.993	0.998 ± 0.003	N.S.	0.008
Whole Body	0.991	1.000 ± 0.003	N.S.	0.016

N.S. is not significant.

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SPATIAL AND TEMPORAL CHANGES IN BONE ARCHITECTURE DUE TO AGING AND OVX IN RAT TIBIAE

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Bone loss due to ovariectomy (OVX) is a well-known rat model for osteoporosis. It is known that local differences exist in spatial and temporal bone changes in this model, though it is not known how these differences arise. In this study we used novel in-vivo micro-CT technology combined with image registration to detect and follow these subtle changes in bone architecture of rats after OVX and aging.

Ten female 10-month old Wistar rats were divided in a sham operated and an OVX group. Over one year post-operation, the right proximal tibiae of the rats were scanned at various time-points, using an in-vivo micro-CT scanner (Skyscan 1076). The animals were scanned at week 0, prior to surgery, and at week 4, 14, 34 and 54 post-surgery. Here we present the data of the first three time-points.

All data sets were repositioned to exactly match the scan at week 0 using image registration software and segmented using an automated local threshold algorithm. For the trabecular bone, we calculated volume fraction (BV/TV) and trabecular thickness (Tb.Th).

In the first 4 weeks the OVX animals had a 31% decrease in BV/TV, which proceeded to 53% after 14 weeks. Bone loss started in the more centrally located trabeculae. Between 4 and 14 weeks, part of the trabecular bone that initially remained intact, was resorbed as well. Besides bone loss, new bone formation occurred at the endosteal cortex. Between 4 and 14 weeks remaining trabeculae increased in thickness (from 139 mm to 153 mm) for all animals and even complete new trabecular structures were formed.

The sham operated rats gave similar results. After 14 weeks BV/TV had decreased 16%, while the thickness of the remaining trabeculae increased, again for all animals.

The changes in bone structure for both groups followed a similar pattern. Decrease in bone volume was followed by an increase in the thickness of non-resorbed trabeculae. The similarity between age related bone loss and bone loss caused by estrogen depletion seems to indicate that similar mechanisms are at work, and that estrogen depletion might have resulted in an increase in the speed at which these mechanisms inflict changes in the ovx group.

The fact that some trabeculae increase in thickness while at the same time their neighbors are being resorbed suggests that bone cells sense spatial differences. We hypothesize that the spatially distributed mechanical loading is the driving force behind these differences in cell behaviour.

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FALL INDEX A HIP FRACTURE PREDICTOR INDEPENDENT OF AGE AND BONE DENSITY

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The risk of fracturing a hip is related to several factors such as bone mineral density (BMD), bone distribution, age, height, and weight. Modern bone-densitometers can measure structural parameters beyond BMD, including cross sectional moment of inertia (CSMI) and cross sectional area (CSA) at the femoral neck. Models have been proposed that combine density, structure, age, height, and weight to produce a Fall Index (FI). FI estimates the ability of a hip to withstand a fall on the greater trochanter, with larger values indicating greater strength and decreased risk (Yoshikawa et al, JBMR 9:1053–1064). In this study, we compared femoral BMD with CSMI, CSA, and FI for assessing hip fracture risk.

A total of 422 women (58 had a prior hip fracture; 364 controls) had a DXA scan using the Lunar Prodigy (GE Medical Systems). For the fracture subjects, DXA measurements were performed on the non-fractured femur. BMD of the femoral neck was determined, as well as CSMI, CSA, and FI using the Lunar Hip Strength Analysis program. Results for fracture cases and controls were compared using an unpaired t-test.

No significant trend was observed with age for the BMD-adjusted CSA, CSMI and FI values. Femoral neck BMD was significantly lower in the fracture group compared to controls. After adjustment for BMD, neither CSMI nor CSA were significantly different between groups. However, FI was significantly lower in the fracture group, consistent with a reduced capacity to withstand a fall. We conclude that femoral neck BMD is an important predictor of femoral fracture. Measurements of femoral geometry, which are based on BMD distribution, did not provide additional predictive power compared to BMD alone. The Fall Index, which combines BMD, geometry, age, height, and weight into a single risk factor, is a significant predictor of hip fracture, even after adjustment for age and BMD.

Table:

	Age	Height	Weight	Neck BMD*	CSMI	CSA	FI
Fract. Group	77 yrs	160 cm	61.7 kg	0.659	10689	149 cm2	1.54**
Control Group	76 yrs	157 cm	62.6 kg	0.748	10284	148 cm2	1.62

**in g/cm2; *Significantly different ($P < 0.01$)

P199

THERAPEUTIC EFFICACY OF RISEDRONATE IN MEN WITH OSTEOPOROSIS ONE YEAR RESULTS FROM 316 PATIENTS

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In postmenopausal osteoporosis clinical studies with Risedronate have shown rapid fracture reduction at vertebral and non-vertebral sites after only 6 months of treatment. This bisphosphonate proved to be equally effective in glucocorticoid-induced osteoporosis and interestingly, the risk reduction observed in male and female subgroups was not different. In the current study, we examine the effects of Risedronate on vertebral fractures and BMD mean change in lumbar spine, femoral neck and total hip BMD only in men with primary and secondary osteoporosis. Secondary endpoints include non-vertebral fractures, height loss, pain, safety, and tolerability. In this single center, open label, matched pair-controlled prospective clinical study, we enrolled 316 male patients with T-score values of lower than −2.5 SD at lumbar spine (LS) and lower than −2.0 SD at the femoral neck (FN) with or without prevalent vertebral fractures (vert-fx). The patients were allocated in pair-wise fashion into two treatment groups. Patients in Group A (n = 158; 81 with, 77 without prevalent vert-fx) received Risedronate 5 mg plus calcium 1000 mg and 800 IU Vit. D daily. Group B comprised equally 158 men. Those with a prevalent vert-fx (subgroup B1 n = 81) were treated with alfacalcidol 1 mg plus calcium 500 mg daily, whereas patients without prevalent vert.-fx (subgroup B2, n = 77) were treated with calcium 1000 mg plus 800 IU plain vitamin D daily. In group A 64 patients (41%) and in group B 66 (42%) had secondary osteoporosis. BMD measurements and x-rays were performed at baseline and 12 months thereafter. After this first year of treatment men receiving Risedronate showed a mean LS-BMD increase of 4.7% compared with a mean increase of 1.0% in Group B patients ($P < 0.001$). The mean change of total hip BMD was 2.7% and 0.4% for groups A and B, respectively ($P < 0.001$). Corresponding changes at the FN were 1.8% and 0.3% for the respective groups ($P < 0.001$). During the 12 months of therapy in 5% (8/158) of patients of Group A and in 12.7% (20/158) of Group B new vert-fx were recorded (RR 0.4, Fisher exact test; $P < 0.028$). The corresponding incidences for patients with new non-vert-fx were 10 and 17, (RR 0.59, n.s due to insufficient power). Both therapies were well tolerated. We conclude that Risedronate therapy reduces the risk of new vertebral fractures by 60% and significantly increases BMD at all measurement sites in men with osteoporosis within one year.

P200

A POOLED ANALYSIS OF THE SAFETY AND EFFICACY OF RISEDRONATE IN PATIENTS WITH REDUCED RENAL FUNCTION

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The objective of this analysis was to investigate the influence of renal function on the safety and efficacy of risedronate in a population of osteoporotic women.

The analysis included osteoporotic women enrolled in the placebo-controlled phase III clinical trials. Patients received either placebo or risedronate 5 mg daily. For each patient, creatinine clearance was estimated using the Cockcroft and Gault method based on baseline serum creatinine, body weight and age. The incidence of AE's, renal function-related AEs, and incidence of new vertebral fractures were summarised for each treatment group based on FDA criteria; Mild ($> = 50 - <80$ mL/min), Moderate ($> = 30 - <50$ mL/min) or Severe (<30 mL/min) renal impairment.

8996 patients were classified as having at least mild creatinine clearance at baseline (Placebo: n = 4500, Risedronate 5 mg: n = 4496). The incidence of AE's, and renal function-related AEs was similar between treatment groups within and across the subgroups. The incidence of fractures for risedronate was similar across all renal impairment subgroups. Statistically significant fracture risk reduction was observed across all three renal impairment subgroups.

In conclusion risedronate significantly reduces vertebral fracture risk in patients with impaired renal function. In addition, patients taking risedronate with renal impairment exhibit no significant increase in the incidence of overall AEs or renal function-related AE's compared to placebo.

Incidence of New Vertebral Fracture
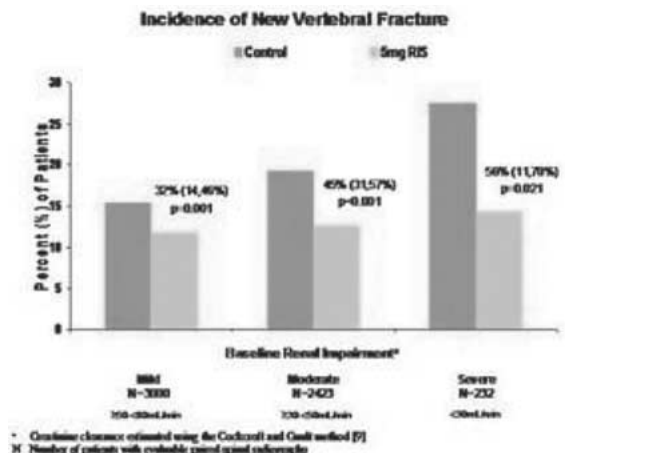
P201

DIFFERENCES IN EARLY DYNAMICS OF SERUM BONE MARKERS IN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS TREATED BY ALENDRONATE OR RISEDRONATE

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Bisphosphonates exert their action on bone mostly by reducing bone resorption. The exact dynamics of serum bone markers after initiation of bisposphonate therapy are unclear and early changes of serum bone markers have not been evaluated. In this study, 50 women with postmenopausal osteoporosis, with a mean age of 55 + 1 years were randomized to treatment with alendronate (AL, 70 mg once a week) or risedronate (Ris, 5 mg/day). Serum levels of C-telopeptides (CTX) and bone alkaline phosphatase (BAP) were determined. Serum samples were obtained for bone markers after 15, 30, 60 and 90 days of therapy. AL treated pts (n = 25) presented a progressive decrease of median serum CTX over 90 days (15days: −17%; 30days: −28%; 60days: −48%; 90days: −54%). While Ris treated patients (n = 25) rapidly reached the nadir by 15 days (15days: −56%; 30days: −57%; 60days: −57%; 90days −58%). The difference in serum CTX between AL and Ris at day 15 and 30 was stat. significant ($P < 0.05$). In AL treated pts, median BAP levels started to change after 60 days (15days: 0%; 30days: 0%; 60days: −8%; 90days: −17%). While in Ris treated pts, median BAP started to decrease after 30 days (15days: 0%; 30days: −16%; 60days: −28%; 90days: −29%). The difference in BAP between AL and Ris was statistically significant at 30, 60 and 90 days ($P < 0.05$). In conclusion, Ris reduces bone turnover more rapidly than AL. The onset of drug action may be important, especially in those with high fracture risk.
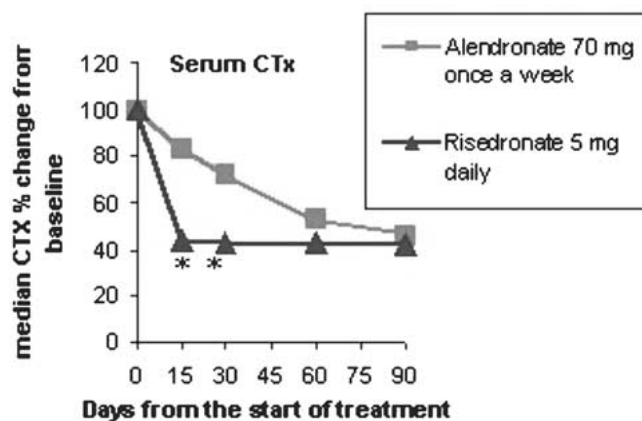
P202

QUANTITATIVE ULTRASOUND VARIABLES ARE SENSITIVE TO CHANGES IN CORTICAL POROSITY

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The main mechanisms of cortical bone loss are endosteal resorption and increases in porosity. In order to distinguish these processes diagnostic measures that show differential sensitivity to changes in geometry and porosity are required. We investigated the sensitivity of speed of sound (SOS) measured by different Quantitative Ultrasound (QUS) methods to changes in cortical geometry and porosity.

53 human proximal finger phalanges were harvested in autopsy courses from female donors. The age range was 62 to 97 years (mean ± SD: 82.2 ± 8.1 years). All phalanges underwent examinations using transverse transmission ultrasound (DBMSonic 1200, Igea, Italy) and axial transmission ultrasound (Omnisense, Sunlight Ultrasound Technologies, Israel). Results were compared with cortical geometry and porosity obtained from Micro Computed Tomography (μ CT, Fan Beam Microscope, Stratec, Germany). Using multifactorial regression models the impact of geometry and porosity on SOS was calculated. Based on these results, we calculated whether SOS is more affected by changes in porosity or geometry (assuming the same change in bone mass).

SOS in transverse transmission (SOStransverse) was best predicted by a combination of cortical cross-sectional area and cortical porosity ($R2 = 0.69$, $P < 0.0001$) while SOS in axial transmission (SOSaxial) was primarily affected by a combination of cortical thickness and cortical porosity ($R2 = 0.52$, $P < 0.0001$). A change in porosity equivalent to a 1% change in bone mass results in a 0.2% change in SOStransverse and a 0.6% change in SOSaxial whereas the same change in bone mass via a change in bone geometry would result in a 0.1% change in SOStransverse and a 0.4% change in SOSaxial. A simulation of ultrasound propagation in the phalanx resulted in similar values of SOS changes.

Our results indicate that SOS is sensitive to changes in bone geometry and porosity. For a given change in bone mass, the sensitivity to changes in porosity as compared to changes in bone geometry is about two times higher for both techniques. However, these results are based on cross-sectional data and they need to be confirmed in longitudinal studies, which could be performed in animals. Our results indicate that QUS methods may help to distinguish endosteal and periosteal changes from changes caused by porosity. Moreover, it appears that QUS is sensitive to detect treatment induced changes in porosity.

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INCREASED PROTECTION FROM NONVERTEBRAL FRACTURE ASSOCIATED WITH LONGER DURATION OF TERIPARATIDE THERAPY

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Profiles of the change in lumbar spine BMD over time show continuous increase for at least 2 years of therapy with teriparatide [rhPTH (1–34)]. More clinically relevant is the extent to which increases in duration of therapy decrease the rate of fracture. Data on the time to first nontraumatic nonvertebral fracture from the Fracture Prevention Trial (Neer NEJM 2001) allow us to quantify this effect. Postmenopausal women with osteoporosis and prevalent vertebral fracture were randomized to placebo (N = 544), teriparatide 20 mcg (N = 541) or teriparatide 40 mcg (N = 552) once daily for a median of 19 months (max 2 years). The rates of patients sustaining fracture diverged throughout the trial (Figure). Cox regression modeling was not able to distinguish significant effects for baseline factors of age, vertebral T-score or the presence of multiple vertebral fractures in predicting nonvertebral fracture. There also was no significant difference per month of therapy (treated as a time-dependent covariate) between the 20 and 40 mcg/day doses. In contrast, the hazard ratio (95% CI) for the combined teriparatide groups versus placebo decreased proportionally 9.1% ($P = 0.002$) for each additional month of therapy, i.e., 0.909 (0.857–0.964). This is equivalent to a 68% decrease per year. We conclude that there is added clinical benefit to increased duration of teriparatide therapy throughout the 2 years studied in this trial.
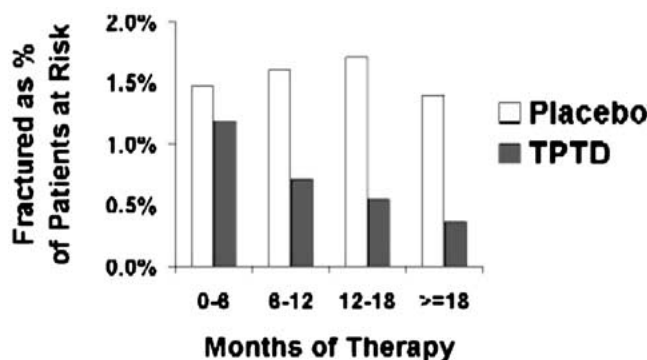
P204

CALCITRIOL (ROCALTROL) IN A THERAPY OF OSTEOPOROSIS

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INTRODUCTION: The calcitriol i.e. rocaltrol is the biological active metabolite of vitamine D3 that increase the absorption of calcium from the gastrointestinal tract and help the reabsorption of calcium in the kidneys.

With this mechanism, calcitriol stimulate the bone mineral density through the bone remodeling.

OBJECTIVE: The purpose of this study was to evaluate the efficiacy and drug safety of calcitriol i.e. rocaltrol in a therapy of corticosteroid induced osteoporosis.

MATERIAL AND METHOD: The open clinical randomized controlled study was designed in this investigation. Total of 43 patients suffering from the inflammatory rheumatic diseases and corticosteroid induced osteoporosis were included. The control group received the calcium 500 mg as evening dosage only. Bone densitometry was performed during the baseline period and at 3, 6 and 12 months after starting the treatment with this drug. Adverse events were detected at each visit using evidence based questioning. Clinical laboratory tests were performed as well.

RESULTS: The results obtained in this study indicate the increase in BMD even after the first 3 months with gradually increase after 12 months. The mean percentage changes in T-score among subjects in calcitriol group were significantly higher ($P < 0.001$) than those among in the control group.

CONCLUSION: These results after one year of therapy with calcitriol in corticosteroid induced osteoporosis resulted in significant and progressieve increase in BMD. Also, calcitriol was well tolerated. No vertebral or nonvertebral fractures occured over 1 year of therapy.

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TANNING IS ASSOCIATED WITH OPTIMAL VITAMIN D STATUS AND HIGHER BONE MINERAL DENSITY IN YOUNG AND MIDDLE AGED ADULTS

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Vitamin D is a steroid hormone that is made naturally in the skin after exposure to sunlight. The increased concern for skin cancer has created a fear of casual sunlight exposure. While prolonged ultraviolet irradiation increases the risk for many skin cancers, decreased sunlight exposure leads to vitamin D deficiency. Adequate vitamin D status is important for optimal bone health. chronic vitamin D deficiency can lead to osteomalacia and osteoporosis in adults. Clinical studies with vitamin D have demonstrated a positive effect on bone mineral density after supplementation with vitamin D compared to non-supplemented groups. Thus, we postulated that adults who use tanning beds containing the vitamin D producing UVB radiation (between 280 to 315nm) should be able to make vitamin D in their skin and raise circulating 25-hydroxyvitamin D levels. We conducted a study to determine the serum levels of 25-hydroxyvitamin D and bone mineral density at the hip and spine in a group of adults who use tanning beds and in a control group of non-tanners. Healthy adults between the ages of 18 and 70 participated in our study. A total of 166 subjects enrolled into the study. One hundred and six subjects were classified as non-tanners or controls and 50 subjects were classified as tanners. Tanning subjects had a higher mean 25(OH)D compared with non-tanning controls, 46.2 ± 3.0 versus 24.1 ± 1.0 ($P < 0.05$). Tanning subjects also had lower PTH values compared to controls, 21.4 ± 1.0 versus 25.3 ± 0.8 ($P = 0.01$). Tanning subjects had significantly higher BMD and z-scores at the hip compared to non-tanning subjects ($P = 0.04$ for both). We found that there was significant positive relationship between 25(OH)D and BMD at both the hip (R = 0.05, $P = 0.003$) and spine (R = 0.06, $P = 0.004$). We conclude that the use of a tanning bed results in higher serum 25(OH)D which may be beneficial to optimal bone health.

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COMPARISON OF OSTEOPOROSIS SCREENING TOOLS THE HEEL ULTRASOUND MEASUREMENT VERSUS THE CALCULATED RISK ASSESSMENT TOOL

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Heel ultrasound can provide low-cost measurements to identify women likely to have osteoporosis at the hip or spine as measured by DXA. Here we determined the specific T-score cutpoint on the Lunar Achilles InSight (GE Medical Systems) that detects 90% of individuals with osteoporosis (T-score £−2.5) at either hip or spine. The Achilles InSight is an imaging heel ultrasonometer with 8–10 second measurement time (the complete examination takes 3–5 minutes). We also compared the performance of heel ultrasound screening to a simple risk assessment tool based on weight and age.

DXA scans of the spine and hip were obtained in 272 women aged 40 to 83 years (mean age 58 ± 7 years) using a Lunar DPX (GE Medical Systems). All subjects also had a heel ultrasound measurement using the Achilles InSight. Osteoporosis was diagnosed if the lowest DXA T-score at spine (L1–L4) or hip (total femur) was £−2.5. The heel T-score cutpoint with 90% sensitivity was determined using ROC analysis. For each subject the value of the osteoporosis risk assessment tool (risk tool in short; defined as (Weight in kg—age)*0.2)) was also calculated.

From the DXA results, 54 of 272 patients were classified as osteoporotic. At a heel T-score of −1.0, sensitivity was 91% and specificity was 46%. Based on ROC analysis, the area under the curve (AUC) for the heel T-score was 0.79, which was significantly greater than the AUC for the risk tool, 0.69 ($P < 0.05$). The AUC for the combination of the heel T-score and the risk tool was 0.81 (0.75–0.87).

We conclude that the Achilles bone ultrasonometer can be used as a valid screening tool for osteoporosis, with over 90% sensitivity at a T-score of −1.0 and good specificity. Moreover, the Achilles performs significantly better in identifying those patients who should be considered for spine and hip bone density assessment compared to the calculated osteoporosis risk assessment tool.

Table:

	AUC	90% Sensitiv. Cut-Off	Sensitivity	Specificity
Heel T-score	0.79 (.72−.85)	−1.0	90.7%	46.3%
Risk Tool	0.69 (.62−.77)	2.4	90.7%	28.9%

P207

THE PRODIGY ADVANCE 10 SECOND QUICKVIEW MODE A PRECISION COMPARISON WITH THE DISCOVERY

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Precision is of primary importance for BMD measurements. Patient BMD changes slowly, hence, precision errors should be minimal to provide an accurate assessment of BMD change over time. On the other hand, there is also the demand for faster measurements. We determined the precision error of the 10 second QuickView mode of the Lunar Prodigy Advance (GE Medical Systems). The precision error of this 10 sec scan mode was compared to the standard 30 sec scan mode and to the 10 sec scan mode of the QDR-Discovery (Hologic). Precision was evaluated using 3 different phantoms: a prototype European Spine Phantom, the Lunar aluminum spine phantom, and the Hologic spine phantom. Each phantom was measured 15 times in each scan mode on both the Prodigy Advance and the QDR-Discovery. The phantom was not repositioned between repeated measurements. Acquisition and analysis were performed according to the manufacturer's instructions for measuring AP spine BMD. The precision error for the Prodigy Advance 30-sec standard mode ranged between 0.29% and 0.35%. The precision error for the Prodigy QuickView 10 sec mode ranged between 0.31% and 0.73% while that of the QDR-Discovery 10 sec mode ranged between 0.65% and 1.47%. Although the Prodigy Advance exhibited the lowest precision error with the 30 sec mode, the 10 sec QuickView precision error, was shown to be consistently lower than the QDR-Discovery 10 sec mode. We conclude that the 10 sec QuickView mode displayed acceptable behavior for BMD assessment.
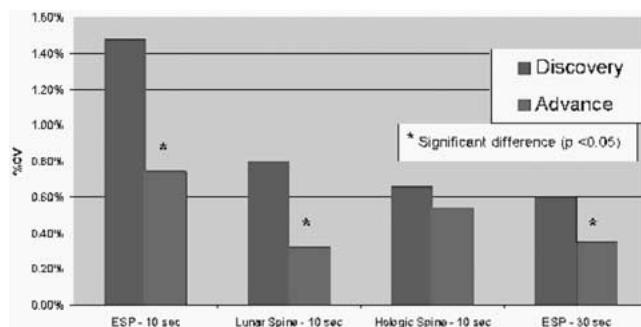
P208

ZOLEDRONATE TREATMENT IN AN ADOLESCENT FEMALE WITH HYPERGONADOTROPIC HYPOGONADISM AND OSTEOPOROSIS SECONDARY TO CHEMOTHERAPY AND IRRADIATION FOR WILMS TUMOR

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Ovarian failure is frequently associated with irradiation and chemotherapy in female patients treated for Wilms tumor. Ovarian protection from irradiation is difficult and inadequate. Estrogen deficiency is closely related to the development of osteoporosis. Adolescent girls are at risk for osteoporosis secondary to estrogen deficiency. Bisphosphonates are the treatment of choice. Zoledronate third generation bisphosphonate is new more potent drug in this class. It is more convenient and easier to administer and more effective in inhibiting skeletal morbidity.

We present 17 2/12 year old female with ovarian failure and osteoporosis secondary to chemotherapy and irradiation for Wilms tumor. She was diagnosed at age 12. She underwent surgical excision of the kidney and adrenal gland followed by a course of radiation and chemotherapy. At age 14.5 she had minimal breast development and no menstrual periods. FSH and LH levels were elevated and estradiol was low. Growth velocity was suboptimal with the normal IGF-1 level. Bone age was delayed. Initial DEXA scan was consistent with the low bone mineral density (BMD) with the L2–L4 Z-score of −3.0. Low dose estrogen therapy was initiated. Calcium supplementation and the increased intake of dairy products were recommended.

Follow up DEXA scan in 6 months indicated worsening of BMD with the L2–L4 Z-score of −3.2. Pubertal progress was noticeable and growth velocity improved. Estrogen dose was increased. Oral bisphosphonate therapy was initiated.

She achieved menarche at age 16 9/12 years. Follow up DEXA scan indicated minimal improvement in BMD with the L2–L4 Z-score of −2.7.

Zoledronate treatment was recommended in order to improve the BMD and for the achievement of peak bone mass. She tolerated Zoledronate infusions well. She had no significant changes in serum calcium levels or side effects related to Zoledronate therapy. She continues with Zoledronate therapy. Follow up DEXA scan is planed in the near future.

Bisphosphonate therapy prevents development and progression of osteoporosis and benefits the achievement of peak bone mass in long term childhood cancer survivors.

Controlled trials are needed to assure long-term safety and efficacy of Zoledronate therapy in pediatric patients with gonadal failure and osteoporosis secondary to chemotherapy and irradiation.

P209

SPINE BMD MEASUREMENTS WITH AND WITHOUT CONVENTIONAL LEG ELEVATION ON THE LUNAR BRAVO

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Measurement of lumbar spine bone mineral density (BMD) using DXA traditionally requires hip flexion and elevation of the legs with a positioning device. This 'legs up' position flattens the lower spine, with the intent of yielding more accurate and precise BMD values. We investigated whether not elevating the legs had a significant impact on accuracy and precision of spine BMD measurement.

Twenty-six subjects were scanned six times at the lumbar spine (Lunar Bravo, GE Medical Systems). Three scans used standard positioning with the legs elevated on the spine block positioner supplied by the manufacturer. The other three scans were done without the spine positioner, with the legs flat on the scan table and feet secured with the dual femur positioner. Subjects were repositioned between the scan positions. Spine BMD (L1–L4) was determined using the manufacturer recommended analysis procedure. The influence of leg position on L1–L4 BMD was compared using paired T-tests, and the precision error (%CV) was calculated for both positions.

There were no obvious visual differences between the scans performed with the legs up or down; disc spaces and vertebral bodies appeared identical. There was a small, but statistically significant, difference of 2.0% in L1–L4 BMD between legs up and legs down values ($P < 0.001$). In the majority of subjects, BMD values were higher with the legs down. Precision (CV) was good in both positions: 1.1% legs up vs.1.5% legs down. Linear regression analysis confirmed that BMD measurements with legs up and down were highly correlated (r = 0.99), with slope near unity (1.01), an intercept near zero (0.035) and an SEE of 0.022. Because of the high BMD correlation, mathematical adjustments within software produced comparable T-scores between the two measurements. After adjustment, T-scores were not significantly different in scans performed in the two positions.

We conclude that differences in BMD that result from measurement of the spine with legs positioned for a femur scan are small. Precision was similar for both positions. With appropriate adjustment, T-scores for spine scans measured with the legs down were equivalent to those obtained with legs up. Use of a legs-down position for spine scans might result in considerable time saved for both patient and clinic.

P210

A STUDY OF BONE STATUS IN AGEING RENAL TRANSPLANT RECIPIENTS

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INTRODUCTION: As renal transplant recipients age they may suffer from involutional osteoporosis and and 25-hydroxy vitamin D deficiency which are common in older populations. Prolonged corticosteroid therapy compounds their abnormal bone quality due to renal osteodystrophy. This study investigates the bone status of these patients.

METHODS: 42 patients >60 years were randomly selected from the transplant list. They underwent DEXA, PTH, 25 (OH)D, creatinine clearance (Cr/Cl using the Cockraft and Gault equation), osteocalcin (OC) bone alkaline phosphatase (BALP), tartrate resistant acid phosphatase (TRACP) and C-telopeptide (CTx).

RESULTS: 22 m and 18 fm participated with a mean ($+$ SEM) age was 65.5 $+ 1.0$ years. Mean bone biochemistry was BALP $15.4 + 1.2$ (ref range <17 mcg/l), OC $69 + 13$ (ref $10–50$ ng/ml), TRACP $4.3 + 0.3$ (ref <5.33 U/L), CTx 0.68 ± 1.0 (ref <1.0 mcg/l), PTH $103 + 12$ ($15–65$ pmol/l). Vitamin D $17.9 + 1.1$ (ref $20–60$ ng/ml) Mean hip BMD was $0.86 + 0.03$ g/cm2 and Cr/Cl was $52.8 + 3.3$ (ref $80–120$ mls/min).

CTx correlated with PTH (r = 0.64, $P < 0.001$) as did OC (r = .33, $P < 0.05$) and OC was the only one to correlate with vitamin D (r = −.38, $P < 0.05$). Both CTx and OC correlated with Cr/Cl (r = −.55, $P < 0.01$ and r = −.54, $P < 0.001$ respectively),whilst BALP and TRACP did not.

Osteoporosis was present in 35% of the group and 65% of females. Hip BMD correlated with CTx (r = −.34, $P < 0.05$), OC (r = −0.47, $P < 0.01$), BALP (r = −.47, $P < 0.01$) and TRACP (r = −.33, $P < 0.05$). Hip BMD correlated with Cr/Cl (r = .43, $P < 0.01$) and PTH (−.39, $P < 0.05$).

Three markers correlated well, CTx and OC (r = .51, $P < 0.01$), CTx and BALP (r = .47, $P < 0.01$), OC and BALP (r = .62, $P < 0.0001$). CTx and TRACP correlated less well (r = .46 $P < 0.05$).

CONCLUSIONS: Osteoporosis was highly prevalent (in 35% of patients). Hip BMD was influenced firstly by renal function and secondly by PTH. Increasing bone resorption was reflected by CTx but not by TRACP. Hypovitaminosis D (<20 ng/ml) was common but neither bone turnover or BMD was influenced by it.

P211

OXIDATIVE STRESS AND BONE HEALTH: ROLE OF THE ANTIOXIDANT LYCOPENE IN THE ACTIVITIES OF OSTEOBLASTS AND OSTEOCLASTS

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The objective of this submission is to review the current state of knowledge on the role of oxidative stress induced by reactive oxygen species (ROS) in bone health, and to present data from some of our studies on the role of the antioxidant lycopene in the activities of osteoblasts and osteoclasts, as well as our current clinical study on the role of oxidative stress and lycopene on bone turnover markers in postmenopausal women. Bone is is continuously being renewed throughout life by the process of bone remodeling. This process involves the coupled events whereby osteoclasts remove old bone and osteoblasts form new bone. A disturbance in the remodeling process is associated with a number of diseases of the skeletal system. There is now convincing evidence to suggest that oxidative stress may contribute to the pathogenesis of the skeletal system in osteoporosis (Rao & Rao, Nut Genomics & Functional Foods 1:35–44, 2003), periodontal disease and rheumatoid arthritis (Rao & Rao, Adv Food Nut Res, submitted, 2003). The evidence will be reviewed. Our preliminary observations showed that hydrogen peroxide-induced ROS inhibited cell proliferation of osteoblasts in a dose-dependent manner, and a generator of ROS, TNF-alpha, inhibited mineralised bone nodule formation in mineralising culture of SaOS-2 cells. We have reported in a recent publication that lycopene, the potent carotenoid antioxidant from tomatoes and tomato products, stimulated cell proliferation and differentiation of SaOS-2 cells in a differentiation-stage dependent manner (Kim et al, J of Med Food 6:79–88, 2003). In another report, we showed that lycopene inhibited the basal and PTH-stimulated multinucleated osteoclast formation in rat bone marrow cultures and the ROS-mediated resorption by these osteoclasts (Rao et al, J of Med Food 6:69–78, 2003). A clinical study is underway in our laboratory to test the role of oxidative stress as a risk factor for osteoporosis in postmenopausal women and to carry out intervention studies with placebo control, lycopene capsules, regular tomato juice, and tomato juice rich in lycopene. In conclusion, there is now strong evidence for the involvement of oxidative stress in bone health, and the results of our previous and current

studies should clarify the mechanisms of the deleterious action of oxidative stress and the beneficial action of the antioxidant lycopene in maintaining bone health.

P212

RAPID RESOLUTION OF THE REDUCTION OF BONE TURNOVER MARKERS AFTER DISCONTINUATION OF RISEDRONATE IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS PREVIOUSLY TREATED FOR 2 YEARS

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Recently, 35 mg risedronate once-a-week (OaW) was shown to have a similar effect on bone mineral density (BMD) and bone turnover markers (BTM) as 5 mg once daily. In the present study, we evaluated the resolution of the reduction of BTM after discontinuation of risedronate.

Subjects were recruited from a group of postmenopausal women who participated in a randomized, double-blind, active-controlled, 2-year study to evaluate the efficacy and tolerability of risedronate OaW (35 mg and 50 mg) compared with risedronate 5 mg once daily in the treatment of postmenopausal osteoporosis (Brown JP et al. C TI 2002; 71:103–112). All were aged > 50 yrs with either a BMD T-score of −2.5 or lower (lumbar spine or proximal femur) or a T-score lower than –2 and at least one prevalent vertebral fracture. Following its termination all subjects were invited to participate to this extension study and those who agreed were randomly (block randomization by centers) assigned to receive risedronate 35 mg OaW (30 mg tablet + 5 mg tablet) or no active treatment for 6 months. All subjects also received 1 g daily of elemental calcium supplementation.

The primary objective was to determine the resolution time of the reduction on bone resorption markers (serum CTx and urinary NTx) after discontinuation of risedronate. The resolution time was defined as the earliest time point where the median percent change (increase) in sCTx or uNTx is > 100% compared to the baseline value obtained after 2 years of oral risedronate. If we assume that the previous treatment lead to at least a 50% reduction in sCTx and uNTx, the resolution time is the time when we achieve resolution of at least 50% of the reduction of bone turnover induced by risedronate.

In conclusion, there is an early resolution (3 months) of the reduction of bone resorption markers after discontinuation of therapy in postmenopausal women with osteoporosis previously treated for 2 years with risedronate.

Table: Median% Changes in Bone Turnover Markers vs. Baseline

	1 mo;% (SEM) N = 44	2 mo;% (SEM) N = 44	3 mo;% (SEM) N = 44	6 mo;% (SEM) N = 42
sCTX Rise	+ 7.3 (7.9)	+ 10.5 (10.5)	+ 18.1 (8.5)	+ 24.4 (24.4)
sCTX Control	+ 54.4 (9.2)	+ 76.9 (9.2)	+ 101.8 (9.5)*	+ 117.4 (14.5)*
uNTX Rise	+ 5.4 (6.4)	+ 7.8 (8.9)	− 0.4 (11.9)	+ 5.8 (8.9)
uNTX Control	+ 28.2 (8.9)	+ 44.2 (10.9)	+ 48.7 (36.5)*	+ 71.3 (19.2)*
BSAP Rise	− 0.7 (2.6)	+ 3.8 (2.6)	+ 3.0 (5.3)	+ 2.4 (3.0)
BSAP Control	+ 0.0 (7.7)	+ 10.3 (2.9)	+ 13.7 (4.2)	+ 22.1 (3.9)*

*p value vs control < 0. 0001.

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CONTINUOUS ADMINISTRATION OF RISEDRONATE FOR 2 YEARS AND BIOCHEMICAL MARKERS CHANGES IN EARLY MENOPAUSAL WOMEN WITH HIGH BONE TURNOVER

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Aim 1) investigating the 2 year effect of daily administration of risedronate to early menopausal women, correcting at the same time 25(OH)2D low levels that ranged from 7.9–21.0 (± 11.0), (normal 9.2–45.2) 2) assessing their quality of life.

MATERIALS - METHOD: 40 early postmenopausal women, 48–53 years old (mean 50), 6 months – 1 year after menopause, with T-score ≤ 2SD in DEXA of the sacral spine, no prior metabolic disorder. Divided in two groups. Group A (n = 30) received 5 mg risedronate, 1 mcg 1α-OH-D3 and 1000 mg calcium bicarbonate daily, while group B (n = 10): 1 mcg of 1α-OH-D3 and 1000 mg of calcium bicarbonate daily, for the first year. During second year, group A received 5 mg of risedronate and 1000 mg of calcium, while group B

received only 1000 mg of calcium. sCTX (normal 250–6114PM) and uNTX (normal 14–74BCE/mM) after a 2-hour fast were measured at 6, 12, 24 months. A questionnaire was completed at 0, 6, 12, 24 months concerning drug tolerance, compliance, mobility, pain, mental health, quality of life. Analysis used student *t*-test.

RESULTS: statistically important decrease in bone markers was observed in group A at 6, 12 and 24 months in relation to baseline values and in relation to group B that demonstrated an increase of the above markers.In group A, a statistically important decrease in sCTX by 11.69% ($P < 0.0005$) at 6 months, by 9.63% ($P < 0.0005$) at 12 months, by 9.65% ($P < 0.0005$) at 24 months was noted. After 2-hour fast uNTX was decreased by 16.95% ($P < 0.0005$) at 6 months, by 8.15% ($P < 0.0005$) at 12 months, by 9.10% ($P < 0.0005$) at 24 months. In group B an increase in sCTX by 8.9% ($P = 0.014$) at 6 months, by 13.3% ($P < 0.0005$) at 12 months, by 16.5% ($P < 0.0005$) at 24 months was observed. Group B uNTX increased by 5.1% ($P = 0.006$) at 6 months, by 10.9% ($P = 0.002$) at 12 months, by 14.5% ($P < 0.005$) at 24 months. A statistically important intergroup difference at 6, 12, 24 months of therapy was observed. Calcium and vitamin D administration during the first year assisted in adequate Vit D levels.

CONCLUSION: The changes observed in markers of bone resorption demonstrated that risedronate resulted in an early decrease of bone loss (as early as 6 months) and assisted in the improvement of bone metabolism after 2 years of therapy. Patients demonstrated good tolerance and compliance to treatment, improved mobility and quality of life as well as better mental health.

P214

PROSTHETIC HIP ANALYSIS AND MARKERS OF BONE METABOLISM IN COMPARISON TO CLINICAL OUTCOME AFTER TOTAL HIP ARTHROPLASTY

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The increasing rate of hip fractures is giving rise to a number of socio-economic problems for the aging community. In addition to being unable to resume their previous living habits, many patients fail to achieve full functional recovery after the fractures. Total hip arthroplasty (THA) is a successful operation for the majority of patients with all forms of hip fractures, being performed increasingly often throughout the world. Revision rates for THA range up to 20% per year. Aseptic loosening is the reason for 75% of the revisions.

In an open study, 40 women and men who had undergone cementless total hip arthroplasty after accidental hip fractures were tested for local bone loss with prosthetic hip analysis by DEXA and one year after operation. Simultaneously the patients received a specific antiresorptive treatment and an analysis of bone metabolism throughout the observation period of one year. The results of this 12-month clinical observation showed a significantly improvment in the clinical outcome of patients following THA, with reduced bone turnover markers by ongoing antiresorptive treatment.

In addition the functional status of this group of patients was improved and their risk of falling was reduced by rehabilitation during the observation period of 12 months.

The regional analysis of prosthetic bone loss after THA seems to be a verx helpfull tool to improve the general clinical outcome after THA.

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BROADBAND ULTRASOUND ATTENUATION COMPARED WITH DUAL-ENERGY X-RAY ABSORPTIOMETRY IN SCREENING FOR OSTEOPOROSIS IN AN ASIAN POPULATION

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Background: Quantitative ultrasound (QUS) of the calcaneus is an alternative technique to dual-energy x-ray absorptiometry (DEXA) for assessing bone mass. Various studies have demonstrated moderate correlation between broadband ultrasound attenuation (BUA) and bone density in the Caucasian population.

Objective: To study the correlation between BUA and bone density, and the potential use of C.U.B.A. Clinical System in screening for osteoporosis in an Asian population.

Methods: 608 subjects had QUS examination of the calcaneus to establish the BUA normative data for the Singapore population. 32 of these subjects had DEXA scans of the hip done. Values for BUA of the calcaneus and bone mineral density of the hip are compared and correlated.

Results: The correlation is moderate with a correlation coefficient of 0.6872 ($P < 0.0001$). This is comparable to results of published series for the Caucasian population.

Conclusions: QUS can be used as a screening tool for detecting osteoporosis in the elderly Asian population.

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HORIZON-PIVOTAL FRACTURE TRIAL UNIQUE DESIGN OF A RANDOMIZED, PLACEBO-CONTROLLED TRIAL TO EXAMINE THE EFFECT OF ANNUAL INFUSION OF ZOLEDRONIC ACID (5 MG) ON HIP AND SPINE FRACTURE REDUCTION

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A single infusion of zoledronic acid has been shown to increase bone mineral density (BMD) at the spine by approximately 5% and decrease bone resorption by about 50% at 12 months compared with placebo. However, the potential to reduce fracture risk is not known. To evaluate the effect of once-yearly zoledronic acid on modifying fracture risk, we are conducting a 3-year, randomized, placebo-controlled trial known as HORIZON-PFT.

7764 women between the ages of 65 and 89 years were recruited from 230 clinical centers in 27 countries, representing a diverse population with respect to socioeconomic, racial and cultural background. Major inclusion criteria were: a femoral neck BMD t-score less than or equal to −2.5, or between −1.5 and −2.5 in the presence of at least one vertebral deformity. Participants were randomized to placebo or zoledronic acid (5 mg delivered in 100 ml volume, 15 minute infusion). Study medication is administered annually, and participants are followed for 3 years. All participants receive 1000 to 1500 mg of elemental calcium plus 400 to 1200 IU of vitamin D per day (minimum background therapy). Co-primary endpoints are hip fractures and new vertebral fractures. Secondary efficacy endpoints include: non-vertebral fractures; change in BMD by DXA; changes in biochemical markers of bone metabolism; and changes in bone density and size by QCT. Safety endpoints include evaluation of adverse events, assessment of bone histology by histomorphometry, and post-dose monitoring for acute changes in renal lab values.

An innovation in this trial is that women who were taking non-bisphosphonate medications for osteoporosis, including HT, SERMS, and calcitonin ("usual care"), were allowed to participate. For the co-primary endpoints, the effect of the study medication on hip fractures will be tested in all women, whereas the effect on vertebral fractures will be tested in the subset (6113) who at baseline were not taking "usual care" medication. The study was designed under the expectation that about 58% of participants would be taking "usual care" medications, but in fact only 21% were actually enrolled into this subset.

Hormones, Including Estrogen, Vitamin D, PTHrP

P217

POOR VITAMIN D NUTRITIONAL STATUS IS COMMON IN AN OUTPATIENT CLINIC FOR INTERNAL MEDICINE IN A RURAL AREA OF THE NETHERLANDS

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Introduction: Gastrointestinal and hepatic disorders are frequently associated with metabolic bone disorders. Malabsorption of vitamin D and calcium contribute to the development of osteoporosis and eventually bisphosphonate therapy may be needed. Vitamin D deficiency is regarded as a major problem for immigrants to the Netherlands, but other parts of the population have not well characterized in terms of 25(OH)D concentration.

Aim of the study: To characterize the prevalence of vitamin D deficiency in a rural area in the Netherlands (Oost Groningen).

Materials and Methods: At an outpatient clinic for internal medicine one physician measured 25(OH)D in all patients at risk and visiting his office between January 2nd till June 30th, 2003. Patients at risk for hypovitaminosis D were systematically checked according to the local guideline (Diagnostisch Kompas 2003). The patients in this study belonged to categories with either a diminished intake, diminished ultraviolet exposure, diabetes mellitus, alcoholism, elevated alkaline phosphatase levels and decreased serum albumin levels.

Reference values in our hospital for vitamin 25(OH)D are: 20–100 nmol/l. Values below 40 nmol/l are classified as insufficient.

Results: 86 patients were severely vitamin D deficient, defined as < 20 nmol/l. Mean age 62 year, standard deviation $\pm$ 21 year, range 21–91 year. Eleven (13%) had a level < 10 nmol/l. Of the 86 patients 15 (17%) were non-western immigrants, 71 (83%) were typically Dutch patients.

Conclusion: Reliable procedures for measuring 25(OH)D can serve as a tool to reduce a major determinant of the healthcare budget. This is also relevant to patients with a white skin and Dutch background. On the 12th Workshop on Vitamin D last summer in Maastricht, dr. Lips published "in treatment or prevention programmes it would be prudent to aim at serum 25(OH)D of 50 nmol/l". Clearly we have a major task at hand to lower the prevalence of vitamin D deficiency in our population.

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EFFECTS OF PARATHYROIDECTOMY ON THE PRODUCTION OF THE BONE RESORPTIVE CYTOKINES INTERLEUKIN-6 AND TNF-ALPHA IN PRIMARY HYPERPARATHYROIDISM

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Background: Increased bone turnover is a prominent feature of primary hyperparathyroidism (PHPT). *In vitro* studies have suggested that the bone resorptive actions of PTH are relayed by enhanced production of cytokines of the TNF and IL-6 families. In the present study, we examined cytokine production in whole-blood cultures from twenty-one patients with primary hyperparathyroidism.

Methods: The LPS induced capacity for cytokine production (TNF-alpha, IL-6 and the soluble receptor sIL-6R) was examined by ELISA before and one year after curative parathyroidectomy (PTX). Changes in BMD and bone turnover markers were also recorded.

Results: LPS induced IL-6 production decreased by 19% ($P < 0.05$), sIL-6R by 12% ($P < 0.05$) and TNF-alpha by 22% ($P = 0.14$) after PTX, indicating increased capacity for cytokine production by leucocytes harvested in the hyperparathyroid state. Further, there was a positive pre-operative correlation between IL-6 production and BMD of the spine(r = 0.49, $P < 0.01$), as well as a strong negative correlation between IL-6 and bone turnover markers (P1CP r = −0.57, $P < 0.01$, P1NP r = −0.56, $P < 0.01$, cross-links r = −0.48, $P < 0.05$ and U-NTX r = −0.48, $P < 0.05$). TNF-alpha showed positive, pre-operative correlation with spine BMD (r = 0.52, $P < 0.01$) and negative correlation with bone turnover markers P1NP (r = −0.44, $P < 0.05$) and U-NTX (r = −0.40, $P < 0.05$).

Conclusions: We conclude that IL-6, sIL-6R and TNF-alpha production by LPS stimulated blood leucocytes is increased in primary hyperparathyroidism, compared with cytokine production after surgical cure. The correlation analyses do not, however, suggest that the effect of PTH on the skeleton is directly mediated through the TNF- or IL-6 cytokine pathways. The increased cytokine production from peripheral leucocytes may not mirror conditions in the bone microenvironment, or increased TNF and IL-6 production could represent a second-line mechanism for relaying resorptive effects to a more PTH-resistant skeleton.

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THE HORMONAL INFLUENCE ON THE DEVELOPMENT OF SLIPPED CAPITAL FEMORAL EPIPHYSIS. AN OLD THEORY REVISITED

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Aims: Slipped Capital Femoral Epiphysis (SCFE), is a relatively rare type of fracture that occurs during the early stages of adolescence. In order to assess the potential pathologic influence of any hormonal disorders or fluctuations on the development of SCFE, we conducted a prospective clinical study with 14 patients.

Methods: Seven boys and seven girls suffering from SCFE were included in this study. The levels of Thyroid Hormones [3.5.3 -Triiodothyronine (T3), Thyroxine (T4) & Thyroid Stimulating Hormone], Testosterone, Estradiol, Dehydroepiandrosterone Sulfate (DHEA_S), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), I-Parathyroid Hormone (I-PTH), human Growth Hormone (hGH), Adrenal Cortex Hormone (ACTH) and Cortisol, along with serum Calcium (Ca), Phosphorus (P) and Alkaline Phosphatase (ALP) levels were measured. All other necessary anthropometric (age, height, weight & sexual development according to the Tanner classification) and clinical (degree and location of slipping) data were also taken into account.

Results: The results showed an increased incidence of pathological values. Fifty two out of a total of 168 hormonal determinations (31%) were pathological (either above or below normal). Serum I-PTH, FSH, LH and testosterone determinations were the ones that revealed the bulk of the pathological values. None of the patients had any clinical findings whatsoever that suggested the existence of an endocrinopathy.

Conclusions: The increased incidence of hormonal disorders that were detected in patients suffering from SCFE together with the recent developments in the understanding of the homeostasis of the growth plate shed new light in the etiology of this disease and actually re-animate the hormonal intervention as a contributing factor in the pathogenesis and development of this multi-factorial disease. We believe that a (possibly) temporary hormonal disorder (and not necessarily a type of endocrinopathy) during the early years of adolescence, may play a potentially significant role (along with others etiologic factors: e.g. obesity, minor or major trauma, growth plate_s planarity and inclination angle, insufficiency of hydrostatic and tensile growth plate s components) in the development of SCFE.

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ORAL VITAMIN D3 SUPPLEMENTATION IN POSTMENOPAUSAL AFRICAN AMERICAN WOMEN

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BACKGROUND: Vitamin D insufficiency is a major problem among African American women especially for those living at higher latitudes during the winter season. Prevention of postmenopausal bone loss could help prevent development of osteoporosis later in life. We conducted a randomized, placebo controlled, double-masked study to test the hypothesis that vitamin D3 supplementation would prevent bone loss in calcium-replete, postmenopausal women.

SPECIFIC AIM: We hypothesize that vitamin D supplementation (intended to increase serum 25-OHD to "optimal" levels) would decrease postmenopausal bone loss in calcium-sufficient African-American women.

METHODS: 208 healthy black postmenopausal women, 50–75 years of age, were randomly assigned to receive either placebo or 20μg daily of vitamin D3. Calcium supplements were provided to ensure a total calcium intake of 1200–1500 mg/d in both groups. After 2 years, the vitamin D3 dose was raised to 50 μg/d in the active group and the study continued for an additional year. BMD of the total body, spine, total hip, and mid radius were measured every 6 months by dual energy x-ray absorptiometry. Bone turnover markers, vitamin D metabolites and parathyroid hormone levels were measured in serum.

RESULTS: There were no significant differences in bone mineral density between the active and control groups throughout the study. There was a transient increase in total body, femur, and radial BMD at 6 months in both groups. Over the 3 years, BMD declined at these sites by .26 to .55%/year. The BMD of the lumbar spine increased slightly in the placebo group and active group, respectively. Serum 25-OHD increased from 47 nM/L to 71 nM/L at 3 months in the active group and by an additional 20 nM/L 3 months after the increase in dose to 50 μg/d. Approximately 40% participants in Ca + D group were still under 80 nM/L in spite of supplementation. There were no persistent changes in serum PTH levels or the markers of bone turnover, although there was a transient decline in PTH in both groups at 3 months. No significant adverse events were attributed to vitamin D supplementation.

CONCLUSIONS: There was no effect of vitamin D3 supplementation on bone loss or bone turnover markers in African-American, postmenopausal women. Further studies are needed to determine if these findings are applicable to other ethnic groups or if higher amounts of vitamin D would have an effect.

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BONE MINERAL DENSITY AND BONE BIOCHEMISTRY IN NUTRITIONAL OSTEOMALACIA

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INTRODUCTION: There is a paucity of data regarding Bone Mineral Density(BMD) & bone biochemistry in osteomalacia and its response to therapy.

AIM: To study changes in BMD & bone formation/resorption markers with therapy in patients with nutritional osteomalacia.

METHODS: This study of 6 months duration included 16 women with nutritional osteomalacia & 10 age & BMI matched controls. Patients were studied as two separate groups, one group received Vitamin D as cholecalciferol, & the other as calcitriol. Both groups received elemental calcium of one gram as calcium carbonate supplement

CONCLUSION: 1) Low bone mineral density might be an indicator of osteomalacia as well as osteoporosis & Elevated S.alk.Phosphatase would be consistent with the diagnosis of osteomalacia.

3) Dramatic increase in BMD is seen on therapy at spine and hip & rate of gain attenuates with time.

4) Restoration of Alkaline Phosphatase and U. Telopeptides would indicate adequacy of Therapy.

5) The effect of calcitriol therapy is seen early and is greater than with cholecalciferol whose effect is more sustained.

Table: RESULTS (mean ± sem)

VIT D	VIT D 0 months	VIT D 3 months	VIT D 6 months	CALCITR 0 months	CALCITR 3 months	CALCITR 6 months	CONTROL
S.25 VD (ng/ml)	4.41 ± .5	72 ± 9.56	72 ± 9.56	7.2 ± 9.5	22.47 ± 3	21.85 ± 2	11.21 ± 1
S.PTH pg/ml	267.6 ± 2	142.2 ± 1	44.4 ± 4.	301.1 ± 5	162.5 ± 3	57.97 ± 1	34.9 ± 3.
Se alk (K.A un	21.1 ± 1.	13.73 ± 1	11.02 ± 1	20.31 ± 1	14.9 ± 2.	12.52 ± 1	5.65 ± .6
Sosteoc (ng/ml	0.8 ± .16	8.73 ± 2.	14.47 ± 3	1.03 ± .0	4 ± .8	8.47 ± 1.	14.21 ± 1
U.CTx mcg/mmo	3428 ± 83	2941 ± 78	3313 ± 10	3181 ± 69	3439 ± 93	4784 ± 14	894 ± 98
%change BMD	0–3 mths	3–6 mths	0–6 mths	0–3 mths	3–6 mths	0–6 mths	
Lumbar spine	78.6 ± 18	12.34 ± 2	46.68 ± 9	99.43 ± 2	35.8 ± 6.	73.35 ± 1	
Hip	82.9 ± 17	19.2 ± 3.	53.29 ± 1	108.58 ±	40.77 ± 9	80.24 ± 1	

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SERUM LEVELS OF 25-HYDROXYVITAMIN D AND FUNCTIONAL RECOVERY AFTER HIP-FRACTURE

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Prevalence of vitamin D deficiency is high in hip-fracture patients, a population characterized by poor functional recovery and high risk of permanent disability. Given the effects exerted by vitamin D in muscle health, it is intriguing that vitamin D depletion may affect restoration of function after hip-fracture. We evaluated the association between serum levels of 25-hydroxyvitamin D and functional recovery in 315 of 350 Caucasian hip-fracture patients consecutively admitted to our rehabilitation hospital. All the fractures were either spontaneous or caused by minimal trauma (trauma equal to or less than a fall from a standing position). None of the patients was currently treated with either vitamin D or its derivatives. The patients underwent 25-hydroxyvitamin D measurement by an immunoenzymatic assay, 21.3 ± 8.1 (mean $\pm$ SD) days after the hip-fracture occurrence. Functional recovery was evaluated by using Barthel Index score.

Low levels of 25-hydroxyvitamin D were found (median = 6.9ng/ml; interquartile range = 8.4 ng/ml). Median Barthel index score was 45/100 before rehabilitation and 90/100 after rehabilitation (interquartile range = 25 and 30 respectively). All the data were non-normally distributed. At Spearman's rank test, a significant positive correlation was observed between serum 25-hydroxyvitamin D and Barthel Index score assessed on both admission to rehabilitation (r = 0.218; $P < 0.001$) and discharge from the hospital (r = 0.198; $P < 0.001$). Linear multiple regression was performed including Barthel index score as the dependent variable (after area transformation, given non-normal distribution). At multiple regression, the association between serum 25-hydroxyvitamin D and Barthel index score was independent of eleven confounding variables: age, sex, hip-fracture type, pressure sores, cognitive impairment, neurologic impairment, infections, time between fracture occurrence and 25-hydroxyvitamin D evaluation, comorbidity, surgical procedure type and previous hip fractures. The percent of the variance of Barthel Index score accounted for by the set of independent variables was 25% before and 31% after rehabilitation. Our data indicate that serum levels of 25-hydroxyvitamin D are positively associated with the functional recovery after hip fracture. It is hypothesized that ensuring an adequate vitamin D intake in the elderly may result in reducing disability after hip-fracture. This issue should be addressed by specific intervention trials.

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BODY COMPOSITION, LEPTIN AND FEMORAL BONE MINERAL DENSITY IN HIP-FRACTURE WOMEN

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Fat body mass (FBM) is a strong predictor of both bone mineral density (BMD) and risk of hip fracture, but the mechanisms responsible are

not completely understood. Several authors investigated the hypothesis that leptin may be the link between FBM and bone, but the published data are conflicting. Recently, leptin has been suggested to exert opposite effects in bone health depending on bone tissue, skeletal maturity, sex and/or signalling pathway: it may actually exert different effects on the bones of different subjects. As a consequence, the relationship among FBM, leptin and BMD must be evaluated in different populations. Our aim was to evaluate whether leptin is the link between FBM and bone in a sample of hip-fracture women, a group of patients not previously investigated. Sixty-two of 74 Caucasian women with hip fracture were evaluated. Serum leptin was measured by radioimmunoassay, 23.4 ± 9.1 days (mean $\pm$ SD) after fracture occurrence. BMD and body composition were assessed by dual-energy X-ray absorptiometry (DXA). In the 62 women, leptin concentration was 26.5 ± 17.4 g/L (mean $\pm$ SD), whereas FBM was 18.198 ± 7.190 g. As expected, a positive linear correlation was found between FBM and both leptin ($r = 0.782$; $P < 0.001$) and femoral BMD measured at five sites (r value ranging from 0.293 to 0.498 depending on the site of the femoral BMD assessment, $P < 0.05$). A positive correlation between leptin and BMD measured at the intertrochanteric area ($r = 0.259$; $P < 0.05$) but not at the other four sites was shown. At linear multiple regression (dependent variable = femoral BMD; independent variables = age, weight, height, body mass index, fracture type, term fracture-DXA, Barthel index score, FBM, lean body mass, serum PTH, serum 25-(OH)vitamin D and leptin), FBM was positively associated with BMD measured at all the five sites. The association between leptin and BMD was inverse and it was significant at four of the five sites of the BMD assessment.

In a sample of hip-fracture women, the positive association between FBM and femoral BMD was not explained by serum leptin. On the contrary, after adjustment for FBM and other confounding variables, an inverse association between leptin and BMD was found. Interestingly, a similar inverse association has been previously shown in samples of both men and premenopausal women: leptin seems to exert unfavourable effects on BMD in various patients, including hip-fracture women.

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LONGER DURATION OF TERIPARATIDE THERAPY WAS ASSOCIATED WITH DECREASED RISK OF BACK PAIN

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In the Fracture Prevention Trial, postmenopausal women with osteoporosis randomized to teriparatide [rhPTH (1–34)] 20 mcg/day (TPTD20) or 40 mcg/day (TPTD40) for a median of 19 months had reduced risk of new or worsening back pain compared to women on placebo (Neer, 2001). To examine whether a longer duration of TPTD therapy was associated with a decreased risk of new or worsening back pain, we analyzed new or worsening back pain data collected as part of adverse event reports. The reduction in risk of new or worsening back pain with additional months of TPTD was analyzed using a Cox regression model. There was no significant difference per month of therapy between TPTD20 and TPTD40. The overall and severe back pain hazard ratios (HR, 95% CI) for TPTD20 versus

placebo were 0.913 (0.893–0.933) and 0.889 (0.837–0.944), respectively ($P < 0.0001$). Thus, for each additional month of TPTD the HR decreased by 8.7% (6.7%–10.7%) for overall back pain and by 11.1% (5.6%–16.3%) for severe back pain. The cumulative HR reductions after 18 months of TPTD were 80.6% (71.5%–86.8%) for overall back pain and 88.0% (64.3%–95.9%) for severe back pain. Time to first back pain with placebo and TPTD20 during consecutive 6-month intervals is presented below. A longer duration of TPTD was associated with a decreased risk of new or worsening back pain in postmenopausal women with osteoporosis. Mechanisms for this effect may include a time-dependent progressive reduction in clinical vertebral fracture risk.

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EFFECTS OF TESTOSTERONE IN A RAT MODEL OF OSTEOPOROSIS COMBINING DISUSE AND ORCHIDECTOMY

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Risk factors for osteoporosis include disuse and reduction in sex steroids. The orchidectomized (ORX) rat is a suitable model for male osteoporosis due to hypogonadism. An IM injection of botulinum neurotoxin (BTX) produces a paralysis and induces a localized bone loss. ORX and BTX were combined to evaluate the preventive effect of testosterone (TESTO) on bone loss.

36 aged male rats were randomized into 3 groups. Animals were either SHAM operated; ORX + BTX (right hindlimb); ORX + BTX + TESTO with subcutaneous injection of TESTO (30 μg/kg/day). ORX and BTX were done the same day; TESTO therapy began on the day of surgery. Animals were euthanazied after one month. DXA was used to measure total and regional BMC and lean masses. Measurements were done separately on both hindlimbs and on excised right vs. left tibia and femur. This model allows a separate study of the effects of ORX on the left limb and ORX + BTX on the right limb. Histomorphometry was done on the tibia to measure BV/TV and trabecular characteristics (Tb.Th, Tb.N, and Tb.Sp). Microarchitecture was analyzed by X-ray microtomography on the femur; BV/TV3D and Structure Modeling Index (SMI) were measured.

ORX induced a decrease in total lean body mass (−14%) and fat content (−6.9%). ORX (on the left side) induced a significant decrease in BMC (−11.2%), BV/TV, BV/TV3D and Tb.N whereas Tb.Sp increased significantly. Tb.Th did not decrease significantly and SMI increased significantly confirming an increased conversion of plates into pillars. The effect of ORX + BTX (on the right side) was cumulative: BMC–33.5%. Histomorphometric changes were maximized on BV/TV, BV/TV3D, Tb.N, Tb.Sp and SMI on the paralyzed side.

TESTO therapy almost prevented the loss of total lean mass (−4.7%) and increased fat content (+7.6%). However, it did not prevent BMC loss (−9.1%) and failed to preserve histomorphometric parameters on the left side. On the right side, TESTO had a minimal effect on BMC (−27.9%) and had no effect on histomorphometric and 3D alterations. TESTO did not prevent the massive and acute bone loss induced by the combination of factors.

Our findings suggest that treatment with testosterone is not sufficient to prevent bone mass decrease and microarchitecture degradation in the aged male rat. Additional therapies must be recommended to prevent osteoporosis and associated microarchitectural evolution.

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SERUM LEPTIN LEVEL IS AN INDEPENDENT PREDICTOR OF BONE TURNOVER IN ADULT GROWTH HORMONE DEFICIENCY (AGHD)

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Leptin has been shown to stimulate osteoblastic cell proliferation, differentiation and mineralisation and inhibit osteoclast generation. However, the association between leptin and markers of bone turnover has not been consistently demonstrated and the aim of this study was to assess whether leptin independently predicts bone turnover.

Leptin and the markers of bone turnover exhibit a circadian rhythm, therefore, we sampled peripheral venous blood at half-hourly intervals in 12 AGHD patients (6 men) over a 24 hour period (1400–1400 h) prior to and after
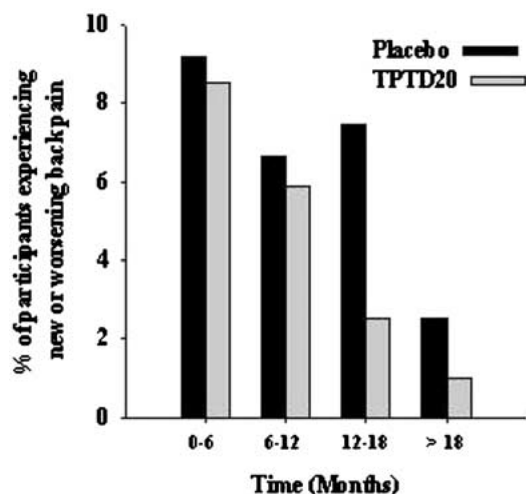
1 and 12 months of GHR. Multiple linear regression was used to identify independent predictors of bone turnover. Leptin, PTH, serum phosphate and serum adjusted calcium were included in stepwise models with type 1 collagen C-telopeptide (CTX: bone resorption) and procollagen type1 amino-terminal propeptide (PINP: bone formation), taken as dependant variables.

At baseline, leptin (regression coefficient (B) = 0.009, standard error (SE) = 0.001, $P < 0.001$) and phosphate (B = 0.08, SE = 0.04, $P < 0.05$) were identified as independent predictors of CTX (goodness-of-fit expressed by adjusted $R^2 = 0.8$) whereas only leptin (B = 0.43, SE = 0.05, $P < 0.001$) and calcium (B = 31.57, SE = 14.47, $P < 0.05$) were found to be significantly associated with PINP ($R^2 = 0.6$). At 1 month, leptin (B = 0.014, SE = 0.002, $P < 0.001$) and phosphate (B = 0.190, SE = 0.062, $P < 0.01$) were associated with CTX ($R^2 = 0.8$) and leptin (B = 0.61, SE = 0.07, $P < 0.001$) alone was associated with PINP ($R^2 = 0.7$). At 12 months leptin (B = 0.021, SE = 0.002, $P < 0.001$) and PTH (B = 0.054, SE = 0.018, $P < 0.01$) were found to be significant predictors of CTX ($R^2 = 0.7$) and leptin (B = 0.689, SE = 0.088, $P < 0.001$) and phosphate (B = 10.60, SE = 4.24, $P < 0.05$) were predictors of PINP($R^2 = 0.6$).

Our results show that leptin is an independent predictor of both, bone resorption and bone formation and may therefore play a role in regulating bone turnover. Following GHR for 12 months, PTH became a predictor of bone turnover, suggesting a possible increase in sensitivity of bone to the effects of PTH, which is in keeping with our studies.

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IN VIVO OSTEOPROTEGERIN (OPG) AND RANKL MRNA AND PROTEIN DETECTION AFTER HIGH DOSE CORTICOSTERONE (CT) AND/OR INTERMITTENT PARATHYROID HORMONE (1–34) (PTH) ADMINISTRATION, AND RECOVERY IN RAT BONE

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Glucocorticoid (GC) and PTH effects on OPG and RANKL are not well defined. OPG decreases in GC-induced osteoporosis, while *in vitro* PTH causes up and down-regulation of RANKL and OPG, respectively. The aim of this study was to detect OPG and RANKL in rat bone after treatment with GC, PTH or both, and after recovery.

Four groups of about 200 g male Wistar rats were treated for three weeks as follows: CT s.c. 10 mg/day; PTH (1:34) s.c. 80ug/Kg, three times a week; CT+PTH; Control (C), s.c. PBS. Two other groups were treated with CT and CT+PTH as above and sacrificed after two weeks of recovery (REC). Distal femurs were embedded in glycolmethacrylate for bone histomorphometry, and the proximal tibiae were paraffin embedded for OPG and RANKL immunodetection and in situ hybridization. Polyclonal antibodies (Santa Cruz Biotechnology) and double human FITC hybriprobes (Biognostik) were used.

Histomorphometric variables of both bone formation and resorption were reduced after CT and increased after PTH; intermediate values were obtained after CT + PTH. Recovery groups showed almost C values. OPG and RANKL mRNA and protein were both co-located in osteoblastic cells, maturative/hypertrophic chondrocytes, a few osteoclasts (OC), part of osteocytes (OS), several bone marrow cells (megakariocytes, adipocytes) and sinusoids. The primary bone matrix, some cement lines and the calcified cartilage-bone interface were OPG positive.Osteoblast vacuolizations were visible in CT and PTH-treated groups, which also showed islets of hemopoietic positive cells next to trabeculae. Numbers of RANKL-positive OS were higher than OPG-positive OS after CT or PTH, and both decreased through C values (72–77%) after recovery. OS numbers decreased in CT and increased in PTH and REC groups.

The inhibitory effect of CT was partly contrasted by PTH. The co-localization of OPG and RANKL was apparently not changed by treatment, in spite of the changes induced in cell numbers and activities. OPG in bone matrix could have a protective action on resorption.

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DIMINISHED CALCITONIN RESERVE IN HEALTHY POSTMENOPAUSAL WOMEN

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BACKGROUND: The increased CT secretion is responsible for the rapid initial decrease in the bone resorption following an acute intravenous calcium load in healthy young women (Zikan V, Stepan J Calcif Tissue Int, in press). Aim: The aim of this study was to assess the CT reserve in postmenopausal women.

METHODS: We evaluated the biological response to calcium load in nine healthy postmenopausal women (mean age, 59 ± 5 years, more than 5 YSM) compared to the young healthy women (mean age, 29 ± 8 years). In addition, 8 women after total thyroidectomy (aged 58 ± 3 years) were tested. After overnight fasting, intravenous infusions of 1,7 mg of elemental calcium/kg body weight over a 10 min period were given. Blood samples for measurements of serum ionized calcium (S-iCa), plasma intact CT and parathormone (PTH) and plasma C-terminal telopeptide of collagen type 1 (beta-CTX) were obtained 3 min before and at 13, 30, 60, 90 and 150 min after the start of the infusion.

RESULTS: A similar increase in S-iCa and decrease in plasma PTH levels were observed in the healthy young and postmenopausal women. However, only in the healthy young women, the plasma CT increased significantly by 13 min ($P < 0.05$) and beta-CTX significantly decreased as early as at 30 min ($P < 0.05$, decrease by 35% as compared with the baseline). In the postmenopausal women the significant decrease of the plasma beta-CTX was observed at 60 minutes (decrease by 26%) and in thyroidectomized women after 150 minutes (decrease by 26%). The response to the calcium load in healthy postmenopausal or thyroidectomized women was significantly diminished throughout the study period as compared with the healthy young women ($P < 0.01$).

CONCLUSION: The delayed decrease in beta-CTX in postmenopausal women may be due to low CT reserve in these women.

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CHANGE IN SERUM FGF-23 AFTER 6 MONTHS GROWTH HORMONE REPLACEMENT IN ADULT GROWTH HORMONE DEFICIENCY

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Fibroblast growth factor-23 [FGF–23] is a recently discovered protein of 30Kda and 251 amino acids the concentration of which can be measured in plasma; it acts as a phosphatonin which either directly or indirectly inhibits phosphate transport through a PTH independent pathway.In adult growth hormone deficiency [AGHD] there is an increase in circulating phosphate [PO4] after growth hormone replacement [GHR]. We have measured FGF–23 and PO4 in fasting blood and urinary PO4 excretion in 24 hours prior to, and 6 months after, GHR in 20 AGHD patients [age 54 ± 12 yr, 12 M, 8F]. There was a significant increase in plasma FGF-23 from 144 ± 80 to 269 ± 213 RU/ml [$P = 0.004$] and PO4 from 1.11 ± 0.18 to 1.23 ± 0.17 mmol/L [$P = 0.005$], the increase in 24 h urinary PO4 excretion from 25.9 ± 9.9 to 30.5 ± 13.8 mmol/24 h was not significant. The increase in plasma FGF–23 after 6 m GHR was >20% in 14/20 [70%] and PO4 increased in 15/20 [75%] of patients. Increase in phosphate clearance [PCl] averaged 7.2% but 11/20 [55%] showed a decrease; the change in the percentage of tubular reabsorbed phosphate [TRP] averaged –0.7% with 13/20[65%] between −10% and +10%. The average decrease in the phosphate excretion index [PEI] was 18.6% but only 12/20 [60%] showed a decrease, statistical significance was not demonstrated in any of these changes. The increase in FGF–23 appears to be an attempt to counteract the alteration in the renal handling of phosphate and subsequent increase in plasma PO4 that is a result of GHR by increasing phosphaturia.

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THE ASSOCIATION OF SEX HORMONE LEVELS WITH POOR MOBILITY, LOW MUSCLE STRENGTH AND INCIDENCE OF FALLS AMONG OLDER MEN AND WOMEN

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The objective of this study was to examine whether low levels of total and bioavailable estradiol and testosterone are associated with impaired mobility, low muscle strength and the incidence of falls in a population-based sample of older men and women.

The study sample included 624 men and 662 women, aged 65–88 years, of the Longitudinal Aging Study Amsterdam (LASA). Levels of estradiol and sex hormone binding globulin (SHBG) were measured in all participants, whereas levels of testosterone were measured only in men. Physical performance, self-reported functional limitations and hand grip strength were assessed cross-sectionally, and a follow-up of falls was performed during three years. Multiple regression analyses and logistic regression analyses, stratified by sex, were performed to analyze the results. Potential confounders included age, level of education, alcohol use, physical activity, body mass index, depression and cognition.

In men, estradiol was not associated with physical performance, functional limitations or muscle strength. Men with the highest quartile of bioavailable estradiol (estradiol/SHBG) had significantly higher physical performance scores than men with the lowest quartile of estradiol (stand beta = 0.103). Continuous levels of testosterone were positively associated with physical performance and muscle strength (stand beta = 0.078 and 0.091 respectively). When using quartiles it appeared that men with testosterone levels in the second and fourth quartile had significantly higher muscle strength compared to men with testosterone levels in the first quartile (stand beta = 0.090 and 0.138 respectively). Bioavailable testosterone (testosterone/SHBG) had a positive association with physical performance (stand beta = 0.099). When using quartiles, men in quartiles 2 and 4 had a significantly higher performance score compared with the first quartile (stand beta = 0.099 and 0.119 respectively). In women, no associations of estradiol and bioavailable estradiol with physical performance, functional limitations or muscle strength were found. In both men and women, sex hormones were not associated with falls after adjustment for potential confounders.

It can be concluded that low levels of sex hormones were associated with measurements of impaired mobility and low muscle strength in men, but not in women. Low levels of sex hormones were not associated with the incidence of falls neither in men, nor in women.

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GROWTH HORMONE MODULATES A FUNCTIONAL RUNX2/CBFA1-STAT3B INTERACTION IN OSTEOBLASTIC CELLS

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Growth hormone (GH) action on bone is mediated either through insulin-like growth factor-1 (IGF-1), whose expression is controlled by GH, or directly by influencing the function of osteoblasts, the bone-forming cells. The present study aimed at exploring the molecular events that underlie the direct biological action of GH on osteoblastic cells, and, specifically, the effects that GH might exert on the function of the osteoblast-specific transcriptional regulator Runx2/Cbfa1.

Untreated and GH-treated human osteoblastic Saos-2 cells (a cell line known to respond to GH) and a variety of techniques such as western immunoblotting, electrophoretic mobility-shift assays, in vivo co-immunoprecipitations, in vitro GST pull-downs, two-hybrid interactions and luciferase assays were employed in our experimental system.

We demonstrated that GH signalling via Stat3/ERK MAPK potentiates the DNA-binding activity of Runx2/Cbfa1 in human osteoblastic cells but, at the same time, attenuates its transcriptional potential. Moreover, a novel physical interaction of Runx2/Cbfa1 with transcription factor Stat3b, that is enhanced by GH in these cells, was documented both in vivo and in vitro. Importantly, this interaction impairs the transcriptional activity of Runx2/Cbfa1 without affecting its DNA-binding capacity.

Our data provide the first evidence that GH modulates the transcriptional function of Runx2/Cbfa1 in osteoblastic cells by promoting its inhibitory interaction with Stat3b, an established target of various signals on bone tissue. Shedding light on such mechanisms will contribute to a better understanding of GH effects on bone homeostasis that may impact on decisions at the clinical level, especially in diseases affecting bone quantity and quality (e.g. osteoporosis).

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REDUCED BONE MASS IN CHILDREN WITH IDIOPATHIC HYPERCALCIURIA

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Idiopathic hypercalciuria (IH) is a common metabolic disease being present in the majority of cases of nephrolithiasis. The pathogenesis of this disorder has been attributed to an increased intestinal absorption of calcium (absorptive hypercalciuria) or to a primary calcium leak from renal tubules with a trend toward a compensatory hyperparathyroidism (renal hypercalciuria). In accordance with the pathogenetic mechanism, patients with renal IH could be expected to have diminished bone mass. In recent years, however, reduced bone density has been described in adults and in children with renal as well as absorptive hypercalciuria.

The aim of this study was to investigate the bone mass and biochemical parameters related to bone metabolism in children with absorptive and renal IH at the time of diagnosis and before any treatment had been started. Thirteen children (eight boys) aged two to eleven years with IH (2 renal IH and 11 absorptive IH) and nineteen healthy children of similar age and sex were studied. Serum intact parathormone (iPTH), bone alkaline phosphatase (bALP), osteocalcin (OC), amino-terminal and carboxyterminal propeptide of type I procollagen (PINP and PICP respectively), c-telopeptide (ICTP) and urine deoxypyridinoline (dPYR) were calculated. Bone mineral density was determined in the patients group by dual x-ray absorptiometry (DEXA) technique. Nine children with IH (1 out of 2 patients with renal IH and 8 out of 11 with absorptive IH) were found to have osteopenia or osteoporosis. Furthermore, the patients with IH had higher serum levels of PICP (marker of bone formation) than the controls (540 ± 196 µg/l vs 385.6 ± 96 µg/l, $P < 0.05$) and marginally higher urine dPYR (marker of bone resorption) than the controls (23.9 ± 15.5 vs 16.7 ± 11.6 nmol/mmol creatinine). The results suggest that an increased bone turnover may be a primary event in children with IH renal or absorptive type leading to osteopenia or osteoporosis.

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HIGH-DOSE OESTROGEN INCREASES APOPTOSIS IN OSTEOCYTES AND OSTEOCLASTS IN CORTICAL BONE IN POSTMENOPAUSAL WOMEN

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The skeletal effects of oestrogen (E) are predominately anti-resorptive. However, high-dose E produces anabolic effects with stimulation of osteoblastic activity in cancellous bone. Recently, increased cortical width has been reported in iliac crest biopsies in women treated with high-doses of E.

To investigate possible mechanisms for this increase we have examined cortical bone from transiliac bone biopsies obtained from 10 postmenopausal women treated with long-term high-dose E and 4 age-matched control women who had received no E treatment. Biopsies were decalcified and paraffin wax-embedded. Sections were immunolocalised for active caspase-3, a marker of apoptosis. Extent of specific protein staining was measured quantitatively by image analysis and expressed as a percentage area fraction of the cortical bone surface.

Within the cortex a 3.6-fold increase in caspase-3 protein expression was seen in the E-treated group (1.62 ± 0.63) compared to the untreated group (0.453 ± 0.51) ($P < 0.05$), particularly in the periosteal half of the cortex. The periosteal and endosteal ratio of caspase staining differed significantly between the two groups (0.77 in the untreated group and 2.18 in the E-treated group ($P < 0.05$). Caspase-3 expression in the E-treated group was seen predominately in osteocytes and cells within Haversian canals adjacent to the periosteal edge. All osteoclasts along the periosteal edge were immunoreactive for caspase-3 whilst osteoblasts were mainly negative. At the endosteal surface occasional osteocytes showed caspase-3 expression whilst osteoblasts were negative. In contrast, sections from the untreated group were less immunoreactive for active caspase-3. Occasional osteocytes adjacent to the endosteal surface and some osteoblasts on the endosteal edge stained positively. Osteocytes adjacent to the periosteal surface were mainly negative whilst some osteoblasts stained positively but osteoclasts showed no caspase-3 activity.

These results indicate that high-dose E increases apoptosis in cortical bone, predominately in osteoclasts and osteocytes in the periosteal half of the cortex. Apoptosis in osteoclasts is consistent with the known anti-resorptive effect of E whilst apoptosis in osteocytes may act as a recruitment signal to osteoblasts leading to increased collagen synthesis. These factors, together with the down-regulation in osteoblast apoptosis at the endosteal surface, may contribute to the observed E-induced increase in cortical width.

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A CROSS-SECTIONAL STUDY OF FINNISH 11-YEAR-OLD GIRLS THE RELATIONSHIP BETWEEN BONE MINERAL DENSITY AND SERUM 25-HYDROXYVITAMIN D

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BACKGROUND: Bone growth accelerates at puberty. It is thought that the bone accretion reaches its maximum 1 to 2 years before menarche. This sets high requirements for diet to provide enough mineral to build the bones. The importance of vitamin D intake in this respect is widely speculated, but previous studies have shown that serum 25-OHD concentration is related to increment of BMD as well as to thicker cortical bone.

OBJECTIVES: We studied the association of serum 25-OHD, PTH, BMD, and osteocalcin in cross-sectional study in early pubertal Finnish girls.

DESIGN: The subjects were 11-y-old girls (n = 196) at Tanner stage 1 or 2, studied between September and March. Their mean (sd) daily dietary intakes of calcium and vitamin D were 1240 mg (700 mg) and 4.7 µg (4.1 µg), respectively. We measured 25-OHD, PTH, and osteocalcin from serum samples. Bone densities were measured by DXA Hologic 4500 at lumbar spine (L1–L4) and left hip.

RESULTS: We found that height, weight and stage of puberty are the most powerful predictors of BMD in these girls. We verified a seasonal variation in the serum 25-OHD. The mean value for 25-OHD was 47.4 nmol/l (16.4 nmol/l), but it differed between months $P < 0.01$. The mean PTH concentration was 3.09 pmol/l (1.06 pmol/l) and for osteocalcin 45.1 ng/ml (9.2 ng/ml). PTH correlated negatively r = −0.325, $P < 0.01$ with S-25-OHD. Osteocalcin correlated with height r = 0.166 $P = 0.023$. The positive role of vitamin D on bone growth was not seen in the less mature girls (Tanner 1). In the peripubertal girls (Tanner 2) we, however, found that those with 25-OHD < 40 nmol/l had lower BMD in trochanter and total hip area ($P = 0.02$) than those with 25-OHD > 40 nmol/l. Interestingly PTH did not differ between these groups.

CONCLUSIONS: As the physical features are the most dominant predictors of BMD in adolescent girls, the impact of vitamin D status is less dominant. However, we found that peripubertal girls with 25-OHD < 40 nmol/l had lower cortical bone BMD than girls with more adequate vitamin D status. Our findings support the importance of vitamin D to bone growth in puberty.

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VITAMIN D DEFICIENCY IN INSTITUTIONALIZED PATIENTS

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Institiuitonalized patients present a special population who have high rates of osteoprorsis and fractures. IN a survey of 210 of these patients using heel desnitomtry, more than n 50% had a heel t-score less than −1.0. In proceeding with further evalauton it was noted that 40% of the patients had serum 25-OH vitmain D levels less than 32 ng/ml depsite supplemtnation with 400–800 IU of vitamin D per day. These data indicate that vitmain D deficieny can be a prevalent contribnutor to poor bone health and should be evaluated and treated prior to initiating treatment for osteoporosis.

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17β-ESTRADIOL ACTS ON FUSION, ADHESION AND APOPTOSIS IN DIFFERENTIATING AND MATURE MURINE OSTEOCLASTS

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Estrogen deficiency induces enhanced bone resorption which might lead to osteoporosis. The mechanism by which osteoclastic bone resorption is reduced by estradiol is not fully understood. We have previously shown that *in vitro* estradiol exerts a direct inhibitory effect on osteoclast activity and β3 integrin expression in human osteoclast progenitors. Therefore, the aim of this study was here to evaluate the effect of estradiol on adhesion and apoptosis in differentiating or mature murine osteoclasts. We used RAW 264.7 cells which were differentiated for 3 (D3, differentiating osteoclasts) or 5 days (D5, mature osteoclasts) with RANKL (30 ng/ml) in phenol red free αMEM supplemented with 10% FCS in the presence (E+) or absence (C) of 10–8 M 17β estradiol. At D5, estradiol reduced the number of TRAP + multinuclear cells by 50% compared to controls (3.1 ± 0.9 vs 6.1 ± 0.4/mm2, $P < 0.01$), as well as TRAP + mononuclear cells (237.6 ± 20.7 for E+ vs 306.4 ± 12.6 /mm2, $P < 0.01$). Moreover, estradiol decreases TRAP + multinuclear/mononuclear cells ratio, indicating that estradiol decreased the fusion of osteoclast precursors. The effect of estradiol on cell adhesion was tested. Differentiating and mature RAW cells cultured in the presence (E+) or absence (C) of estradiol were seeded *in vitro* nectin pre-coated plates and adhesion was assessed by crystal violet method. Estradiol, added for 2 hours during adhesion time of mature osteoclasts, induced a decreased cell adhesion by 35% compared to controls (1.01 ± 0.35 vs 1.33 ± 0.83 respectively). Apoptosis was then investigated through the caspase 3 activity by spectrofluometry method an expressed as AU/µg proteins. Caspase 3 activity increased in a serum-dependent manner (5.85 ± 0.58 for 10% FCS, 9.30 ± 1.38 for 1% FCS, 15.04 ± 0.89 for 0% FCS). Caspase 3 activity was evaluated in differentiating cells (D3) and in mature osteoclasts (D5) after 48 h exposure of estradiol. In differentiating cells, estradiol promoted caspase 3 activity (12.33 ± 2.35 vs 9.30 ± 1.38 in the presence of 1% FCS; 22.26 ± 3.89 vs 15.04 ± 0.89 at 0% FCS) as well as in mature osteoclasts (5.31 ± 0.72 vs 3.87 ± 1.04 with 1%FCS).

In conclusion, 17β estradiol decreased osteoclast resorption through several mechanisms by decreased fusion of precursors, adhesion of cells and promoting apoptosis in differentiating and mature osteoclasts.

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BONE MASS VARIES IN MAMMALS ACCORDING TO SERUM LEPTIN LEVELS.

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Leptin is a powerful inhibitor of bone formation by osteoblasts *in vivo*. This antiosteogenic function involves leptin binding to its receptors on ventromedial hypothalamic (VMH) neurons, the autonomous nervous system and b-adrenergic receptors on osteoblasts. However, the mechanisms whereby leptin controls the function of VMH antiosteogenic neurons remain unclear. In this study, we compared the ability of leptin to regulate body weight and bone mass and show that leptin antiosteogenic and anorexigenic functions are affected by similar amounts of leptin. Using a knock-in of LacZ in the leptin locus, we failed to detect any leptin synthesis in the central nervous system. However, increasing serum leptin level through transgenesis reduced bone mass, regardless of the amplitude of this increase. Conversely, reducing serum free leptin level by overexpressing a soluble receptor for leptin increased bone mass. Congruent with these results, the high bone mass of lipodystrophic mice could be corrected by restoring serum leptin level, suggesting that leptin is an adipocyte product both necessary and sufficient to control bone mass. Consistent with the high bone mass phenotype of lipodystrophic mice, lipodystrophic patients display an advanced bone age, a reflection of premature bone formation. Taken together, these results indicate that adipocyte-derived circulating leptin is a determinant of bone formation and suggests that leptin antiosteogenic function is conserved in mammals.

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CORRELATION BETWEEN DEFICIENCY OF 25 (OH) VITAMIN D AND LIVER DAMAGE IN ADULT TALASSEMIC SUBJECTS

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We have recently showed an absolute or relative deficiency of 25(OH) vitamin D in adult talassemic subjects compared to controls. To evaluate if this deficiency is often due to liver damage in talassemic patients, we have measured serum levels of 25(OH) vitamin D on 24 adult talassemics (range of age 20–41aa.). Serum levels < 15 ng/ml indicated relative deficiency of vitamin D; serum levels < 10 ng/mls indicated absolute deficiency of vitamin D.

Than, we have also evaluated circulating levels of ferritine, trasferrine, GT and hepatitis B or C infections.γTGO/P, 13 adult talassemic subjects showed a relative deficiency of vitamin D (54%), 4 adult talassemic subjects showed absolute deficiency. Compared to controls, adult talassemic subjects showed low bone mineral density (L1-L4 Z score: −2.71 vs −2.4 Ds) and highter levels of ferritine (991 ng/ml vs 550 ng/ml), TGP (36,4 ui/l vs 24 ui/l). Adult talassemic subjects didn't present statistically significative differences in GT, compared to controlss γserum levels of TGO and 20 adult talassemic subjects (83%) presented HBV infection and 8 HCV infection.

Our data confirmed an absolute or relative deficiency of 25(OH) vitamin D on adult talassemic subjects compared to controls. This deficiency increse with age. It' is unclear if cronic liver damage, due to iron deposition, causes this deficiency. Other studyes are necessary to demostrated this ipothesis.

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EFFECT OF IN VIVO INHIBITION OF OSTEOBLASTIC MMPS ON BONE LOSS INDUCED BY OVARIECTOMY

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Matrix metalloproteinases (MMPs) play a key role in bone matrix remodeling. We have shown previously that transgenic mice (TG) overexpressing TIMP-1 (tissue inhibitor of matrix metalloproteinase) specifically in osteoblasts exhibit transient increase in bone mineral density and bone volume. This phenotype has been shown to be associated to trabecular bone in one-month-old female mice.

The aim of our present study was to evaluate in vivo the role of MMPs on the bone loss induced by estrogen deficiency. Thus, we have ovariectomised or sham-operated adult wild type (WT) or transgenic mice (4 months). One group of ovariectomised mice were treated with estradiol (15 μg/kg/day) during 30 days. Animals bone mineral density was measured in total body, femur and lumbar vertebrae by DEXA (PIXIMUS) at day 0 (before ovariectomy) and at day 30 (after ovariectomy).

Our results show a significant overall bone loss in WT and TG ovariectomized mice. Interestingly, our results show also that the decrease in bone mineral density observed in TG mice is significantly lower than in WT mice in femur ($P < 0.014$) and in the caudal vertebrae ($P < 0.012$). Estradiol treatment allows to correct bone loss in both genotype.

In conclusion, these preliminary data indicate that in vivo inhibition of osteoblastic MMPs partially prevents bone loss induced by estrogene depletion. But at this time of the analysis, we can not exclude that the positive effect of MMPs inhibition observed on bone mineral density is secondary to increased bone remodeling induced by estrogene.

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CORRELATION BETWEEN DEFICIENCY OF 25 (OH)-VITAMIN D, BONE MINERAL DENSITY AND MARKERS OF BONE TURNOVER IN SICILIAN POSTMENOPAUSAL WOMENS.

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High prevalence of vitamin D deficiency has been reported in many countries independently of sunlight exposure. In this study we assessed the prevalence of vitamin D deficiency between sicilian post-menopausal women. Two hundred consecutive unselected postmenopausal women (aged 48–65 0.4 yrs), coming for a screening evaluation to a ± years, mean age 56.7 menopausal center, were studied.

The 25 OH- vitamin D serum levels were measured and compared with those of a selected (nurses and doctors) group of 45 postmenopausal women who had normal bone mass (T-score between + 0.5 and #0.9) and similar age and body weight (control subjects). Mean serum levels of 25 OH-vitamin D were similar in the 2 groups of women. The 12.8% of unselected post-menopausal women had an absolute deficiency of 25 OH-vitamin D (values lower than #2 SD of control women, < 17.8 ng/ml) while 17% had relative deficiency of vitamin D (values between mean #1 SD and mean #2 SD of control women, < 22.6 ng/ml and > 17.8 ng/ml). A relative deficiency 25 OH-vitamin D was also found in 2 control women (4.4%). Compared to controls and to postmenopausal women with normal vitamin D values, post-menopausal women with vitamin D deficiency had significantly ($P < 0.01$) lower bone mass (by dual X-ray absorptiometry) and higher serum parathyroid ormone, bone alcaline phosphatase and C-telopeptides. In conclusion, vitamin D deficiency is common also in a region of Sicily with sunlight exposure. Genetic defects or diet may be more important than sunlight exposure in determining vitamin D values of post-menopausal women.

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MODULATION OF THE T3-INDUCED ACTIVITY OF THE MOUSE OPG-PROMOTER IN NIH3T3 BY RUNX2

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Osteoprotegerin (OPG), a small glycoprotein expressed by osteoblasts (OB) as well as in cells of other tissues, inhibits the differentiation and activity of osteoclasts via interception of RANKL. Recently we have shown that thyroid hormone (T3) regulates OPG mRNA expression in the osteoblasitc cell line MC3T3-E1 [1]. Usually the effect of T3 is mediated by a complex of T3 with thyroid hormone receptors (TR), which bind to a sequence of the promoter-DNA, a so-called thyroid hormone responsive element (TRE). In addition interactions of steroid hormone receptors with bone specific transcription factors are reported, i.e. vitamin D receptor (VDR) with the transcription factor Runx2 in ROS 17/2.8 cell line [2]. Runx2 is an osteoblast-specific transcription factor, which contributes to the expression of osteoprotegerin [3] and exists in several isoforms [4].

METHODS: We isolated a 0.6 kb mouse OPG fragment by genome walking. Cloning and computer analysis of this fragment revealed the presence of one putative TRE- and two putative Runx2 binding elements suggesting a possible regulation of mOPG by T3 and Runx2. We evaluated whether Runx2 in 2 isoforms (Runx2 type I and Runx2 type II) [3] could affect the basal and T3 regulated activity of the OPG promoter in transient transfection assays in NIH3T3. Additionally we transfected TR for being independent of intracellular TR.

RESULTS: We showed that T3 stimulated the activity of the 0.6-kbp OPG-promoter fragment. Runx2 affected the OPG-promoter activity by attenuating the basal expression and by inhibiting the T3-stimulation, but there seemed to be no difference between the two types of Runx2. By mutation of the two putative Runx2 binding sites we were able to attenuate the T3-induction. In summary the T3-induction of mOPG promoter activity in NIH3T3 was affected by Runx2.

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LOW CREATININE CLEARANCE ASSOCIATED HIGH RISK OF FALLS AND ITS TREATMENT WITH ALFACALCIDOL

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Aims: The number of fallers and falls in elderly can significantly be reduced with a treatment with D-hormone analogues. Impaired renal function is detrimental to the activation of calcitriol (D-hormone). Therefore, we determined the cutoff levels of creatinine clearance (CrCl) at which D-hormone serum levels decline and searched for other low D-hormone associated factors. We also investigated in post hoc analyses of a double-blind randomized study, using the determined cutoff, if CrCl is associated with the risk of falls and whether treatment with Alfacalcidol can reduce this risk.

Methods: For 36 weeks 378 community-dwelling elderg Alfacalcidolμly men and women received randomly 1 TEVA) or placebo daily. Serum calcidiol, D-hormone and iPTH were® (Alpha-D3 regularly measured by radioimmunoassay. Falls were assessed by a questionnaire. We analyzed the risk of becoming a faller and the risk of falling in multivariate-controlled logistic regression models

according to a CrCl cutoff at 65 ml/min and according to treatment groups. The results are from ITT analyses.

Results: We found a significant multivariate-adjusted association between CrCl and D-hormone serum levels ($P <$ .0001), which steadily declined below a CrCl of 65 ml/min. Low D-hormone serum levels were in multivariate-controlled analyses significantly associated with a CrCl of <65 ml/min, the use of diuretics and a diagnosis of adult onset diabetes ($P = 0.0008$, $P = 0.001$ res. $P = 0.003$). In the Placebo group we observed significantly more fallers in participants having a CrCl of <65 ml/min compared to 65 ml/min (OR 4.01, 95% CI 1.48 −10.98, $P = 0.006$). In ≥ participants with a CrCl of participants with a CrCl of <65 ml/min the 36 weeks of treatment with Alfacalcidol was, compared to placebo, associated with a significant reduction in the number of fallers (OR 0.26, 95%CI 0.08 −0.80, $P = 0.019$), and a reduction of the number of falls (OR 0.29, 95% CI 0.09 −0.88, $P = 0.028$). No clinically relevant hypercalcemia were observed.

Conclusion: A reduced CrCl of <65 ml/min is significantly associated with low D-hormone serum levels and associated with a significant increased risk of falls. In a community-dwelling elderly population with a CrCl of <65 ml/min, treatment with Alfacalcidol can significantly and safely reduce the low CrCl associated increased number of fallers and the high risk of falls.

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Withdrawn

Cancer and Metabolic Bone Diseases other than Osteoporosis

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SECULAR TRENDS IN EXTENT AND ACTIVITY OF PAGET'S DISEASE

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Evidence from several countries suggests that severe Paget's disease, as judged by total plasma alkaline phosphatase activity (ALP) at presentation, is becoming less common. We have examined new referrals to our Paget's disease clinic over a 30 year period (1973–2002), to determine whether the fall in ALP has resulted from a reduction in disease extent or disease activity. Of 1487 patients 832 (56%) had scintiscans. From the scintiscan the proportion of the skeleton involved with Paget's disease (E, %) was determined by the method of Coutris et al. Pretreatment total ALP activity was measured (normal <120 u/l) and the average disease activity of pagetic bone was estimated, assuming that a typical total ALP in this age group would be 80 u/l, to which liver and bone isoenzymes both contribute 40 u/l. Thus the bone-derived components (pagetic and non-pagetic bone, respectively): (E)A + (E - 1)40 = Total ALP- 40, from which the average disease activity of pagetic bone (A) could be calculated. The values for A ranged from 0 to 370 arbitrary units, with a log10 distribution and geometric mean of 62. Disease extent was negatively correlated with year of birth (r = −0.18, P,0.0001) and year of referral (r = −0.25, P,0.0001), but not with age (r = 0.04, $P = 0.22$). Disease activity was also negatively correlated with year of birth (r = −0.09, P,0.011) and year of referral (r = −0.11, P,0.001), but not with age (r = 0.01, $P = 0.79$). However the correlation coefficients were lower and the statistical significance weaker for disease activity. The absolute number of subjects with $>20\%$ skeletal involvement declined significantly across deciles of year of birth ($P = 0.003$) but the number of subjects with disease activity of >74 units ($P = 0.82$) did not. We conclude that both disease extent and average activity of pagetic bone have declined significantly over the past three decades, but that the decline in extent predominates.

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USE OF CALCITONIN AND ETIDRONATE IN HIV-HCV-COINFECTED WITH BONE MASS LOSS

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Introduction: Hepatitis C virus (HCV) is an RNA virus is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Owing to shared routes of transmission, HCV and human immunodeficiency virus (HIV) coinfection are common, affecting approximately one-third of all HIV-infected persons. Low bone mineral density may be yet another common adverse effect of protease inhibitor combination therapy.

Objective: to determine if the combined use of calcitonin and etidronate influences on bone mass loss in hiv-hcv-coinfected patients with bone mass loss

Material and method: We studied for 10 months 22 women who were 45 to 67 years old at base line, were within 1 and 14 years of menopause, and had a bone mineral density at the lumbar spine between 144 mg/cc and 75 mg/cc measured by the QBMAP system with a spiral CT Picker PQ-S densitometer at L2, L3, L4 and L5. Of all the women, 12 were assigned to 400 mg of etidronate for 15 days in each 90 days, 800 IU of vitamin D3 and 1 g of calcium carbonate supplementation. 10 were treated with 400 mg of etidronate for 15 days in each 90 days, 100 UI of intranasal calcitonin, 800 IU of vitamin D3 and 1 g of calcium carbonate supplementation. The SPSS programme was used for statiscal analysis.

Results: The characteristics of the women recruited for both groups were similar. Mean mineral bone density at the lumbar spine was between 1 and 4 DS below the mean value for 25 years old normal premenopausal women. After a treatment of 12 months no statistically significant difference was found among both groups as for the bone mineral density at the lumbar spine.

Conclusions: It is necessary to carry out a wider and longer study, on HIV-HCV-coinfected patients with bone mass loss.This results can be interesting for VIH-HCV coinfected, who have a lot of drugs.

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EXTRACORPOREAL SHOCK WAVE THERAPY FOR THE TREATMENT OF CHRONIC CALCIFYING TENDONITIS OF THE ROTATOR CUFF—A RANDOMIZED PLACEBO CONTROLLED MULTI-CENTER TRIAL

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Context: Extracorporeal shock wave therapy (ESWT) is increasingly used for calcifying tendonitis of the shoulder, but limited evidence supports its use.

Objective: To determine whether fluoroscopy guided high energetic extracorporeal shock wave therapy improves function, reduces pain and diminishes deposits in patients with chronic calcifying tendonitis of the shoulder.

Design: A double-blind, randomized, placebo-controlled multicenter trial conducted between February 1997 and March 2001

Setting: Study participants were recruited multicentric from referring primary care physicians, orthopedic surgeons and sports physicians of 7 different orthopedic departments in Germany and Austria.

Participants: 164 patients were screened and 144 enrolled. 144 completed the treatment and 134 completed follow up six month after intervention. Entry criteria included symptomatic calcifying tendonitis, symptoms of at least 6-months duration, completed and failed conservative treatment, deposit size of at least 5mm, deposit type 1 or 2 (Gärtner), signed informed consent, age greater than 18 years.

Interventions: Patients were randomly assigned to receive two extracorporeal shock wave therapies within two weeks (Group I: 1,500 high-energy shocks; Group II: 6,000 low-energy shocks) for a total dose of 0.960 J/mm^2 or identical placebo (Group III).

Main outcome measures: Primary outcome measurement six months after ESWT: Improvement from baseline measured on the Constant and Murley Score (CMS) after 6 months; Secondary endpoint: reduction of size of calcareous deposit (mm^2).

Results: Primary outcome measure: Compared to placebo Group III there was a highly significant improvement of shoulder function on the CMS in Group I (mean change [SD], 31.0 pts [14.6], $P < 0.001$) and also a significant improvement of shoulder function on the CMS in Group II (mean change [SD], 15.0 pts [16.4], $P < 0.019$). Secondary outcome measure: Six months after intervention complete disappearance of the deposit was found in 60% (Group I), 21% (Group II) and in 11% in the placebo group.

Conclusions: We found evidence to support a beneficial effect of high-energy ESWT over placebo on shoulder function and disintegration of the symptomatic calcifying tendonitis six months following treatment.

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REAL TIME RT-PCR AMPLIFICATION OF HUMAN PARATHYROID RELATED PEPTIDE AND PARATHYROID HORMONE RECEPTOR MRNA'S IN TUMORS

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Parathyroid hormone related peptide (PTHrP), originally isolated from cancers inducing the humoral hypercalcemia of malignancy (HHM), has been

reported to be expressed by neoplams not associated with hypercalcemia. Beside its endocrine action, PTHrP could play a role in tumor development and metastatic potential. We present here the work up of an assay to amplify the mRNA's of PTHrP and its receptor by real time two steps RT-PCR. GAPDH was used as housekeping gene.

Method:Total RNA was extracted with TRIzol reagent (Gibco BRL). 1mcg of total RNA was reverse transcribed in the presence of Random Hexamers after digestion by 1U/mcg DNase1. Primers and probes, designed using software Primer Express v2.0 (Applied Biosystems), were added for the amplification.The following primers pairs and probes were used: PTHrP(Exons2-3) rev: CTgATgTTCAgACACAgCTCTTTT-fwd: gATgCAgCggAgACTggTTC-probe: CggCggCTgAgACCCTCCACC.Receptor (Exons M1–M2): rev: gCAgCATgTAAggAC AggAACA fwd: ATgATTTACACCgTggg CTACTC-probeTCCCTggCgTCCCTCAC CgTAgC.GAPDH (Exons 4–5) rev:T gg ACTCCACgACgTACTCA-fwd: gAAATCCC ATCACCATCTTCCAg-probe: CgCCAg CATCgCCCCACTTgATTT. All the probes were labelled with FAM and HBQ1. A renal tumor was used as positive control, water and samples without reverse transcriptase as negative controls. Serial extractions, reverse transcription and amplification of the renal tumor were studied to evaluate the variability of the results.

Results: 1-In terms of the Ct ratios of GAPDH/PTHrP on the renal tumor, the variability of 34 amplifications with 12 extractions and 34 reverse transcription was 0.99 ± 0.07 (CV:7%). The mean ratio was 0.72 ± 0.07 (CV = 9.5%) for 27 measurements of PTH receptor expression. 2-For a same extraction and different reverse transcriptions, the CV was 4.3% for the mRNA of PTHrP (n = 14) and 7.6% for its receptor (n = 10).3- Results obtained in tumors are presented in the table.

Conclusions: 1-The method allows quantitative determination of PTHrP and its receptor.2- mRNA extraction and RT contribute equally to variability.3- The level of expression of PTHrP and its receptor in tumors is quite variable, and a prospective study of final outcome should be done. 4. Unexpectedly, both mRNA's were found to be expressed in a benign lipoma.

Table: Expression of PTHrP and its receptor in tumors

Type of tumor	n	CtG/CtP	CtG/CtPR
Breast	5	ND0-0.8	ND-0.9
Stomach	3	ND-0.8	ND-0.7
Lipoma	1	0.6	0.5

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RISEDRONATE 30 MG/DAY IS SAFE AND EFFICACIOUS IN PATIENTS WITH A WIDE RANGE OF RENAL FUNCTION

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BACKGROUND: Risedronate 5mg/day up to 3 years has previously been shown to be safe and effective in patients with varying degrees of renal insufficiency treated for postmenopausal osteoporosis. The objective of this analysis is to investigate the influence of renal impairment on safety and efficacy in patients with Paget's disease of bone taking risedronate 30 mg/day.

METHODS: 276 male and female patients, enrolled in Phase II and Phase III clinical trials for Paget's disease of bone and took 30 mg/day of risedronate for 2–6 months were analyzed. Creatinine clearance was estimated using the Cockcroft-Gault method. The incidence of AEs and changes from baseline in serum creatinine, alanine and aspartate aminotransferase (ALT, AST) and serum alkaline phosphate (ALP) were summarized for patients with a wide range of renal function.

RESULTS: The mean age (SE) of this population was 68.1 (0.55) years and the mean (SE) creatinine clearance was 65.9 mL/min (19.6), the range spanning from normal (>80 mL/min) to severely impaired (<30 mL/min), according to FDA criteria. The average duration of drug exposure was 106 days. There was no observed relationship between the incidence rate of AEs and the degree of renal impairment (R2 = 0.016). Likewise, there was no correlation between baseline creatinine clearance and % changes in serum creatinine (R2 = 0.034), AST (R2 = 0.005), ALT (R2 = 0.001). Treatment response as measured by changes in ALP after 6 months, was also not affected by renal function (R2 = 0.0).

CONCLUSION: Based on controlled clinical trial experience, this analysis shows, that risedronate 30 mg/day over periods of two to six months is both safe and efficacious across a wide spectrum of renal impairment in patients with Paget's disease of bone.

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OSTEOPROTEGERIN SERUM LEVELS AND BONE DISTURBANCE IN KIDNEY TRANSPLANT RECIPIENTS

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Bone disturbances occur very often in kidney transplant recipients despite good renal function. Bone metabolism disturbances developing before transplantation may persist after transplantation and new factors occur that have negative impact on the bone, i.e. immunosuppressive agents. In order to investigate the role of osteoprotegerin in bone disturbances in 28 kidney transplant recipients (15 male, 13 female, aged 25–66 years, creatinine clearance 55 mL/min), the serum osteoprotegerin levels were measured 3-224 months posttransplant, as well as those of iPTH, bone alkaline phosphatase, telopeptide, 25(OH)D3, Ca and Pi. Urinary calcium excretion in 24 hours was also measured. Bone mineral density (BMD) was estimated in the lumbar spine, femoral neck and distal third of radius using dual energy absorptiometry (DEXA).

Telopeptide and bone alkaline phosphatase serum levels were significantly (i.e. $P < 0.05$) higher if posttransplant period at estimation was 12 months (N = 10), than in those in whom this period was longer (N = 18). The time spent on dialysis correlated significantly negatively with femoral neck and distal radius bone mineral density, and positively with bone alkaline phosphatase levels. Posttransplant period duration correlated significantly negatively with bone alkaline phosphatase and telopeptide values. IPTH values correlated significantly positively with bone alkaline phosphatase, osteoprotegerin ($P < 0.01$), Ca, and negatively with Pi and 25(OH)D3 values. Bone alkaline phosphatase correlated significantly positively with telopeptide and Ca, and negatively with Pi and 25(OH)D3. Telopeptide correlated significantly positively with Ca. Osteoprotegerin correlated significantly negatively with Pi ($P < 0.05$). Femoral neck Z score correlated significantly positively with Pi, and distal radius bone mineral density significantly negatively with calcium excretion. P values <0.05 were considered significant.

Conclusions. Dialysis duration is a risk factor for bone mineral density reduction. Bone turnover rate decreases after kidney transplantation. Serum osteoprotegerin levels were influenced by PTH; the higher PTH secretion, the higher serum osteoprotegerin levels. No correlation between osteoprotegerin serum levels and bone mineral density was found in this patient group.

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EFFECT OF BISPHOSPHONATE THERAPY ON SERUM CONCENTRATIONS OF RANKL AND OPG IN MALIGNANT HYPERCALCEMIA

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Background: Recent research points to a crucial role of RANK, RANK-ligand (RANKL) and osteoprotegerin (OPG) system in the physiopathology of neoplastic bone involvement: Tumor cells stimulate osteoclast-mediated bone resorption by directly expressing RANKL or increasing the expression of RANKL on osteoblasts or bone marrow stromal cells.

Aim of the study: We evaluated whether treatment with the nitrogen-containing bisphosphonate ibandronate affects the serum concentration of OPG or soluble RANKL.

Patients and methods: 16 hypercalcemic cancer patients (10 females, 6 males; median age 68.5 years) received one infusion of 6 mg ibandronate on day 0. Serum levels of soluble RANKL, OPG, creatinine and calcium were determined before ibandronate treatment and daily until day 5. Both soluble RANKL and OPG were measured by commercially available enzyme immunoassays (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria). These assays measure the total (free and bound) amount of OPG ciruclating in blood and the concentration of free, soluble RANKL.

Results: Ibandronate treatment led to a decrease in the median serum calcium concentration (day 0: 3.06 mmol/l, day 5: 2.41; $P < 0.0001$) whereas the serum concentrations of OPG (day 0: 8.45 pmol/L, day 5: 8.59 pmol/L; $P = 0.05224$), of soluble RANKL (day 0: 0.2950 pmol/L, day 5: 0.2475 pmol/L; $P = 0.2017$) and of creatinine (day 0: 0.5 mg/dL, day 5: 0.3 mg/dL; $P = 0.0776$), as well as the soluble RANKL/OPG ratio (day 0: 0.028, day 5: 0.023; $P = 0.1692$) did not change.

Conclusion: Bisphosphonate treatment of neoplastic bone disease does not appear to affect the RANK, RANKL, OPG system.

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IGF-IR AND OSTEOCLAST ACTIVITY IN SKELETAL METASTASES OF NEUROBLASTOMA

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The insulin-like growth factors IGF-1 and IGF-2 are highly concentrated in bone, and are essential for normal skeletal growth. IGFs are actively synthesized by osteoblasts and stored within the skeletal matrix, therefore interacting with osteoclast after being released during bone resorption. By using osteoclast precursor monocyte-like models (human FLG29.1 cells and murine RAW264.7 cells), and human osteoclasts from peripheral blood mononuclear cells (PBMC) as a model, we demonstrated that IGF-IR is expressed and phosphorilated in fully differentiated osteoclasts as well as in osteoclast precursors. In osteoclast precursors, IGF-IR was also responsible for a mitogenic signal. By Western blot, IGF-IR phosphorilation was null in monocyte precursors, increased after 2 days of culture under differentiating conditions, followed by a silencing phase (day 4), and finally, in fully differentiated osteoclasts (day 7), by the highest phosphorilation level. Interestingly, most cancers that usually metastasize to bone (breast and renal carcinomas, neuroblastoma) produce high levels of IGF-1 or IGF-2, and in these neoplasms the IGFs system is fundamental for tumor mitogenesis and survival. Although the role of other paracrine networks, such as the Osteoprotegerin/RANKL/RANK system cannot be excluded, tumor IGFs might also be useful in the bone microenvironment for neoplastic cells to interact with osteoclast precursors and induce osteolysis. In fact, among different carcinoma cell lines, the highest levels of IGF-2 were found to be produced by SH-SY5Y neuroblastoma cells, that also showed the most powerful ability to induce osteoclast differentiation from PBMC. Moreover, the addition of different molecules to inhibit IGF-IR activation, such as antisense oligonucletides, suramin, or α-IR3 monoclonal antibody, to the conditioned media of SH-SY5Y cells were able to effectively block tumor-induced osteoclast formation and resorption. These results suggest that in the bone microenvironment of metastatic neuroblastoma the expression of IGF-2 plays an important role in osteolysis induced by tumor cells and that IGF-2 directly stimulates osteoclast recruitment and activation. Targeting IGF-IR combines the advantage of interfering both with tumor cell survival and proliferation and the osteolytic activity triggered by neuroblastoma cells. Supported by grants from the Italian Association for Cancer Research and the Italian Ministry of Health.

P252

MUTATION AT CODON 404 IN EXON 8 OF SEQUESTOSOME 1 GENE CREATING A METHIONINE TO VALINE SUBSTITUTION SEGREGATES WITH PAGET'S DISEASE OF BONE PHENOTYPE IN AN ITALIAN FAMILY.

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Paget's disease of bone (PDB) is a metabolic bone disorder affecting up to 3% of Caucasian populations over 55 years of age. The disease is characterized by focal and disorganized increase of bone turnover and genetics factors are important in its pathogenesis involving at least 8 different human chromosomal loci. Mutations in the gene encoding sequestosome 1 (SQSTM1) were identified as a common cause of sporadic and familial PDB in French Canadian, British descendent and US patients. All identified mutations localize to exons 7 and 8 of the gene, affecting the highly conserved ubiquitin-binding domain (UBA). We originally performed mutational analysis of exon 7 and 8 in 62 PDB Italian patients, identifying one "classical" P392L and two novel mutations, M404 V and G425R, at exon 8, the latters consisting, respectively, of A > G and G > A transversion. Patient exhibiting M404 V substitution was from a large family, whit several members affected by PDB. We performed mutational analysis in 20 individuals, 4 affected, including the proband, and 16 unaffected members. Affected subjects were clinically evaluated both by biochemical and imaging tests. The M404 V mutation was found in 8 individuals: 3 with clinically diagnosed polyostotic PDB and 5 "asymptomatic" offspring (age range from 41 to 53 years) of three affected patients. Not mutated individuals did not exhibit any clinical evidence of PDB. Mutation M404 V consists of a highly conservative amino acid substitution, and Methionine residue at position 404 is highly conserved among other species, rat and mouse, suggesting an important role in the functionality of the SQSTM1/p62 protein. This familial segregation of M404 V mutation with PDB phenotype strongly supports the hypothesis that this mutation is involved in PDB pathogenesis, according to a possible dominant negative mechanism of action. Moreover, its location at exon 8 level confirms the evidence of a clustered mutational area at this level in this disorder, supporting the role of the UBA domain in the biological properties of SQSTM1/p62 protein. Both instrumental and biochemical evaluation of the 5 "asymptomatic carriers" will potentially provide new important acquisitions on the pathogenesis of this metabolic disorder of bone.

P253

LIPOPHILIC STATINS INDUCE APOPTOSIS IN HUMAN OSTEOSARCOMA CELLS THROUGH HMG-COA REDUCTASE INHIBITION

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p53 is a tumor suppressor gene that acts by inducing growth arrest or apoptosis. Resistance to chemotherapy associated with p53 deficiency remains a major mechanism responsible for the failure of osteosarcoma treatment. We hypothesized that statins, which act as HMG-CoA reductase inhibitors and induce cell death, may promote apoptosis in human osteosarcoma cells independently of p53 expression. To test this hypothesis, SaOS2 (p53-deficient) and OHS4 (functional p53) human osteoasarcoma cell lines were treated with hydrophilic or lipophilic statins (0.1–100 μM) (kindly provided by Bristol-Myers Squibb, USA). In these two cell lines, statins induced apoptosis in a dose-dependent manner, as shown by a -fold increase in the number of TUNEL-positive stained cells and marked increase (10-fold) in the activity of effector caspases−3, −6, −7. Lipophilic statins (Cerivastatin, Atorvastatin, Simvastatin) were more efficient to induce apoptosis than the hydrophilic statin Pravastatin. Both in SaOS2 and OHS4 cells, the pro-apoptotic effect of Cerivastatin, Atorvastatin, Simvastatin (10 μM) was abolished by mevalonate (1 mM) or by geranylgeranyl pyrophosphate (10 mM), a mevalonate metabolite, indicating that inhibition of small GTPases prenylation is involved in statin-induced apoptosis in osteosarcoma cells. In addition, biochemical analysis showed that apoptosis induced by Atorvastatin (10 μM) in both Saos2 and OHS4 cells was associated with inhibition of MAPK phosphorylation. We conclude that lipophilic statins are potent inducers of apoptosis in osteosarcoma cells independently of p53 expression and that the pro-apoptotic effect of statins may involve a MAPK-dependent pathway. These data suggest that lipophilic statins may be effective in inducing chemoresistant osteosarcoma cell death and reduce tumor burden.

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HUMAN BREAST CANCER CELLS INDUCE OSTEOCLASTOGENESIS AND ENDOTHELIAL CELL PROLIFERATION *IN VITRO* AND ARE AFFECTED BY INHIBITION OF C-SRC ACTIVITY

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Bone represents the principal site of metastasis for breast cancer. Recent evidence suggests the involvement of c-Src in the development and metastases of several carcinomas, including mammary cancers. To elucidate whether c-Src could be pharmacologically targeted to reduce cancer cell activity, the human breast cancer cell line MDA-MB231 was treated with two c-Src inhibitors belonging to the pyrrolopyrimidine classes, or were stably transfected to overexpress wild type (MDA-MB231-SrcWT). The ability to induce osteoclastogenesis was investigated treating mouse bone marrow cultures with conditioned medium from these cells. As expected, a significant increase of TRAP-positive multinucleated osteoclasts and resorption pits was observed in the presence of conditioned medium, suggesting direct stimulation of osteoclast activity by paracrine factors, which may include the osteoclast-stimulating cytokines, IL-6 and IL-1beta, as evidenced by their transcriptional expression in the cancer cells. Treatment of osteoblast primary cultures with MDA-MB231 cell conditioned medium induced a selective transcriptional up-regulation of IL-6, IL-1beta, and RANKL, but not OPG, suggesting a further indirect stimulation of osteoclastogenesis and bone resorption via the osteoblast lineage. c-Src inhibitors caused a concentration- and time-dependent reduction of MDA-MB231 cell proliferation, adhesion, spreading and migration. In agreement with these results, MDA-MB231-SrcWT cells showed increased proliferation, migration and invasion relative to the parental cell line. To clarify whether breast cancer cells affected endothelial cell behavior, the human EAHy926 endothelial cell line was treated with MDA-MB231 conditioned medium. We observed a significant increase of EAHy926 proliferation and migration relative to untreated cells, while invasion was unaffected. Moreover, conditioned medium arising from MDA-MB231-SrcWT significantly enhanced endothelial cell proliferation relative to medium from parental cells. In conclusion, these data demonstrate a complex role for c-Src in breast cancer cell activity, suggesting potential application of c-Src-targeted treatments as therapeutics against breast cancer tumor growth and development of distant metastases.

P255

WHAT CAUSES RECURRENT FRACTURES IN CHILDHOOD?

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Background: Fractures are frequent in childhood with the incidence peaking around the time of peak height velocity. Previous reports have indicated a variety of potential contributors to fracture risk including low bone mineral content and density, milk avoidance, lack of habitual physical activity, asthma, high body mass index, and a high consumption of carbonated beverages.

Aims: We wished to test the hypothesis that children who sustained fractures in childhood had a lower bone mass than those who remained fracture-free, and that children suffering recurrent fractures had different underlying risk factors from those who had fractured only once.

Methods: We studied 150 children aged 4–16 years; 50 who had suffered multiple fractures, 50 who had fractured for the first time, and 50 fracture-free controls. Children were seen within two days of their fracture. Subjects underwent assessment of bone mineral content and density by total body and lumbar spine DXA. Anthropometry and grip-dynamometry were carried out, and information about factors possibly relevant to fracture aetiology such as milk intake, physical activity levels, asthma prevalence and carbonated beverage consumption was recorded using questionnaires.

Results: Children who had sustained one or more fractures had a significantly lower BMC and aBMD at all sites than controls (L2-4 BMC $P = 0.0002$; L2-4 aBMD $P < 0.0001$; TB BMC $P < 0.0001$; TB aBMD $P < 0.0001$). There was, however, no difference in bone mass adjusted for body size between children with one or multiple fractures. The factors which, in combination, were associated with an increased risk of recurrent fracture were increased body mass index, reduced consumption of milk and independently increased consumption of carbonated drinks. Lack of exercise was also found to increase recurrent fracture risk, and parental attitudes to physical activity dictated children's activity patterns.

Conclusions: Children with fractures have lower bone mass for body size than children without fractures. The factors which in combination predict multiple fractures include modifiable items such as diet and exercise. There are important public health implications of this work given the current trends to lower physical activity and increased body mass index at the time when fracture incidence is at its highest.

P256

VITAMIN D DURING PREGNANCY AND GROWTH IN EARLY INFANCY

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BACKGROUND: Studies in vitamin D deficient pregnant women have shown that supplementation influences postnatal growth with infants of supplemented mothers being longer and heavier at the age of one year irrespective of postnatal vitamin D supplementation.

AIMS: The aims of this study were to determine whether endogenous variation in maternal vitamin D as assessed by cord blood 25 hydroxy vitamin D (25 OHD) concentrations was associated with variation in body size during the first six months of life.

METHODS: 110 pregnant women were recruited into the study. Cord blood vitamin D was measured in 101 and the infants measured at birth, 3 (n = 87) and 6 (n = 89) months. Details concerning maternal history, parental heights and weights, mode of feeding and postnatal vitamin D supplementation and ethnicity were recorded. 88 mothers were white Caucasian.

RESULTS: Serum 25 OHD levels for the group as a whole were low (mean median 15.4 nmol/l) with 71% being below 20 nmol/l, one of the suggested thresholds for vitamin D insufficiency. Ethnicity and prenatal vitamin D supplementation were associated with differences in cord blood 25 OHD. The only growth parameter which showed a relationship with cord blood 25OHD was head circumference z score at six months of age.

CONCLUSIONS: No definite association between body size at birth, 3 or 6 months and cord blood 25 OHD could be shown. The values recorded for 25 OHD in this study were generally lower than those recorded previously and approximately one third of those of healthy, non-pregnant women using the same assay. These low values may indicate a general increase in the population prevalence of vitamin D insufficiency possibly relating to the increased use of sun screens and covering up during the summer months. Low vitamin D intake in infancy is associated with reduced bone mass and an increased risk of type 1 diabetes in later life. Vitamin D supplementation studies are warranted in pregnant women in the UK.

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ACTIVE IMMUNOTHERAPY USING CYTOTOXIC DENDRITIC CELLS IN A RELEVANT RAT MODEL OF OSTEOSARCOMA

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Osteosarcoma is the most frequent primary bone tumor that develops mainly in the young (median age: 18 years). Current strategy for treatment of high-grade osteosarcoma based on neo-adjuvant chemotherapies leads to pulmonary metastasis apparition. Despite recent improvements, the rate of survival remaining around 55 to 70% after 5 years, new therapeutic approaches need to be developed, such as active immunotherapy using dendritic cells (DC). Recently, a sub-population of CD11b+ CD103+ CD4- DC has been shown to exhibit direct cytotoxic activity in vitro against tumor cells but not normal cells. The aim of this study was to assess the effects of active anti-tumor immunotherapy using these CD4- DC, in a transplantable rat model of radio-induced osteosarcoma, which parallels human clinical data. The cytotoxic activity of CD11b+CD4- as well as CD11b+CD4+ DC towards osteosarcoma cells (OSRGa cell line) was assessed using an in vitro Cr51 release assay. CD4- but not CD4+ DC exhibited a rapid cytolytic activity against OSRGa cells. In vivo studies confirmed this direct anti-tumor activity. Fresh spleen CD4+ and CD4- DC were prepared from naïve SPD rats and were cultured overnight in the presence or in the absence of live OSRGa cells, and then re-purified. Fragments of osteosarcoma were implanted in SPD rats at contiguous to the right tibia. Animals were weekly vaccinated with the DC for 5 consecutive weeks starting on day +11 after tumor grafting. In animals vaccinated with CD4- DC cocultured with OS-RGa, a diminution of tumor burden was observed together with an increase of the survival rate as compared to control groups. In some cases the tumor totally disappeared without recurrence. Moreover, a number of immunized rats acquired resistance towards the osteosarcoma tumor as a subsequent implantation of osteosarcoma did not lead to tumor development. These preliminary results offer a promising therapeutic approach in bone tumor diseases treatment by cellular therapy using cytotoxic DC. Further investigations are currently performed to define the cytotoxic mechanisms involved and the potential implications of these cells in the presentation of tumor antigen and T cell activation, as well as in direct anti-tumor activity.

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IN VITRO BLOCKADE OF RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA B LIGAND PREVENTS OSTEOCLAST DIFFERENTIATION INDUCED BY NEUROBLASTOMA CELLS

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Bone is one of the target organs of metastasis in advanced neuroblastoma, and metastatic osteolysis depends on osteoclast activity. Proliferation and differentiation of osteoclasts are mediated by a cytokine system that includes the receptor activator of NFkB ligand (RANKL), which binds two types of receptors: RANK, expressed in osteoclasts, and osteoprotegerin (OPG), a soluble decoy receptor. RANKL binding to RANK activates the cascade of intracellular events of osteoclast differentiation. RANKL binding to OPG limits its biologic actions. The role of OPG/RANKL/RANK network in the pathogenesis of bone metastasis in stage IV neuroblastoma has been analyzed. RANKL and OPG expression was investigated in different neuroblastoma cell-lines (LAN1, SH-SY5Y). All the cell lines had a large amount of mRNA for OPG and RANKL. Nevertheless, while the RANKL protein was released in the culture medium, OPG was very low or even undetectable. We investigated the paracrine activity of neuroblastoma cell lines in inducing the differentiation of osteoclast precursors obtained from PBMCs. The culture medium of SH-SY5Y and LAN1 induced the expression of markers of osteoclast differentiation, including RANK, c-src, c-fos, cathepsin K and a strong tartrate-resistant acid phosphatase positive reaction. The crucial role of RANKL in inducing the osteoclastogenesis was confirmed by using a neutralizing anti-RANKL antibody, which inhibited the expression of RANKL-dependent genes and the generation of multinucleate TRAP giant cells. Biological modifiers of RANKL activity, as antisense oligonucleotides (ODNs) and small interfering RNAs, were evalutated for their ability to inhibit osteoclast differentiation. We have observed a decrease in both mRNA and protein, as well as a strong inhibition of osteoclastogenesis. Our findings confirm that neuroblastoma cells are able to induce the osteoclastogenesis via RANKL, and suggest that the RANKL expression, associated with the lack of the decoy receptor OPG could be the key mechanism by which neuroblastoma cells are able to colonize bone and to induce bone metastases. RANKL could be a relevant target in adjuvant therapies of bone metastasis and antisense strategies could be employed to prevent the osteoclastogenesis induced by tumor cells.

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BONE METASTATIC RENAL CARCINOMA CELLS ENHANCE ANGIOGENESIS AND OSTEOCLAST DIFFERENTIATION *IN VITRO*

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Renal cell carcinoma (RCC) is well vascularized, both in the primary tumor and the metastasis. Endothelial cells, after stimulation with interleukin-1, endotoxin, or TNF-a, produce bone-resorbing cytokines and growth factors, including RANK-L. If tumor cells, in addition to the endothelial cell proliferation and tube formation, induce also the expression of a favoring-osteolysis phenotype, bone metastasis could be supported. The renal adenocarcinoma ACHN cell line and a cell line from a bone metastasis of renal carcinoma (CRBM) were investigated for the angiogenetic and the osteoclast-activating effect. These cell lines express mRNA specific for TGF-b1, IL-6 and FGF-2, but do not express RANK-L. Both ACHN- and CRBM-conditioned media stimulated significantly the migration, the proliferation and the tubular structure formation of BBE bone endothelial cells. In addition, ACHN medium, but not CRBM, induced the expression of the osteolytic cytokine M-CSF in endothelial cells. The ability of renal carcinoma cell lines to activate osteoclasts was tested by using cultures of peripheral blood mononuclear cells (PBMC), isolated from buffy coats, co-cultured with CRBM, ACHN, or BBE, or incubated with the conditioned ACHN or CRBM or BBE medium, or with BBE-conditioned medium previously stimulated with ACHN or CRBM medium. After 8 days, the TRAP activity (measured with an enzymatic method) and the number of multinucleated cells were determined. ACHN and CRBM co-cultures or conditioned media, added directly to PBMC, did not induce osteoclast differentiation. Non-stimulated BBE-conditioned medium or the co-culture with non-stimulated BBE induced higher TRAP levels. The co-culture of PBMC with BBE previously stimulated with CRBM-conditioned medium produced the highest TRAP levels. The bone endothelial cells induced the differentiation of pre-osteoclasts, which was further increased by stimulation with medium conditioned by CRBM, but not by ACHN. Therefore, CRBM cells increase the effect of BBE on osteoclast differentiation. In conclusion, ACHN and CRBM have an angiogenetic effect. With regards to the effect on osteoclast differentiation, they have little direct effect, but appear to act through the endothelium in different ways. While ACHN induce endothelial cells to express M-CSF, CRBM, isolated from an osteolytic metastasis, release factors into the medium that induce endothelial cells to favor the differentiation of osteoclasts.

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BISPHOSPHONATES POTENTIAL THERAPEUTIC RELEVANCE IN PRIMARY BONE TUMORS

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Current therapeutic strategies of primary bone tumors are based on neo-adjuvant chemotherapy, delayed en-bloc wide resection and adjuvant chemotherapy. Unfortunately, recurrence associated with osteolytic processes as well as an absence of response to anti-tumour drugs are often observed, leading to the development of metastases and ultimately to the death of the patients. Therefore, development of new therapeutic approaches is needed. The efficacy of a bisphosphonate (BP) of the third generation, zoledronate (ZOL) was assessed in two experimental models of primary bone tumors in rats: osteosarcoma and chondrosarcoma. Bisphosphonates have been used successfully for many years to reduce skeletal complications associated with a wide spectrum of benign and malignant bone diseases characterized by enhanced osteoclastic bone resorption. A model of transplantable rat osteosarcoma initially radio-induced that mimics the development of human osteosarcoma at the temporal and physiologic level was used, together with a rat model of Swarm chondrosarcoma. Treatments with ZOL (100 microg/kg, twice a week) began 7 days (chondrosarcoma) or 14 days (osteosarcoma) after tumor implantation. Clinical and radiological parameters were assessed during the 4 week-treatment. In osteosarcoma model, ZOL not only reduced significantly bone resorption with absence of cortical degradation, but also inhibited the tumoral progression by 46% and increased the survival rate, as compared to non treated animals. Complementary *in vitro* analyses showed an anti-proliferative effect of 10-6 M ZOL (−72%) specific of osteosarcoma cells as compared to osteoblasts. In the chondrosarcoma model, ZOL treatment beginning 7 days after tumor implantation induced a 50% decrease of tumor volume as compared to non treated animals. In another protocol, ZOL-treated rats had a recurrence rate of 50% versus 100% of recurrence after intralesional curettage without any treatment. When recurrence occurred, the volume of the tumor was 62% lower in treated group in comparison with control group. These results demonstrate for the first time an anti-tumor effect of BPs in two separate experimental model of primary bone tumors, thus allowing to consider these two molecule as potential therapeutic agents in clinical trials of tumoral bone pathologies.

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TUMOR INFILTRATING LYMPHOCYTES FROM OSTEOSARCOMA CHARACTERIZATION AND THERAPEUTIC INTEREST

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Osteosarcoma is the most frequent primary bone tumor that develops mainly in the young (2nd and 3rd decade). The current treatment consists on a tumor resection associated with very toxic neo-adjuvant chemotherapy. Unfortunately in many cases, recurrences associated with osteolytic process and lack of response to anti-tumor drugs lead to metastasis development and to patient death. In this context, it appears necessary to develop less toxic and more effective new therapeutic approaches of osteosarcoma targeting bone tumor development and pulmonary metastasis. The purpose of the present work was to develop passive immunotherapy protocols based on the utilization of cytotoxic lymphocytes specific of tumor cells.

Cytotoxic tumor infiltrating lymphocytes (TIL) were isolated from two types of tumor tissue: biopsies from patients suffering from osteosarcoma and tumor tissue originate from a rat transplantable osteosarcoma model. This rat model mimics the temporal as well as physiological development of human osteosarcoma. These different tumor fragments were cultured in suitable medium contained IL2 for 10 days. TIL were then collected, amplified before characterization consisting on phenotypic analysis by flow cytometry and functional analysis against allogenic cells by cytotoxic tests with 51Cr. TIL isolated from osteosarcoma biopsies were mainly CD3 + CD8 + (> 70% for human and > 60% for rat) and possessed an increased cytotoxic activity compared to the peripheral blood leukocytes. Indeed, human TIL cytotoxic activity against MG63 or SaSO2 osteosarcoma cell lines is increased 4–5-fold compared to the activity of peripheral blood lymphocytes. Similarly, 20% increase of rat TIL cytotoxic activity against ROS or UMR106 osteosarcoma cells is also observed compared to peripheral blood lymphocytes.

These results demonstrated that TIL obtained from human or rat osteosarcoma fragments expressed a cytotoxic phenotype higher than autologous peripheral blood leukocytes. Passive immunotherapy based on TIL expansion technique then represents a potential new therapeutic approach of osteosarcoma. Efficacy of TIL will be now analysed in rat transplantable osteosarcoma model (tumor evolution, metastasis development, etc.).

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EFFECT OF BISPHOSPHONATE THERAPY ON BONE MARKERS IN PAGETIC PATIENTS WITH SKULL INVOLVEMENT

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Previous studies have shown that pagetic patients with skull involvement frequently display a marked increase of bone turnover. However, the extent to which this influences response to therapy is unknown. The aims of this study were to evaluate response to therapy in disease activity in pagetic patients with and without skull involvement and to compare the usefulness of new bone markers in the evaluation of these patients.

Methods: 37 patients with Paget's disease (9 with skull involvement) treated with tiludronate (400 mg/d × 3 months) and 26 controls were included. Serum total alkaline phosphatase (TAP), bone alkaline phosphatase (BAP), PINP and urinary α-α CTX, β-β CTX and NTX were measured at baseline and at 1 and 6 months afterdiscontinuation of therapy. Quantitative bone scan was performed at baseline and at 6 months, and an index of disease activity (SAI) was obtained. Patients were classified into three groups: patients with skull-involvement (Sk group), patients without skull-involvement and patients without skull-involvement but with similar SAI to those with skull-involvement.

Results: All groups of patients showed higher baseline values in all markers compared to controls. At baseline, Sk group showed significantly higher values in all markers when compared to patients without skull-involvement. In addition, there were no significant differences between Sk group and patients with similar SAI except for NTX, which was higher in the first group. The α-α CTX was the marker with the highest values in Sk group (46 times higher than normal values in Sk group vs 20 times higher in patients without skull-involvement). Six months after therapy the percentage of patients with markers within the normal range in patients without skull-involvement were: 71% for TAP, 71% for BAP, 63% for PINP, 54% for NTX, 48% for α-α CTX and 52% for, β-β CTX; in patients with similar SAI to Sk group the values were 60% for TAP, 60% for BAP, 50% for PINP, 33% for NTX, 31% for α-α CTX and 38% for, β-β CTX, whereas in Sk group they were: 33% for TAP, 22% for BAP, 0% for PINP, 13% for NTX, 17% for α-α CTX and 17% for, β-β CTX.

Conclusion: Pagetic patients with skull involvement showed a marked increase in bone turnover and a lower response to bisphosphonate therapy. Moreove, α-α CTX is the marker with the highest increased values in these patients. The results suggest that these patients probably need to be treated with higher doses or more potent bisphosphonates.

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LONG-TERM RESPONSE AFTER BISPHOSPHONATE THERAPY IN PAGET'S DISEASE (PD). PROPOSED INTERVALS FOR MONITORING TREATMENT

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Objective: 1- to monitor the long-term evolution of PD activity after treatment with tiludronate by using serum total alkaline phosphatase (TAP) and more sensitive markers such as bone alkaline phosphatase (BAP), PINP and urinary NTX; 2- to analyse the predictors of long-term response to therapy and; 3- to study the most appropriate intervals of time for monitoring the response to therapy.

Methods: 32 patients with PD were included. All received 400 mg/d of tiludronate for 3 months. 21 patients completed the study. TAP, BAP, PINP and urinary NTX were measured at baseline and at 1, 6, 12 and 24 months after discontinuation of therapy. Quantitative bone scan was performed at baseline and at 6 and 24 months obtaining an activity index (SAI). Patients were classified into two groups depending on the long-term response to treatment: Group 1, patients who presented a persistent and significant decrease in disease activity at 24 months, n = 12 (57%) and Group 2, patients who presented a relapse in the activity of the disease at this time, n = 9 (43%). The relapse of disease activity was defined as a significant increase of SAI ($>13\%$) between 6 and 24 months after the end of treatment whereas the response to therapy was defined as a significant reduction in SAI ($>13\%$) at 6 months. In addition, these results were compared to the evolution of bone markers.

Results: Bone markers and SAI decreased significantly after therapy and the nadir response was observed at 6 months. At this time 100% of patients responded to therapy. Persistent long-term response was associated with lower baseline indices of bone turnover (BAP <60 ng/mL or TAP <600 U/L). The intervals of time for monitoring depended on the marker used: no patient from Group 1 presented a biochemical relapse in TAP at one and two years after the end of treatment whereas 33% and 45% of these patients showed relapsed BAP at these points-time. Moreover, all patients from Group 2 presented a biochemical relapse of BAP at two years whereas in only 33% of these patients TAP relapsed at this time.

Conclusion: most of the pagetic patients treated with tiludronate presented a long-term response which persisted 2 years after the end of treatment. The nadir response to treatment was observed 6 months after discontinuation of therapy whereas the relapse of disease activity was already observed one year after the end of therapy and depended on both the baseline disease activity and the bone marker used in the evaluation.

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STRONTIUM AND OSTEOMALACIA IN DIALYSIS PATIENTS

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Strontium is a trace element that affect calcium metabolism in end stage renal failure and dialysis patients. Strontium may cause renal ostedystrphy and particularly osteomalacia. We report a prospective study on 30 dialysed patients and 30 controls and compare serum strontium level and we study the correlation with osteomalacia. There was 13 womans and 17 men, middle age 52 years, dialysed at mean since 92 months. serum strontium level in dialysed patients was

104.03 ± 21.48 microgr./l, and in control group 12.36 ± 6.16 microg/l; the difference was highly significative.

Clinic and radiographic analysis found 5 patients with osteomalacia, the mean level of strontium was 100 microg/l and there was no difference with dialysis group. Our preliminary analysis confirm high level of strontium on dialysed patients but could not demonstrate the hypothesis of strontium-induced osteomalacia.

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THE HEPARAN SULFATE PROTEOGLYCAN, SYNDECAN-2, INDUCES APOPTOSIS IN OSTEOSARCOMA CELLS

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Syndecans are cell surface heparan sulfate proteoglycans that serve as co-receptors and modulate the actions of a large number of extracellular ligands.We transfected MG63 cells with the intact or modified sequence of Syndecan-2 (Synd2) to overexpress Synd2 or Synd2 lacking the carboxy terminal motif (CterSynd2) or Synd2 lacking its whole cytoplasmic domain (Dsynd2). We show here that Synd2 overexpression induces apoptosis in MG63 cells. Indeed, most Synd2 overexpressing cells were found to be TUNEL positive. Analysis on agarose gel confirmed the DNA fragmentation in cells transfected with Synd2. We also found that effector caspase activity was increased in Synd2, CterSynd2 and Dsynd2 expressing cells, compared with control cells transfected with the empty vector. Treatment with sodium chlorate, an inhibitor of glycosaminoglycan sulfatation, induced high inhibition of the caspase-3 like activity. This indicates that heparan sulfate chain activities are responsible for the caspase induction. However, PARP a caspase substrate, was cleaved only in cells overexpressing Synd2 or CterSynd2 and not in Dsynd2 transfected cells. Moreover, overexpression of CterSynd2 and Dsynd2 did not induce DNA fragmentation. Dsynd2 even increased life span of MG63 cells. Thus, caspase activation was not sufficient to kill the cells. The cytoplasmic domain of Synd2 seems to be involved in the activation of the mitochondrial apoptotic pathway since a release of cytochrome C from the mitochondria to the cytosol was associated with caspase 9 activation in cells overexpressing Synd2 or CterSynd2. Despite the activation of mitochondrial pro-apoptotic events, CterSynd2 expressing cells survived. These cells displayed a high proliferation rate, as measured by cell counting, that might balance the cell death induced by CterSynd2 proteoglycan. Finally, we show that overexpression of Synd2 (but not CterSynd2 nor Dsynd2) induced an increased phosphoJNK and an increased Bax expression that was abolished by SP600125 a specific JNK inhibitor. Thus, Synd2 may induce MG63 cell apoptosis through the JNK-Bax pathway.

In conclusion, Synd2 modulates apoptosis in MG63 cells. The different domains of this protoglycan, heparan sulfate bearing ectodomain, cytoplasmic domains, and Cterminal motif, might be involved in distinct levels of the apoptosis tunning, such as extracellular ligands binding, kinase recruitment or activation, and interactions with scaffolding or cytoskeleton proteins, respectively.

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ERK1/2 ACTIVATION IS PIVOTAL FOR ESTROGEN RECEPTOR-POSITIVE BREAST CANCER CELL PROLIFERATIVE RESPONSE TO CLODRONATE IN STEROID-FREE MEDIUM

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Aromatase inhibitors (AIs) induce complete estrogen deprivation and could replace tamoxifen as the first line endocrine therapy for breast cancer in the metastatic and adjuvant setting. Nevertheless, AIs lead to clinically significant bone loss. Several trials are ongoing to combine AIs with bisphosphonates, even more that adjuvant clodronate (Clod) has been shown to reduce the incidence of bone metastases in the adjuvant setting. We thus examined the effects of Clod on the growth of MCF-7 breast cancer cells cultured in steroid-free medium (SFM), an *in vitro* condition that mimics the effects of AIs. In SFM, 10^{-4} M Clod stimulated MCF-7 cell growth by up to two-fold (crystal violet staining assay), whereas it had no detectable mitogenic activity in complete medium. 17β-estradiol (10^{-4} M) also stimulated MCF-7 cell proliferation in SFM. Partial (4OH-tamoxifen, 10^{-7} M) and pure antiestrogens (fulvestrant, 10^{-7} M), simultaneously added with Clod, completely suppressed the mitogenic effects of the bisphosphonate, suggesting that it is mediated by an activation of estrogen receptor (ER). In accordance with this view, Clod induced ER downregulation, weakly increased progesterone receptor (PgR) expression, and stimulated the transcription of an estrogen-responsive reporter gene. Moreover, we investigated the mitogen-activated protein kinase pathway as a new potential target of this bis-

phosphonate in MCF-7 cells. Clod increased extracellular signal-regulated kinase (ERK1/2) phosphorylation and its mitogenic effect was prevented by ERK kinase inhibition. Furthermore, sodium pyrophosphate and sodium orthovanadate, two classical phosphatase inhibitors (10^{-5} M), also stimulated MCF-7 cells proliferation in SFM. Hence, Clod, due to its analogy to pyrophosphate, could interfere with the activity of phosphatase and cause subsequent accumulation of phosphorylated key proteins such as ERK1/2. In conclusion, we report a previously unknown stimulating effect of Clod on ER-positive breast cancer cells growth in SFM, a condition that is potentially relevant to the use of AIs for breast cancer. Our data indicate that ER specifically mediates these effects of Clod on cell growth and that ERK1/2 activation plays a key role in the cell proliferation. Finally, our results suggest that Clod could act as a phosphatase inhibitor promoting ERK1/2 phosphorylation, which in turn could activate ER inducing gene transcription and cell proliferation.

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ACTIVATION OF ESTROGEN RECEPTOR BY EXTRACELLULAR CALCIUM IN BREAST CANCER CELLS

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The skeleton is the most common metastatic site of breast cancer, especially when these cancer cells express estrogen receptor alpha (ER). Metastatic cells induce extensive osteolysis, leading to the release of growth factors that enhance cancer cell proliferation (the "vicious cycle"). Large quantities of Ca^{++} are released during tumor-induced osteolysis (TIO); whereas extracellular Ca^{++} concentration is about 1 mM, it can reach up to 40 mM at resorption sites. However, the effects of Ca^{++} on breast cancer cells have been little studied. We examined the effects of Ca^{++} on ER expression and transcriptional activity and determined the role of the calcium-sensing receptor (CaR) in the effects. Ca^{++} (15–20 mM) markedly downregulated ER protein and stimulated ER transcriptional activity in MCF-7 breast cancer cells stably transfected with an estrogen-responsive luciferase reporter gene. Lower Ca^{++} concentrations (2–5 mM) significantly increased progesterone receptor (PgR) levels, a classical marker of ER activation. 17 β-estradiol, a positive control, decreased ER levels, transactivated the reporter gene, and enhanced PgR expression, while 20 mM Mg^{++}, a negative control, had no effects. The pure antiestrogen ICI 182,780 (Faslodex) completely suppressed transactivation of the reporter gene and induction of PgR expression, indicating that Ca^{++} may specifically activate ER. Furthermore, increasing intracellular Ca^{++} with the calcium ionophore A23187 did not affect ER activation, suggesting that Ca^{++} acted at the cell surface. Thus, we examined the involvement of the CaR that is expressed in MCF-7 cells. NPS R-467, a selective CaR activator, decreased ER levels, increased ER transcriptional activity and, like Ca^{++}, enhanced PgR expression. In contrast, a highly-selective CaR antagonist suppressed (by 32–51%) the effects of 20 mM Ca^{++} on ER downregulation and on ER transcriptional activity. In summary, our results indicate that Ca^{++} acts as a weak "estrogenic" compound that activates ER in breast cancer cells. This activation is mediated, at least in part, by the CaR. Our data thus suggest that Ca^{++} released during the process of metastatic bone resorption could participate in the pathogenesis of osteolysis, leading to a further amplification of the vicious cycle of bone destruction.

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CHEMOATTRACTION OF OSTEOCLAST PRECURSORS BY STROMAL CELL DERIVED FACTOR-1 IN HUMAN OSTEOLYTIC BONE TUMORS

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Introduction: Giant cell tumor (GCT) of bone is a unique bone lesion that is characterized by an excessive number of multinucleated osteoclasts and severe destruction of bone. GCT consists of neoplastic stromal cells, multinucleated osteoclasts and their precursors – thus serving as a naturally occurring human disease model for the study of osteoclastogenesis. It still remains unclear how stromal cells of GCT recruit osteoclast precursors. One of candidate molecules is a chemokine. Stromal cell derived factor-1, that is produced by stromal cells. In the present study, we determined chemoattractant role of stromal cell-derived factor-1 (SDF-1) in osteolytic human bone tumors.

Materials and Methods: We established GCT cultures from 4 patients after IRB approval. We characterized cellular components of GCT and confirmed the presence of CD14 ± monocytes/CD68 ± macrophages and CD34 ± hematopoetic stem cells that express CXCR4, a specific receptor for SDF-1. The SDF-1 gene expression and SDF-1 protein were confirmed by real time RT-PCR, in situ hybridization, ELISA, and immunohistochemistry in the GCT tissue and cultured cells. The chemoattractive function of conditioned media was assessed by monocyte migration assay.

Results: GCT cells expressed genes for RANKL and TNF alpha. Conditioned media from GCT culture induced osteoclastogenesis in monocyte culture. SDF-1 positive cells were arranged in the stroma as well as around the blood vessels suggesting the active role in chemoattraction of osteoclast precursor cells. In situ hybridization showed SDF-1 gene expression by stromal cells of GCT. SDF-1 was present at 25–50 ng/ml in the conditioned media from GCT, which is in the range of physiological chemotactic concentration. Monocyte migration was 2.5-fold higher using the GCT conditioned media compared to the control media. The migration was inhibited by an average of 36% with anti-SDF-1 neutralizing antibody or competing recombinant SDF-1.

Discussion: These results suggest that SDF-1 is one of significant chemoattractant factors involved in the recruitment of hematopoietic osteoclast precursor cells during tumor-induced osteoclastogenesis. Stromal cells of locally aggressive bone lesions have capacity to induce osteoclastogenesis as well as chemoattraction of osteoclast precursors. Not only osteoclasts but stromal cells should be targeted in order to prevent recurrence of osteolytic lesions.

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TREATMENT OF BENIGN OSTEOLYTIC BONE LESIONS WITH TOPICAL ANTIRESORPTIVE AGENTS: PART I. A SCIENTIFIC RATIONALE

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INTRODUCTION: Tumor-induced osteclastogenesis is triggered by mesenchymal stromal cells and excessive osteolysis may lead to pathologic fracture and pain. We determined the therapeutic effect of bisphosphonates on the benign aggressive osteolytic diseases prior to possible clinical applications.

MATERIALS AND METHODS: (1) Cell Culture: We established cultures from 4 giant cell tumors (GCT), 5 unicameral bone cysts (UBC) and 1 nonossifying fibroma (NOF) that showed extensive bone destruction on radiographs. 30 nM and 100 nM of zolendronate and pamidronate were added to the GCT and UBC cultures. Cells were prepared for flowcytometry, RT-PCR and western blotting. Normal bone marrow stromal cells and fibroblasts were used as controls. (2) RT-PCR, Western Blotting and Flowcytometry: Real-time RT-PCR was performed using primers for RANKL, TNF alpha, cbfa-1, osterix, osteocalcin and SDF-1. Flowcytometry was performed using samples from day 1 and 3 cultures. Western blotting was performed to confirm the activation of caspase 3 and degradation of poly (ADP-ribose) polymerase (PARP). In addition, GGOH and FFP were added to determine the therapeutic targets.

RESULTS: Conditioned media from GCT, UBC and NOF cultures induced osteoclastogenesis in the human monocyte culture in the presence or absence of high concentration of osteoprotegerin. RANKL, TNF, cbfa-1 and SDF-1 were consistently expressed by stromal cells while Osterix gene expression was barely detectable or absent. Bisphosphonates induced death of neoplastic stromal cells in a dose and time dependent manner. Bisphosphonates induced activation of caspase 3 and degradation PARP suggesting activation of apoptosis. GGOH blocked bisphosphonate-induced apoptosis in UBC cultures not in GCTs. Bisphosphonates did not affect expression of genes for RANKL, SDF-1 and other adhesion molecules.

DISCUSSION: These findings indicate that stromal cells of aggressive osteolytic lesions are of early osteoblastic lineage with maturational arrest and are capable of inducing osteoclastogenesis and actively recruiting ostoclast precursors. Bisphosphonates effectively and selectively induced apoptosis in the stromal cells of the human osteolytic diseases. The specific pahramacologic action may be different among different types of tumors. Our data provide a scientific rationale for the use of bisphosphonates for benign osteolytic diseases in addition to known inhibitory effects on osteoclasts and osteoclastogenesis.

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TREATMENT OF BENIGN OSTEOLYTIC BONE LESIONS WITH TOPICAL ANTIRESORPTIVE AGENTS: PART II. PRELIMINARY CLINICAL RESULTS

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INTRODUCTION: Giant cell tumor (GCT), unicameral bone cyst (UBC), aneurysmal bone cyst (ABC) of bone are locally aggressive benign bone lesions that are characterized by extensive bone destruction and a high recurrence rate. Many nonspecific local adjuvant therapies such as phenol, hydrogen peroxide or liquid nitrogen have been used but specific adjuvant therapies to target osteoclastogenesis have not been described. The purpose of our retrospective clinical study is to determine the safety and therapeutic efficacy of topical bisphosphonates for aggressive osteolytic lesions.

MATERIALS & METHODS: Ten benign osteolytic lesions were treated with conventional surgical curettage in conjunction with topical pamidronate or

zolendronate. The lesions were 4 GCTs with extensive bone destruction, 4 UBCs in the femur, 1 aneurysmal bone cyst (ABC) in the proximal tibia, and 1 extensive fibrous dysplasia in the femur. Two of 4 UBCs recurred three times prior to presentation. There were four pathologic fractures. After surgical curettage, the cavitary lesions were soaked with 30 mg of pamidronate or 1 mg of zometa for 1 minute and then packed with allograft bone chips that were soaked with the same amount of bisphosphonate solution. The cases were followed with serial radiographs for one year.

RESULTS: Clinical data showed no recurrences of GCT or UBC after the use of topical bisphosphonates one year postoperatively. There was one case of ABC that showed focal recurrence at the periphery of the lesion. Overall, bone graft incorporation and fracture healing were not affected by bisphosphonates and the integrity of the allografts appeared to be better maintained. Seven patient developed postoperative fever that resolved.

DISCUSSION: To our knowledge, this is the first clinical series on the safety and therapeutic efficacy on the topical use of bisphosphonates for the locally aggressive osteolytic disorders such as GCT and UBC. Topical application is a logical therapeutic approach for the localized aggressive osteolytic disorders without affecting any other uninvolved skeletons. The preliminary results were very encouraging based on the preclinical scientific data as well as clinical results. Patients with recurrent, aggressive osteolytic lesions in the weight-bearing bones would benefit from the specific pahramcologic agents that can target an autocrine loop of osteoclastogenesis among neoplastic stromal cells and hematopoietic osteoclast precursors.

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BSP GENE TRANSCRIPTIONAL REGULATION THROUGH A CAMP RESPONSE ELEMENT IN BREAST CANCER AND OSTEOBLASTIC CELLS

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Bone sialoprotein (BSP) is a secreted glycoprotein primarily found in the mineral compartment of developing bones. BSP is detected in a variety of human cancers and particularly those that metastasize to the skeleton. High expression of BSP in breast and prostate primary carcinomas is associated with progression and bone metastases development. This discovery led us to study the regulation of BSP transcription in human breast cancer cells compared with cells from the osteoblastic lineage. We focused our study on MCF7 breast cancer cells and Saos-2 human osteosarcoma cells which constitutively express BSP. We first constructed a series of promoter deletions cloned upstream of a luciferase reporter gene that were transiently transfected in both cell lines. We found that a -84 bp human BSP promoter-luciferase construct is sufficient for maximal reporter gene expression in MCF-7 breast cancer cell line. This promoter region analysis allowed us to identify a CRE element (TGACATCA) and an E-box (CACCT) overlapping the CRE. Using mutated promoter constructs, we evaluated the impact of each of these elements on the activity of the human BSP promoter. CRE is an important regulatory element in both cell lines and is responsible for the high transcriptional activity observed with the -84 bp construct. However, the E-box element doesn't seem to be functional. Mobility shift assays using probes containing the CRE element in the presence of MCF-7 or Saos-2 nuclear extracts revealed a major DNA-protein complex that we attributed to the CRE element. Our study demonstrated that three transcription factors, CREB-1, Jun D and Fra-2 interact with the CRE in MCF-7 cells as well as in Saos-2 osteoblastic-like cell line. Interestingly, Jun D and Fra-2 have been shown to be key transcriptional factors during osteoblastic cells maturation for the expression of osteoblastic proteins such as osteocalcin. Moreover, we showed by Western blot that MCF7 cells expressed a high level of Jun D and Fra-2 as in Saos-2 cells. As such, BSP transcriptional regulation through the CRE element represents a new demonstration of a common regulatory mechanism utilized by both osteoblasts and breast cancer cells and supports the osteomimetism hypothesis. Indeed, our results suggest that the expression of critical transcription factors, such as Fra-2 and Jun D in breast cancer cells, confers to these cells a phenotype that mimics that of osteoblasts.

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DIFFERENTIAL EFFECTS OF BASIC CALCIUM PHOSPHATE CRYSTALS ON ARTICULAR CHONDROCYTE ACTIVATION

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Basic calcium phosphate (BCP) crystals including hydroxyapatite (HA), octocalcium phosphate (OCP) and carbapatite (CA), have been implicated in the pathogenesis of certain degenerative types of arthritides, such as the Milwaukee shoulder syndrome and osteoarthritis. BCP crystals have heterogenous physicochemical properties and proinflammatory effects, as shown in *in vitro* and *in vivo* studies. However their role in the pathogenesis of articular destruction is still poorly known. This study was aimed to evaluate the direct and distinct effects of

BCP crystals on chondrocyte activation. Sterile synthetic BCP crystals were characterized by X-ray diffraction and infrared spectroscopy. Bovine articular chondrocytes were cultured in non adherent conditions with OCP, HA and CA crystals or IL-1b as a positive control. Nitric oxide (NO) and iNOS expression were assessed by the Griess reaction and RT-PCR respectively.

Chondrocyte activation by BCP crystals was related to the crystal type. OCP and CA stimulated NO release in supernatants and induced iNOS and IL-1b mRNA expression in a time-course and dose-response fashion. In contrast HA have no effect on NO production and iNOS expression. OCP crystal-induced iNOS activation was markedly inhibited by the transcriptional inhibitor, actinomycin D and also the translational inhibitor cycloheximide suggesting regulation at both transcriptional and translational levels. Signaling studies using pharmacologic inhibitors showed that at least OCP crystals induced iNOS activation and NO production through p38 and JNK mitogen-activated protein kinases (MAPK) whereas Erk1/2 MAPK was not involved. iNOS stimulation was not dependent on IL-1b since IL-1ra did not inhibit NO production by OCP crystals. OCP crystals induced p38 and JNK MAPK phosphorylation.

In conclusion, our data suggest that different BCP crystals exert distinct chondrocyte activation, and can explain the clinical features. Specifically NO, an important mediator of cartilage degradation, can be directly produced by OCP and CA crystals in articular chondrocytes. Chondrocytes might play a direct role in the pathogenesis of articular destruction triggered by microcrystals.

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INCREASED EXPRESSION OF RECEPTOR ACTIVATOR OF NF-KAPPA B, ITS LIGAND RANKL AND THE DECOY RECEPTOR, OSTEOPROTEGERIN, IN THE COLON OF CROHN'S DISEASE PATIENTS

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Receptor activator of NF-kappa B (RANK) is expressed by osteoclast precursors but also in the immune system by mature dendritic cells (DC). Together with its ligand RANKL expressed on T lymphocytes, RANK plays a critical role in DC-T lymphocytes interaction, particularly influencing T lymphocytes and DC survival as well as T cell activation. Osteoprotegerin (OPG), a decoy receptor for RANKL may interfere with this interaction. RANK, RANKL and OPG levels of expression have never been studied in the gut. Our aim was to check for mRNA expression of RANK, RANKL and OPG in human colon and to describe their protein levels in CD, a chronic inflammatory disease associated with bone loss.

Material and methods: Total RNA was extracted from 5 normal colon samples from patients operated for colonic cancer. RANK, RANKL and OPG mRNA expression was studied by specific RT-PCR. Fixed colonic samples from 14 patients with CD and 4 controls were used to localize and quantify RANK expression by immunostaining and immunofluorescence. Supernatants of cultured colonic biopsies from 15 CD patients and 7 controls were analysed by immunoassays for RANKL and OPG production and their correlation to pro- and anti-inflammatory cytokines was studied.

Results: mRNA expression of RANK, RANKL and OPG was confirmed in human colon. RANK was mainly expressed in colonic mucosa by CD68+ activated macrophages and s100+ DC, as determined by immunostaining. The number of RANK+ cells was significantly increased in CD colon, particularly in inflamed area. Production of RANKL and OPG by cultured colonic biopsies was also significantly increased in CD. OPG production was significantly correlated with histological inflammation and the levels of pro- and anti-inflammatory cytokines while RANKL production was not significantly different between inflamed and uninflamed area.

Conclusion: RANK, RANKL and OPG are expressed in the human colon. RANK is mainly expressed by mucosal activated macrophages or DC and is overexpressed in CD. RANKL and OPG are also produced in larger amount by colonic mucosa in CD. The role of these molecules in the regulation of chronic intestinal inflammation is certainly worth investigating and might be related to the loss in bone mass observed in CD.

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QUANTITATIVE ULTRASOUND MEASUREMENTS OF BONE MASS IN LIVER AND CARDIAC TRANSPLANTATION PATIENTS: ARE THEY USEFUL?

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Background: Osteoporosis is one of the most frequent long-term complications of transplant recipients, and severely affects quality of life in these patients. However, there are little information about the diagnostic utility of heel

quantitative ultrasound measurements (QUS) in this group of patients. The aim of our study is to investigate if QUS is a valid method to detect low bone mass in transplant recipients.

Methods: We have cross-sectionnally evaluated 52 patients, 32 with orthotopic liver transplantation and 20 with cardiac transplantation. There were 34 men and 18 women, with a mean age of 55 ± 12.6 years, and mean time since transplantation of 53.4 ± 47.8 months. Bone mineral density was assessed (DXA) at spine (L1–L4) and hip with an Hologic QDR 4500 densitometer. In addition, all subjects had calcaneal QUS measurements using a Sahara device (Hologic Inc, MA, USA). The quantitative ultrasound index (QUI) and the estimated heel BMD were calculated from speed of sound (SOS, m/s) and broadband ultrasonic attenuation (BUA, dB/MHz) as has been described elsewhere. QUI T-scores were obtained from Spanish normal values.

Results: From DXA measurements, 75% of the subjects had osteopenia (T score < −1) and 24% had osteoporosis (T score < −2.5). A significant correlation was observed between QUS parameters and DXA measurements: QUI was positively correlated with lumbar BMD ($r = 0.379$, $P < 0.01$), femoral neck BMD ($r = 0.342$, $P < 0.05$), intertrocanteric BMD ($r = 0.441$, $P < 0.01$) and trocanteric BMD ($r = 0.501$, $P = 0.001$); heel BMD was also correlated with lumbar BMD ($r = 0.395$, $P < 0.01$), femoral neck BMD ($r = 0.339$, $P < 0.05$), intertrocanteric BMD ($r = 0.439$, $P < 0.05$) and trocanteric BMD ($r = 0.496$, $P = 0.001$). In order to investigate the ability of QUS to identificate the subjects with low bone mass (osteopenia), the sensitivity and specificity for different cutoff values of QUI T-score were calculated (see Table). Conclusion: Low bone mass is very common in liver and cardiac transplant recipients. Although a positive correlation exists between lumbar and femoral BMD and QUS parameters, quantitative ultrasound is not useful as a diagnostic tool to identificate transplanted patients with low bone mass.

Table: Diagnostic performance of QUI T-score

QUI T-score Cutoff	Sensitivity	Specificity
−0.25 SD	91.6%	33.3%
−0.50 SD	83.3%	33.3%
−1.0 SD	61.1%	58.3%
−1.5 SD	38.8%	75%
−1.8 SD	13.8%	83.3%

Bone development and Tissue Engineering

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RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN IN TREATMENT OF MAXILLOFACIAL OSSEUS DEFECT

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The osteoinductive properties of rhBMP-7 have been studied in bone defect on animal models. Six rabbits underwent reconstruction of unilateral hemimandibulectomy stabilizing with mini plate. Three animal received human recombinant BMP-7 in colagen carier mixed with bone marrow from crista iliaca. Control group of other three rabbits received only autolog bone transplant from crista iliaca in defect.

Reconstructed segments were evaluated by ALP activity, C-T and BMD analysing 30 postoperative days and histologic and clinical inspection after 60 postoperative days.

Results: ALP activity were significant higer in group with rhBMP-7 after 14 days then in group with bone transplant 30 days. C-T and BMD (bone mineral density) analysing after 30 days showed average value of new formed tissue in rhBMP-7 group 471−437 mg/cm³ and controul group 534−520 mg/cm³ . Clinical inspection after 60 days on rhBMP-7 group showed totally bridging defect with new formed bone and incorporation on two of three than control group showed no integration of bone transplant on all animals. Histological analysing showed abundant proliferation of vesels and new bone tissue (osteoblas, osteocit) . Controul group showed necrosis, died bone tissue.

Conclusions: This result indicate that rhBMP-7 in colagen carrier mixed with bone marrow induced new bone formation across critical size mandibular defects successfully integrated with existings host bone and creating stable union on two

of three. (64%) Control group showed no integration bone transplant. This date indicated that rhBMP-7 have potent osteoinductive effect in relation with autolog bone transplant alone.

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HISTOLOGIC AND HISTOMETRIC EVALUATION OF OSTEOGENESIS INDUCED BY OCTACALCIUM PHOSPHATE (OCP) COMBINED WITH BONE MATRIX GELATIN (BMG) IN RAT SKULL DEFECTS

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The purpose of this histologic and histometric study was to assess the osteogenic potential and determined the quantity of new trabecular bone formation after implantation of OCP and BMG alone and in combination with together into the cranial defects in the rat. For this reasons we used 110 young male Sprague Dawley rats (5–6 weeks age and 120–150 gr weight). After the rats were divided in fore groups randomly, a full thickness standardized trephine defect, 5mm in diameter, was made in the rats parietal bone and 5 mg of OCP, BMG alone and combination of OCP/BMG (in ratio 1/4) were implanted into the defects. No OCP and BMG particles were implanted in control group that was otherwise treated identically. On the 5th, 7th, 14th, 21st and 56th days after implantation, the rats were killed and bone samples collected. After processing the samples by routine histological procedures, 5 μm thick sections of bone were cut and stained with Haematoxyline & Eosin (H & E) and studied histologically and histometrically by using light microscope and eyepiece graticule. The amount of newly formed bone was quantitatively measured by the use of histomorphometry methods.

As the results showed, the new bone formation was initiated from the margin of defects during the 5–7 days after implantation. In addition to bone formation from the margin of defects toward the center, interstitial growth of new bone was seen locally around the implanted materials. During the 14–21 days after implantation, bone marrow cavities and bone marrow tissues in newly formed bone were seen. By the end of study, the newly formed bone increased and relatively was matured and almost all of the implanted materials were absorbed. In control group, at the end of study, a few clusters of new bone were seen near to the defect margins and host bone. The histomorphometric analysis indicated statistical significant differences in the amount of new bone between the experimental and control groups ($P < 0.05$).

In conclusion, implants of OCP/BMG appear to stimulate bone induction and new bone growth in bone defects greater than the another groups and these biomaterials could be used in the repair of cranial bone defects in clinical situations.

Key words: Octacalcium phosphate, Bone matrix gelatin, Bone induction, Parietal bone, Rat.

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OUR EXPERIENCES WITH TREATMENT OF LARGE DEFECTS OF ARTICULAR CARTILAGE WITH AUTOLOGOUS CHONDROGRAFT TRANSPLANTATION

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Background: Large defects of articular cartilage have a poor regeneration capacity and often are leading to osteoarthritis. Authors are presenting experiences with treatment of large cartilage defects on a load bearing areas in large joints, with use of autologous chondrografts, prepared on the base of fibrin glue.

Materials and methods: From December 1999 to November 2002 were treated in facultative surgery hospital 19 patients (16 men, 3 women) with large defects on a load bearing areas of bearing joints using autologous chondrografts transplantation. 16 patients had defects of the femoral condyle, including 2 double defects, and had defects of the trochlear tali. During diagnostic arthroscopy of the defect there is a segment (weight 300 mg) obtained from nonbearing area of articular cartilage to cultivation. After 3 weeks autologous chondrograft is prepared from suspension of autologous chondrocytes and fibrin glue Tissucol (fa immuno). 1 mm 3 of chondrograft contain 5−6 millions chondrocytes. Size and thickness of graft is specified in accordance with defect during arthroscopy. After removing of the subchondral sclerotic bone to the cacelouse bone until bone is appeared, chondrograft is posted in the place of the defect using fibrin glue Tissucol.

Results: Results of healing were checked by followed arthroscopy with biopsy specimens harvest after 3 months and by MRI 4 months after transplantation. Immediately after transplantation in all patients pain, swelling, synovial exudates and joint locking were reduced. One year after transplantation in all patients 10 patients underwent examination 9 patients had excellent results, only one patient with transplant on talus was reported owing to impingement syn-

drome on malleolus lateralis. Histology showed appearance and structure hyaline– like cartilage.

Conclusions: Cultured autologous chondrografts can be used for treatment of large cartilage defects bearing joints. Mosaic plastic is excluded if area of defect is above 2 cm.

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PERIPHERAL OSTEOMA OF THE MANDIBLE: A CASE REPORT

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Osteoma is a benign osteogenic lesion of bone tissue that is characterised by very slow contiuous growth. It can be developed as a central, peripheral or extraskeletal type.

Osteomas are found commonly in the skull, facial and jaw bones. Peripheral osteomas of the mandible are very rare. Only very few cases -not related to Gardner syndrome-have been reported in the English literature the past 40 years.

Although it is most common in young adults, it can arise at any age. A new case of a peripheral osteoma of the mandible is presented. A 23 year old female was admitted to the ward due to a swelling of the right mandibular body. The patient was afebrile, in good condition and without any other general or local clinical signs. Her medical history imcluded no significant trauma that she could remember and she had first noticed a small mass in the area 3 years ago. The size of the mass had gradually increased causing an obvious face asymmetry. Physical examination showed a painless, hard immobile lession in the region of the first molar. The panoramic radiography showed -not very clearly-a round opaque mass while the CT scanning revealed a well-circumscribed radiopaque mass 1.5cm × 1 cm × 0.5 cm. The mass was removed surgically under an intraoral approach. Histologic examination of the extracted tissue confirmed the diagnosis of the peripheral osteoma. A 5year follow-up showed no recurrence.

CONCLUSION: An important new case of mandibular osteoma without evidence of facial trauma is described with an excellent outcome and a very good prognosis.

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MITRAL ANNULUS CALCIFICATION IN PATIENTS WITH CHRONIC RENAL FAILURE

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Mitral annular calcification (MAC) is a degenerative process associated with left ventricular hypertrophy (HLV) and progressive atherosclerosis, characteristic of the older age groups. The aim of investigation was to determinate the MAC frequency in patients on hemodialysis and to try to find the correlation between MAC intensity and the duration of hemodialysis, age, sex, Ca/P metabolism, level of parathormone and atherogenic factors. Our study included 40 subjects, 24 men and 16 women, aged 20–67 years (X = 47 ± 32). All patients were included in the chronic intermittent hemodialysis program three times a week for four hours. Dialysis was performed on dialyzers with membranes made from regenerated cellulose acetate. At the beginning of dialyses, blood samples were taken for laboratory analysis of calcium, phosphate, alkaline phosphatase, parathyroid hormone, total cholesterol, HDL and triglycerides. In all patients twelve-lead ECG was recorded and 2-dimensional echocardiography and M-mode were performed. The following MAC seventy scores, for M-mode and 2-D echo are used, mild, 3 to 5 mm (I segment score 1); moderate, 6 to 7 mm (II segment score 2); and severe, more than 8 mm (III segment score 3). In this study the presence of MAC was echocardiographically demonstrated in younger age group patients on hemodialysis in an attempt to explain the pathogenesis of MAC development. Myocardial calcifications are known to lead to hemodynamic disorders of cardiac function, which can directly cause frequent incidents and cardiac deaths in hemodialysis patients. Cardiac calcified syndrome could be a sequel of MAC causing conduction disturbances, valvular stenosis or insufficiency, and arterial emboli or endocarditis. A group of 40 patients on hemodialysis (aged 20 to 67, 26 men and 24 women) were divided into two groups: group 1 without MAC, N = 17 (42.5%), X = 3.5, SD = 3.1; and group 2 with MAC, N = 23 (57.5%), X = 6.2, SD = 2.4. M-mode and 2-D echocardiography were performed in all patients. Group 2 was divided into three subgroups according to MAC quantitation: mild N = 16 (70%), severe, N = 4 (17%), moderate, N = 3 (13%). Study results showed positive correlation between MAC and serum values of Ca and P ($P < 0.05$). Increased values of HDL cholesterol, statistically significant of the level $P < 0.05$ were observed. Study results showed the correlation between MAC and time factor, i. e. duration of dialysis treatment to be statistically significant ($P < 0.05$).

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Withdrawn

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DIETARY CALCIUM SUPPLEMENTATION IN SUCKLING RATS: SHORT-TERM BENEFIT TO BONE?

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Our previous investigation showed that dietary calcium supplementation during suckling period increases skeletal calcium and does not affect bone mineral density in adolescent rats. The present study aimed at evaluating how long does the increase in bone calcium persist after withdrawal of dietary calcium supplementation.

Forty-eight female suckling Wistar rats were supplemented with 6% calcium (as hydrogenphosphate) in cow's milk from day of birth 6 through 14, seven hours per day. Controls were administered cow's milk. After daily treatments the pups were returned to their mothers. Skeletal calcium was measured three times: on day of birth 15 (at the end of supplementation), on day of birth 21 (at weaning), and on day of birth 27 (after one-week 0.33% calcium diet). Calcium in carcass (whole body after removal of all internal organs and skin) and trace essential elements (iron, zinc, copper) were analysed in the liver, kidneys and brain by flame atomic absorption spectrometry.

As found earlier, 3–4 times higher calcium intake during suckling period increased calcium in carcass in comparison to the control. However, this difference in bone calcium disappeared one week after cessation of dietary calcium supplementation. No side effects on growth and other tissue essential elements were found (except a transient liver zinc decrease).

Summing up, the increase in bone calcium due to dietary calcium supplementation during earliest period of life has no long-term effect on bone; furthermore it disappears already at the time of weaning.

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A NEW MODEL FOR FEMORAL OSTEOTOMY STABILIZATION IN RAT USING AN OSTESYNTHESIS PLATE COATED WITH GROWTH FACTORS (IGF-I AND TGF-β1)

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Introduction: Previous studies successfully used a biodegradable poly(D,L-lactide) coating as a local drug delivery system for growth factors from intramedullary implants (Schmidmaier 2001). In this study we developed a new rat model for plate osteosynthesis, coated with PDLLA as a local drug delivery system for the growth factors IGF-I and TGF-β1. Titanium plates were used for stabilization of a 0.6 mm osteotomy gap. The plates were coated with 50 µg IGF-I and 10 µg TGF-β1 incorporated in PDLLA. Biomechanical tests and histomorphological analyses were performed.

Methods: A standardized osteotomy (0.6 mm gap) of the right femora of rat was performed. The osteotomy was stabilized using a coated or uncoated four hole titanium plate and 1.3 mm titanium cortex screws. X-ray examinations (p.a. and lat.) were performed throughout the experimental period of 42d. After sacrifice both femora were dissected for biomechanical testing using a material testing machine. For histological and histomorphometric analyses 5 µm sections were stained with Safranin O/light green and v. Kossa. The histomorphometry of the callus was investigated using an image analysing system. Statistic: ANOVA, Bonferroni.

Results: The radiological evaluation revealed a callus formation in all investigated groups without complete consolidation of the osteotomies 42 days after operation.

A significantly higher maximum load was measured in the growth factor group compared to the uncoated control group.

Indirect healing occurred with intramembranous ossification and enchondral ossification. No callus showed a complete mineralized bridging in the histology. Comparing the callus composition a significantly higher callus density was assessed in the growth factor treated group compared to the control. The percentage of cartilage in the callus was in the PDLLA-group significantly higher than in the control.

Discussion: This study clearly demonstrates, that locally applied growth factors IGF-I and TGF-β1 were able to enhance the biomechanical stability and the callus mineralization of the healing femur in comparison to the control

group. Although, the osteotomy is not completely healed due to the growth factor treatment at this time point, acceleration in healing can be measured. These results are in accordance with a previous study investigating the effect of local growth factor release from intramedullary titanium implants (Schmidmaier 2001).

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MID FEMORAL NECK CORTICAL THICKNESS AND STABILITY CHANGES WITH AGE

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Introduction: Femoral neck (FN) fragility has been attributed to age-related bone loss, with increased loss in women. Mechanical loading, compared to inactivity, is largely protective over age 50, and is mediated through the inferior cortex principally by walking and stair climbing. The purpose of this study was to identify the age-related changes that take place in the FN cortical thickness and their implications for its mechanical stability in the different regions around the mid FN cross-section.

Materials and Methods: Measurements were taken from peripheral quantitative computed tomogram (pQCT) images of a sub-group of 87 cadaveric femurs (F 7, 21–95, and M 13, 20–84). The mid FN cross-section was segmented radially into eight regions and the cortical bone stability ratio (CBSR) determined by the distance of each region to the FN cross-section's centroid divided by the region's mean cortical thickness (CT).

Results: The differences in regional CT between men and women, was less than 11% (Female: 3.03 ± 1.102 m; Male: 2.7 ± 1.063 mm (mean ± SEM) $P > 0.3$). However, there were differences in CT and CBSR between the young under fifty, (Un50, n = 9) and the old, (Abv50), (ANOVAs for young vs old: CT $P = 0.01$; CBSR $P = 0.006$). These effects were substantially attributable to differences in the inferior region, where there was an increase in thickness of the cortical bone. Abv50 29% (Abv50: 3.15 ± 1.074 mm; Und50: 2.45 ± 1.05 mm. $P = 0.01$). There was a corresponding improvement of 39% in CBSR in this region (Abv50: 3.39 ± 0.48; Und50: 5.58 ± 0.51, $P = 0.0059$) and 35% in the inferoanterior region (Abv50: 3.92 ± 0.54; Und50: 6.02 ± 0.62; $P = 0.018$). However there was a deterioration in CBSR posteriorly, −39% (Abv50: 12 ± 1.1; Und50: 8.5 ± 1.0; $P = 0.014$) and superoposteriorly, −26.1% (Abv50: 15 ± 1.0: Und50: 12 ± 1.03; $P = 0.03$).

Conclusions: A more uniform cortical thickness, seen in the young, would optimise fracture resistance to overloading from unusually loaded directions. Ageing was associated with a thickening of the inferior cortex and thinning of the cortex elsewhere, with corresponding effects on mechanical instability and potentially fracture when load is applied eg through the greater trochanter. This might reflect the hip's predominant loading exposure of walking in mid to late adult life. The hypothesis is being tested in the remaining 67 femurs.

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MERGING OF TRABECULAE INTO CORTICAL BONE UNDER THE GROWTH PLATE DURING GROWTH IS REGULATED BY MECHANICAL LOAD TRANSFER

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Longitudinal growth occurs because the growth plate produces new trabeculae, later resorbed or merged into the cortical shell. This process implies transition of trabecular metaphyseal sections into diaphyseal ones. We hypothesize that the development is governed by mechanical stimuli, and that trabecular and cortical bone share the same regulatory mechanisms for adaptation to mechanical loads. To test these hypotheses, we monitored the 3D development of the tibial cortex in growing pigs, using μCT. Specimens were sawn from the posterior cortex at three levels: just below the growth plate, at one-third of tibial length, and at one-half of tibial length. We then tested if regulation mechanisms for trabecular bone adaptation can also explain cortical bone development. For this, the tendency of cortical bone development was simulated using our mechanical stimulation theory, which could explain bone (re)modeling of trabecular bone [1]. A 3D FE-model was created for a section of cortical bone and loaded in longitudinal direction with a, from endosteally to periosteally, distributed increasing load.

The main results showed that from the growth plate towards the diaphysis, the pores of the trabecular structure gradually filled in, so density increased and a cortex developed. The computer model, largely predicted this morphological development (fig).

We conclude that merging of metaphyseal trabeculae under the growth plate into cortex is likely to be governed by mechanical stimuli. Further, diaphyseal cortex development of growing long bones can be explained as a form of trabecular bone adaptation, without need of different regulation mechanisms for cortical and trabecular bone.

Reference: [1] Huiskes et al., Nature, 405:704–706, 2000.
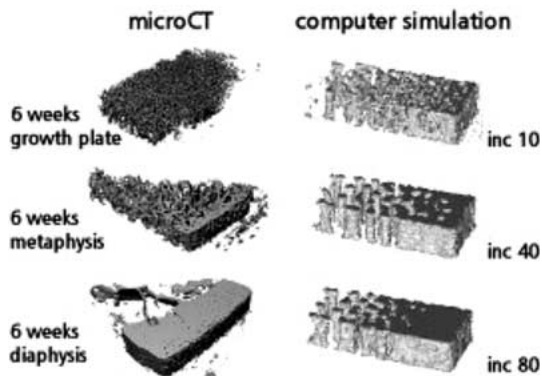
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BONE MINERAL DENSITY OF CHILDREN WITH GROWTH HORMONE DEFICIENCY BEFORE, 1 YEAR AND 7 YEARS AFTER CESSATION OF THERAPY

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Growth hormone deficient children have reduced bone mineral density (BMD) due to delayed bone maturation. Growth hormone is essential for normal growth during childhood and adolescence and influences bone mineralization and body composition.

The aim of the present study was to evaluate the bone mineral density of growth hormone deficient children before, after 1 year of treatment with rhGH as well as 7 years after cessation of therapy.

Patients and Methods: 20 children (16 boys and 4 girls) with documented (in two provocative tests) growth hormone deficiency participated in this study. Bone age was delayed with respect to the chronological age with a mean of 2.2 ± 0.5 yrs. The patients were divided into group A, which comprised 10 patients whose BMD was measured at the beginning and 1 year after discontinuation of rhGH therapy and group B, that consisted of 10 patients whose treatment had ceased 7 years ago. Mean group A and B ages were 9.5 ± 3.5 yrs and 20 ± 3.5 yrs, respectively. BMD was measured at the lumbar spine (L1–L4) level, using dual energy x-ray absorptiometry (DEXA). BMD values were compared to sex and age matched healthy control All children had normal calcium intake and normal physical activity.

Results: Although mean pretreatment BMD values were significantly different from those of the normal control group ($P < 0.05$), they went on to increase so that after 1 as well as 7 yrs after cessation of rhGH therapy, no statistically significant differences were noted. When BMD was corrected for bone age similar results were found, with no noticeable differences existing between boys and girls.

Conclusion: Growth hormone deficient children who have completed rhGH therapy and whose BMD might have been compromised, appear to have normal BMD values 7 yrs after cessation of therapy when compared to the normal population.

Table:

	BMD (g/cm^2)		
	Group A: Pret	Group A:1 yr	Group B: 7 yr
	n = 10	n = 10	n = 10
Patients	0.643 ± 0.132*	0.690 ± 0.159**	0.991 ± 0.137**
Control group	0.680 ± 0.134*	0.684 ± 0.070**	0.920 ± 0.054**
Boys	0.646 ± 0.147**	0.666 ± 0.173**	0.993 ± 0.112**
Girls	0.631 ± 0.371**	0.670 ± 0.445**	0.899 ± 0.143**
difference	* $P < 0.05$	** $P > 0.05$	

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BONE MINERAL DENSITY OF CHILDREN WITH INFLAMMATORY BOWEL DISEASE AND OTHER CONDITIONS REQUIRING ENTERECTOMY

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Retardation of growth and skeletal maturation are common in children with IBD and children who underwent enterectomy. Osteopenic children run a higher risk of fractures in childhood and adult age due to failure in achieving their optimal peak bone mass. The aim of the present study was to measure and compare the bone mineral density (BMD) of children with IBD as well as other diseases of the alimentary tract that required enterectomy with normal controls.

Patients-methods: A total of 19 patients (4 with Crohn's disease (mean age at the time of the study 11.25 ± 9.46 yrs) and 15 that underwent enterectomy (6 with Hirschsprung's disease (mean age 12.28 ± 5.23 yrs), 4 ileoectomized for intussusception (mean age 11.22 ± 1.27 yrs) and 5 with necrotizing enterocolitis (mean age 5.2 ± 1.22 yrs) were studied. The duration of symptoms ranged from 1 month to 10 years. None of the children with Crohn's disease had received corticosteroids before BMD measurement, while some had been treated with sulfasalazine. BMD evaluation was conducted 5 yrs after the surgical procedure and 5 yrs after the diagnosis of IBD. The BMD was measured by dual energy X-ray absorptiometry at the lumbar spine level (L1–L4) and compared to age and sex matched control values.

Results: The mean BMD values of children with Crohn's disease, Hirschsprung's disease, ileoctomized and children with necrotizing enterocolitis (NEC) did not significantly differ from those of normal controls (patients 0.808 ± 0.209, 0.696 ± 0.150, 0.650 ± 0.028, 0.603 ± 0.005 vs. control group 0.893 ± 0.115, 0.718 ± 0.096, 0.692 ± 0.033, 0.682 ± 0.015, respectively $P > 0.05$).

Conclusion: Our results suggest that the BMD values of patients that required enterectomy for different reasons did not significantly differ from those of the normal control group within 5 yrs. Although this could be related to dietary habits, longitudinal studies (> 5 yrs) are needed in order to investigate whether these children will finally attain their peak bone mass or whether they may benefit from additional calcium and vitamin D supplement.

Table: Comparison of BMD in patient (p) and control group (c)

		BMD (g/cm^2) p	BMD (g/cm^2) c
Crohn's dis	n = 4	0.808 ± 0.209*	0.893 ± 0.115*
Hirschsprung	n = 6	0.696 ± 0.150*	0.718 ± 0.096*
Ileoectomy	n = 4	0.650 ± 0.028*	0.692 ± 0.033*
NEC	n = 5	0.603 ± 0.005*	0.682 ± 0.015*
		* $P > 0.05$	insignificant

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BIOMATERIAL FOR BONE RECONSTRUCTION STUDY OF THE DEGRADABILITY OF POLY (2-HYDROXYETHYL) METHACRYLATE

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Synthetic materials are commonly used in biomedicine, however only a few of them are biodegradable. Studies of biomaterials surface are usually observed by technique providing information on dehydrated materials; however, they do not reflect the materials in biologic conditions of hydration. We have evaluated the potential biodegradability of a bone substitute, poly (2-hydroxyethyl) methacrylate (pHEMA), in contact with J774.2 macrophagic cells. We have determined the surface topography and roughness of the hydrated and dehydrated biomaterial.

Two groups of pHEMA cylinders and pellets were prepared by bulk polymerization. They were cultured in contact with J774.2 cells during 21 days. The first group was linear pHEMA, prepared without addition of a cross-linker. The second was cross-linked pHEMA (prepared with 2.5% (w/w) of ethylene glycol dimethacrylate-EGDMA). After cell culture, pHEMA cylinders and pellets were collected, prepared for scanning electron microscopy (SEM) and Atomic Force Microscopy (AFM), embedding in Lowicryl and sectioned. AFM measurements were done in contact mode. For imaging the surface of pellets incubated in saline by AFM, a lab-made liquid containing device was used. Controlled air-dried pellets were imaged as usually.

Cross-linked pHEMA disks, observed air-dried, exhibited a smooth and regular surface topography (Ra = 14 nm) similar to that of hydrated disks (Ra = 5 nm). Cells were affixed onto the cylinder's surface. The contact surface between cells and polymer was smooth and regular and did not differed from

the cylinder's surface before cell incubation. AFM measurements showed a relatively smooth surface topography (Ra = 153 nm) similar to the topology (Ra = 151 nm) before cell culture. For linear pHEMA, pellets observed air-dried showed a smooth and regular surface, whereas, pellets observed in saline showed a highly rough and irregular surface topography (respectively 26 nm vs. 296 nm). Cells were enclosed in the hydrogel mass. The surface of contact between cells and cylinders was rougher and irregular compared to surface before incubation. Few cells have engulfed polymer debris. AFM measurements showed a rougher surface topography after cell culture confirming polymer erosion.

Linear pHEMA appears to swell in saline and to be degradable by J774.2 macrophage cells. The surface in contact with the cells is more irregular and rougher than one observed air-dried. Cross-linking by EGDMA appeared to hamper resorption and swelling.

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EFFECTS OF CYCLIC RGD PEPTIDE FUNCTIONALIZATION ON THE BONE INGROWTH PROCESS IN POROUS CERAMICS IMPLANTED IN RABBIT CONDYL: A QUANTITATIVE HISTOMORPHOMETRY BASED APPROACH

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The aim of this work was to exploit quantitative histomorphometry approach to evaluate the effects of a functionalization by cyclic RGD peptides of porous ceramics on the bone ingrowth process in an experimental rabbit model. Twelve rabbits were maintained in our animal colony, and for each of them two porous ceramics (HA-TCP, 40/60%) were prepared for implantation. Only one of the two ceramics was functionalized by grafting of cyclic RGD peptides, and both of them were pre-cellularized with autologous osteoprogenitor cells from bone marrow. Functionalized and pre-cellularized ceramics (+ RGDc) were implanted in the left femoral condyl, while pre-cellularized ceramics (−RGDc) were implanted in the other side. Then, the rabbits were maintained until they reached 2 (n = 6) and 4 weeks (n = 6) respectively. At the time of euthanasia, right and left implants were removed and prepared for histomorphometry analysis. For each sample, 4 non-adjacent coloured slices were digitized with a dedicated high-resolution scanner. Image analysis algorithms were developed in order to evaluate the total surface of the implant (S), the total surface of the material (M), and the total surface of the newly formed bone (B). The global bone formation parameter was evaluated as F = B/(S−M). Local analysis was performed by using appropriate algorithms in order to evaluate the bone formation ratio in each pore of the material. This local analysis permitted the evaluation of the distribution of the bone formation ratios. After 2 weeks, global bone formation was significantly higher with RGDc than without: F = 0.12 $\pm$ 0.05 versus F = 0.21 $\pm$ 0.06 (P = 0.02; paired t-test). No significant difference appeared after 4 weeks: F = 0.21 $\pm$ 0.05 versus F = 0.18 $\pm$ 0.03 (P = 0.3). However, at the same time, the local analysis showed different distributions of bone formation ratios. In the + RGDc group, the distribution was more Gaussian-like waith a stabilisation of the bone formation ratio around 0.14, while such stabilisation did not appear in the -RGDc group after 4 weeks of implantation. In this study we have quantitatively showed the potential of a functionalization with cyclic RGD peptides for increasing the osteoconduction properties of porous ceramics in terms of speed and homogeneization of the bone ingrowth process. Histomorphometry approach coupled with local analysis should be a useful quantitative tool for the study and the optimization of bone implants.

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LYSINE DOSE-RESPONSE ON BONE MINERAL STATUS, BONE TURNOVER AND APPARENT CALCIUM ABSORPTION IN GROWING RATS

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There is early evidence to suggest that lysine plays a role in generalised growth, bone mineral accretion and calcium balance. However, no studies have looked at the dose-response of lysine on bone mineral status, bone metabolism and calcium balance under protein-replete conditions. The aim of this study was to determine the optimum dietary intake of lysine in growing rats, and to investigate if lysine supplementation above this optimum dosage confers extra benefits for bone growth. Diets were formulated to contain 20%, 60%, 80%, 120% and 160% of lysine requirement (based on National Research Council

requirements) and were equivalent in all other respects. Three-week old rats were randomised by weight to one of five groups (n = 10/group). Bone mineral content (BMC), bone mineral density (BMD), and bone area were measured by dual-energy X-ray absorptiometry (Piximus Densitometer, USA) at three time points (at 5 weeks, 9 weeks and 14 weeks) from the start of the study. Blood samples were also collected at the same time for measurement of insulin-like growth factor 1 and serum osteocalcin. Rats were also transferred to metabolic cages at 3 time points for determination of apparent calcium absorption. Calcium in the diet and faeces was analysed using flame atomic absorption spectrometry. Apparent calcium absorption was calculated as the difference between calcium in the diet consumed and that in faeces. Preliminary results from the first time point showed that rats fed 20% lysine had significantly lower BMC ($P < 0.01$) and BMD ($P < 0.01$) for both femur and vertebrae compared to the other groups. Bone area was also significantly lower at both sites ($P < 0.01$). The apparent calcium absorption was also significantly lower in this group compared to the rest of the groups ($P < 0.01$). No significant differences in BMC, BMD, bone area and apparent calcium absorption were observed among the other groups. These preliminary results indicate that lysine deficiency compromises bone growth, and possibly bone mineralisation. Whether a dose-response of lysine is apparent at 9 and 14-week time points will be determined. Underlying mechanisms of IGF-1 on bone growth will also be examined.

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OMOLOGOUS BIOACTIVATED BONE IN ACETABULAR BONE LOSS FOR HIP REVISION SURGERY

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Hip revision used to be a rescue-surgery ("act and then think"), becoming an anatomo-functional surgery later ("think and then act"), with growing care to the following objectives: filling the bone defect (anathomy), restoring rotation center of the hip (biomechanics), restoring limb length and hip function (clinics). Recently, through research on growth factors and stam cells, authors proposed a "fourth objective": to obtain a bioactive graft with better capability to osteo-integrate and restore a good bone stock, as for the amount of bone and the quality of bone (tissue engineering). This objective can be achieved only through cooperation between orthopaedic surgeons, hematologists and laboratory operators. Concerning the biomechanical and clinical objectives, we experienced several implants, with almost one thousand hip prosthesis revisioned in our center. As for the anatomical objective, omologous bone grafts from tissue banks are the most used mean to fill a bone defect, and we used it extensively together with rings. Recently it has been introduced in practical clinic the use of platelet gel and bone marrow-derivated cells to enhance bone graft osteointegration: a way to achieve also our "fourth objective". We analyze our experience of an omologous bone graft specific indication (AAOS tipe III bone defect, cranial to the acetabulum ring), comparing the simple bone bank graft to a bioactivated graft. We also developed a method of our own to produce platelet gel in our center, a simple and cheap way to obtain autologous concentrated growth factors, without any discomfort for the patient. We present the results of a randomized clinical trial that compares grafts in similar situations (same implant, same bone loss, same surgical procedure), by several radiografic means to study the evolution of the graft itself, to assess both quality and amount of osteointegration.

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THE RELATIONSHIP OF BODY WEIGHT AND HEIGHT TO ULNAR BENDING STIFFNESS, AN INDEX OF BONE STRENGTH, IN SEDENTARY YOUNG WOMEN

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The relationships of impact load to bone density and mechanical strength are clear for bones in the lower extremities that support body weight but may be regulated by other factors in the upper extremities (Ruff 1995, Looker 2002).

Purpose: The purpose of this study was to determine the relationship of body weight (WT) and height (HT) in young sedentary women to ulnar and tibial bending stiffness (EI), an index of bone strength.

Methods: We recruited 133 young sedentary women who were eumenorrheic (> 9 menstrual cycle/yr), currently not pregnant and non-smokers for this study. WT and HT were measured using a clinical scale to the nearest one tenth of kg or cm, respectively. Subjects were separated into 3 groups based on WT: low = 51 ± 5 kg (LO, N = 43), medium = 59 ± 5 kg (MO, N = 46), and high = 75 ± 13 kg (HI, n = 44). Density (BMD) of the distal forearm (wrist, WBMD) and Os Calcis (heel, HBMD) was measured with a Lunar PIXI, and

BMD of the leg (LBMD) and the entire arm (ABMD) with DXA (Hologic, model QDR 4500 W). Lean body mass (LBM), fat-mass (FM) and percent body fat (%BF) were assessed using whole-body DXA scans. We use a mechanical response tissue analyzer (MRTA), a noninvasive measure of bone bending stiffness (EI, Nm^2) to assess bone strength in the ulna and tibia. Bone stiffness is the product of a material property known as the Young's modulus of elasticity (E) and the cross-sectional moment of inertia (I) all of which reflect the composite effects of the geometric characteristics of bone, matrix properties and the distribution of bone mass about its bending axis (Steele 1988, McCabe 1991). For multiple group comparisons the Bonferroni t statistics were used (i.e., statistical significance was set with alpha = 0.05/2 or $P < .025$).

Results: The LO group were slightly younger than the HI. LBM, FM, %BF, HT, WBMD, HBMD, LBMD, ABMD and tibia EI were not significantly different among the groups. Ulnar EI was less in the LO compared to the HI group (20 ± 5 vs 25 ± 7 Nm^2, $P < .025$) and there were no differences between the LO and MO groups.

Conclusion: We found no differences in BMD in the heel, leg, wrist or arm in young sedentary women grouped according to body weight but greater EI in a bone of the upper extremity of the group with highest body weight. This suggests that factors other than impact load have a significant influence on biomechanical properties of bones of the upper extremity. (Supported by NIH Grant #5 S06 GM05393306 and NASA #SAA2-401535).

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DELIVERY OF TGF-BETA 3 PLASMID DNA VIA COLLAGEN GEL IN RAT CALVARIAL OSTEOBLAST CULTURE

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Studies directed toward using different forms of collagen as a carrier for naked plasmid DNA have shown the potential of these carrier matrices in therapeutic gene delivery and tissue engineering. The objective of this study was to determine the suitability of a dense collagen gel as a vehicle for sustained delivery of plasmid DNA in calvarial osteoblast culture. Fetal rat calvarial osteoblasts were grown in DMEM supplemented with 10% fetal calf serum and antibiotics. On the day prior to transfection, cells were seeded at a density of 50,000 cells/well into 48-well plates. Plasmid DNA, encoding transforming growth factor-beta 3 (Tgf-beta 3) combined with Geneporter (Gene Therapy systems, Inc.) was mixed with either collagen gel (32 mg/ml, NeuColl, Inc.) or media. Collagen with DNA (1.0 ug/ml) or media with DNA (0.25 ug/ml) was added to the wells then supernatants were collected at various time points up to 17 days. DNA release from the gel was measured by spectrophotometry and Tgf-beta 3 production was quantified by ELISA. DNA release data indicated a gradually increasing release of the plasmid from collagen over time, up to 17 days. Plasmid DNA released from the collagen gel was able to transfect calvarial osteoblasts which resulted in an up-regulation of Tgf-beta 3 protein production. Although Tgf-beta 3 production was observed in both experimental groups, cells exposed to collagen with DNA demonstrated elevated Tgf-beta 3 production levels. Tgf-beta 3 production peaked at approximately 10 days in contrast to the 2 day peak measured for DNA in media. In the collagen group Tgf-beta 3 up-regulation was measured as late as 17 days compared to 4 days in the media group. These results indicate that collagen gel can provide sustained release of viable plasmid DNA which can result in prolonged osteoblast transfection and elevated growth factor production. This suggests that use of collagen gel as a vehicle may provide a strategy to mediate localized and controlled gene delivery *in vivo*.

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OSTEOBLASTIC DIFFERENTIATION OF MESENCHYMAL STEM CELL AND BONE FORMATION BY RETRO VIRUS-MEDIATED BMP2 GENE TRANSDUCTION

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The stromal cells of bone marrow, also called mesenchymal stem cells (MSC), have the capacity to differentiate into a variety of mesenchymal phenotypes including osteoblasts, chondrocytes, fibroblasts and myoblasts. In this presentation, we report *in vitro* osteogenic differentiation of rat MSC by retrovirus-mediated gene transduction of bone morphogenetic protein 2 (BMP2), and *in vivo* bone formation by implantation of BMP 2-gene transduced MSC.

The rat MSC were isolated from femur of 5 weeks-old male Wistar rat and cultured in a-MEM supplemented with 10% FBS. Gene transduction with conditioned medium of retroviral producer cells was performed once per day for 6 hours for 7 days from the day after cells were harvested. BMP2 transduced MSC with scaffold were implanted into subcutaneous site of 5 weeks-old syngenic rats. These implanted were harvested at 8 weeks postimplantation, and prepared for histologic analysis.

Alkaline phosphatase activity was significant higher in BMP2-transduced cells than that in non-transduced cells or LacZ-transduced cells as controls. BMP2-transduced cells led to significant formation of mineralized nodules stained with Alizarin red-S, but nodule was not observed in non-transduced cells or LacZ-transduced cells. Forced expression of BMP2 in BMP2 transduced cells was confirmed by RT-PCR, and the expression of osteoblastic marker, osteocalcin and PTHR (parathyroid hormone receptor) were upregurated in BMP2-transduced cells. In histologic analysis, newly formed bone structures were observed at implanted site.

These results indicated that retrovirus-mediated gene transduction of BMP2 induce osteogenic differentiation of MSC, and BMP2 transduced MSC have the potential to regenerate bone tissue.

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A NEW NON-VIRAL VECTOR DELIVERED IN A FIBRIN GLUE MATRIX FOR LOCAL OSTEOBLAST TRANSFECTION AND GENE THERAPY

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Introduction: Several problems occurred using viral vectors for gene therapy like dramatic immune response up to cases of death. Only few non-viral vectors have been used in orthopaedics so far. In this study we investigated a new developed non-viral copolymer-protected gene vector (COPROG) and its ability to transfect osteoblasts with a reporter gene *in vitro*.

Materials and Methods: COPROGs were prepared as described by the working group containing plasmids with firefly luciferase as a reporter gene.

The fibrinogen component of fibrin glue (TISSUCOL Baxter, Germany) was mixed with the gene vector and 42 µl/well were dispensed on a 12-well plate. Clot formation was initiated by addition of 8 µl thrombin solution (4 IU). Primary rat osteoblast (1E5/well, 3wells/group) were seeded onto the coagulated clots and incubated in MEM/F12 + 10% FCS. The following groups were investigated:

I Fibrin glue without vector (Control)
II Fibrin glue with Luciferase vector (DNA 10 µg/well)

Cells were harvested on days 6, 14, 21, 28, mechanically homogenized and a luciferase assay (Promega, Germany) was performed according to the manufacturer. Luciferase expression was measured by relative light units/10s. Background was subtracted from the reported values.

Results: Luciferase expression was highest on day 6, decreasing over the time but was still detectable on day 28 (Fig.). The fibrin clot was degraded after 21 days in all groups.

Discussion: The results demonstrate that the COPROGs were able to transfect osteoblasts *in vitro*. Further studies will investigate long term effects of the gene vector and the *in vivo* behaviour with growth factor encoding plasmids.

Luciferase Transfection of osteoblasts in vitro
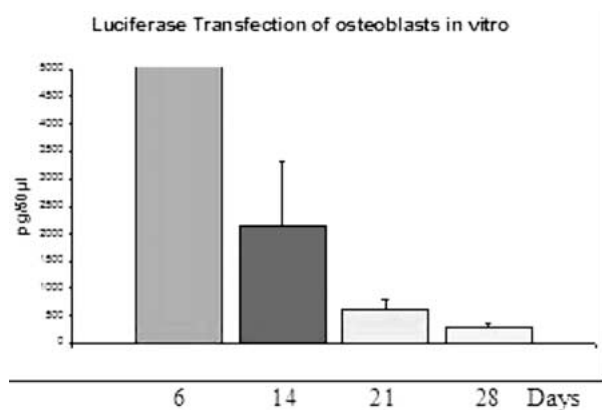
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GROWTH AND FORMATION OF RAT TIBIAE AFTER METADIAPHYSIS BONE DEFECT PLASTICS

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For the experiment we took 175 rats and divided them into 5 groups: K - intact animals; D - rats with defects on the proximal metadiaphysis (2,2 mm); A - rats with similar defects filled with ceramic hydroxyapatite (CHA); B - rats with defects filled with demineralized bone matrix (DBM); C - rats with defects filled with 2:1 combination of DBM and CHA. All rats were males with initial weight of 130–150 g.

D-group. This group exhibited accelerated longitudinal and appositional bone growth. MSAP exceeded control values by 5.63% (the 15 day), by 5.08% (the 30 day) and by 6.04% (the 60 day). ML changed less significantly however in the period up to 60 day there was a clear tendency to growth. Acceleration of apposition is probably due to periosteal response to perforation, while longitudinal growth gets accelerated due to processes of reparative regeneration when blood circulation increases and bioactive substances accumulate in perforation site which mediates functioning of epiphyseal cartilages.

A-group. In this group longitudinal growth rate values were indistinguishable from analogous of D-group. MSW in the period from the 30 to the 90 day was lower than analogous of D-group by 11.83%, 8.15% and 7.73% respectively. Retardation of appositional growth rate coincides with the most active processes of bioresorption of CHA i.e. when large amount of Ca ions is released.

B-group. By the 15 and the 30 day tibiae ML was lower than in D-group by 3.84% and 5.05% respectively. Appositional growth rate also tended to decrease, however MSAP was significantly lower than that of D-group only by the 30 and 90 days - by 10.75% and 10.83% respectively. It's probably due to DBM morphogenetic proteins which accelerate differentiation and growth of bone tissue.

C-group. Here longitudinal growth rate also retarded. The values of ML were lower than analogous of D-group by 3.81% (the 15 day), by 3.02% (the 30 day), by 4.98% (the 60 day) and by 3.24% (the 180 day). Under these conditions appositional growth rate was also inhibited and diaphysis MSAP was lower than in D-group during the whole experiment by 7.87–14.35%. Filling the defect with composition of materials leads to increased of both calcium and bone morphogenetic proteins. As we showed in our previous studies reparative regeneration under these conditions is the most optimum, however the whole bone responds by retardation of growth processes, probably due to changes in structure of epiphyseal cartilage.

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THE BIOTRANSFORMATION OF CALCIFIED COLLAGEN INTO THE CARTILAGINOUS TISSUE

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The native bone regeneration (of the apatite-water-protein system) by the calcified collagen compound is undoubtedly obvious [1–3]. The polysaccharide bioobject (cartilage) by the collagen-protein implant is highly conjectural as it has no theoretical prerequisites. The object of the investigation was to study the substitution effectiveness of the tetragonal cartilage which was removed in the course of a septum-operation with the use of the calcified material "LitAr", which was used first by us for the total substitution of the cartilaginous tissue. "LitAr" is material which is used commonly for replacing bone tissue defects. This is a xenogenetic collagen-hydroxyapatite compound with a density of 20–60 H. The lysis of "LitAr" in the organism takes place for 15–20 days. The native protein structure regeneration by the collagen compound is demonstrated early. The polysaccharide bioobject regeneration by the protein implant is in our opinion highly conjectural as it has no theoretical prerequisites. Nevertheless we used "LitAr" in rhinosurgical practice (septum-operations) that is to say for the hialine septal cartilage recovery. There have been operated 11 patients with the observation time period up to 2 years. The removed cartilage fragments have been replaced by the compound "LitAr". The material biotransformation in the defect zone was checked by computed tomography from 2 weeks to 5-6 months after performing the operation. In the plane of the sagittal section the nasal septum in the zone of the substitution by the material in 2 weeks has had a density of 50–80 H, and it conformed with the cartilage density in the no-resected parts. In the remains of the lyzed material the density was higher: 120–150 H. Checking in 5 months has revealed the total septal cartilage recovery in accordance with the normal anatomical constitution. Thus, the conducted investigation has revealed that the lysis of the calcified material "LitAr" and the product of its biotransformation is determined most likely by not a substitution site, but the cellular regeneration mechanism which is realized for the tissue type the defect of which is substituted by the material "LitAr".

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DOES THE SEROLOGICAL COURSE OF BONE TURNOVER PARAMETERS REPRESENT THE HISTOLOGICAL PATH OF BONE HEALING AT THE FRACTURE SITE?

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Clinical studies suggest that bone turnover markers (BTM) monitor or even predict the course of fracture healing since their serum levels represent the matrix formation at the fracture site. Although the serology of BTM may offer a chance to obtain a dynamic healing picture no direct validations for the observed serological changes exist. Therefore, the aim of this study was to evaluate correlations between the histological and serological course of fracture healing. 32 female merino sheep received a tibial osteotomy which was distracted to 3 mm and stabilized by a standardized external fixator, to achieve secondary fracture healing. The sheep were divided into 4 groups representing 2, 3, 6 and 9 weeks of fracture healing. Callus slices were stained immunohistologically for collagen I, II and III. Collagen areas were determined. Blood samples were taken pre- and postoperatively in weekly intervals. PICP, skeletal alkaline phosphatase (sALP), osteocalcin (OC) and PIIINP were measured by radioimmunoassays. All parameters showed broad inter-individual variations. 2 weeks after surgery the callus was composed of 80% collagen I and 19% collagen III. Only a small amount (1%) of collagen II was detected. At 3 weeks collagen I has decreased to 63% and collagen III has slightly raised. 13% of the callus were formed by collagen II. In the 6th week collagen I has increased to 70%. Collagen II and III were slightly reduced. At 9 weeks the callus consisted of 96% collagen I, 3% collagen II and 1% collagen III. The levels of all BTM decreased within the first week after surgery. Thereafter, PICP, sALP and OC fluctuated around their preoperative levels during the entire healing period. The PIIINP level increased between the 4th and 7th postoperative week. The observed changes in the BTM levels did not correlate with the histological findings at the fracture site. It remained unclear if the initial decrease of the PICP, sALP and OC levels represented a local or systemical shutdown of the osteoblast activity which may have caused the decrease of collagen I between the 3rd and 6th week. Further it could not be determined whether the increased PIIINP level was a delayed reflection of the formation or indicated the degradation of collagen III. It remains controversial if the serology of BTM directly reflects the course of fracture healing or a systemic response. Thus it might be questionable if BTM are able to serve as predictive parameters for fracture healing.

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ALLOLOGOUS CULTURED CHONDROCYTE PELLET STIMULATES NEW BONE FORMATION AT BONE TENDON JUNCTION

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Background: Bone-tendon junction (BTJ) is a unique structure composed of a fibrocartilage transitional zone, characterized with calcified fibrocartilage connecting to bone and non-calcified fibrocartilage connecting to tendon. This unique structure provides a gradual transition in stiffness and diminishes stress concentrations at the bone tendon interface. The biological healing in BTJ is slow, and the transitional zone often cannot be regenerated. We examined the feasibility of using allologous cultured chondrocyte pellet (CCP) to enhance BTJ healing in a rabbit partial patellectomy model.

Methods: Partial patellectomy was performed in 18 week old New Zealand white rabbits. After removal of the distal 1/3 patella, the patellar tendon was reattached to the proximal patella by intraosseous sutures, and protected by a figure of eight wiring. Chondrocytes isolated from the cartilaginous ribs of six week old New Zealand white rabbits were cultured for fourteen days into CCP. CCP was placed at the healing interface during repair in the study group (CCP group). The control group received no interposition. The patella-patellar tendon complexes were harvested at 8, 12 and 16 weeks after operation for histological studies (n = 4 for each time point). Two additional rabbits with CCP were harvested at 2, 4 and 6 weeks for early changes.

Results: There was no evidence of immune rejection of the allologous CCP. The 2, 4, 6 week samples showed progressive differentiation of the CCP with chondrocyte hypertrophy and columnar alignment. The CCP then gradually transformed, and could no longer be identified by 12 weeks. Earlier structural

integration at the BTJ in the study group with CCP interposition was observed compared with controls at 8, 12 and 16 weeks. Formation of a fibrocartilage zone like structure was seen in the later phase of healing in the CCP group. Endochondral ossification was evident adjacent to the patella bone cut surface. Toludene blue stain showed new bone formation with high trabecula density. The amount of new bone formed was significantly more in the CCP group at 12 and 16 weeks.

Conclusion: Allologous cultured chondrocyte pellet has a stimulatory effect on bone tendon junction healing. It may be used in the future to induce better regeneration of a damaged fibrocartilage transitional zone and replace the lost bone segment.

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INFLUENCE OF MECHANICAL LOADING ON BONE MINERAL DENSITY AND MICROARCHITECTURE OF THE MANDIBLE IN GROWING RATS

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The influence of mastication on alveolar bone is still poorly understood. Insertion of a bite-opening appliance (continuous light force on the mandible) and changes in food consistency (intermittent reduction of the forces on mandible with soft diet) were used to investigate the influence of mastication on mandibular bone in young growing rats. Thirty-six 4 weeks old male rats were divided into two equal groups, which were fed with either a hard or soft diet. After 2 weeks, half of the animals in both groups had their upper molars fitted with an upper posterior bite block, a device similar to that used in clinical orthodontics. The remaining animals served as controls. After another 4 weeks the animals were sacrificed and their left hemi-mandibles excised. Bone mineral density (BMD) and bone microstructure parameters of the alveolar process were measured using dual-energy x-ray absorptiometry (DXA) and micro-computed tomography (micro-CT), respectively. The alveolar process width was also measured. Soft food resulted in a reduction of BMD ($P < 0.05$) and a significant decrease of trabecular bone volume (0.33 ± 0.05 vs 0.39 ± 0.04, mean $\pm$ SD, $P < 0.05$) and thickness (150.7 ± 16.1 vs 170 ± 16.8, $P < 0.01$). The architectural modifications induced by the soft diet were partially prevented by the insertion of the bite block. The bite block did not significantly influence the alveolar process BMD but led to a significant increase of mandibular cortical thickness in rats fed the soft diet (3.85 ± 0.05 vs 3.74 ± 013, $P < 0.01$), together with an inhibition of alveolar process vertical growth and a significant increase of alveolar width. Body weight was similar in the 4 investigated groups, confirming a similar food intake. This rat model could prove to be a useful tool for the *in vivo* investigation of the role of muscular forces on the shape and structure adaptation of mandible.

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COMPUTER AIDED BONE TISSUE ENGINEERING

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Computer-aided technologies are introduced in tissue engineering for the design and manufacturing of scaffolds intended for implantation as defined three-dimensional tissue substitutes. In this study we tested whether autologous osteoblast-like cells cultured *in vitro* on individualised scaffolds can be used to induce bone regeneration in mandibular defects. For this purpose, bone defects were surgically created in the mandibles of 4 minipigs and the scaffold of the defect site was modelled by image processing of computed tomography data. Autologous bone cells from porcine calvaria were harvested from the periosteum of minipigs and grown in culture. Cells were seeded on scaffolds generated by rapid prototyping of polylactite/polyglycosite copolymers based on an stl. data set. Cells grew in the scaffold construct and remained viable and differentiated during culture time. The defects were then reconstructed by implanting the cellular hybrid material. The intraoperative situation as well as the postoperative radiographic control demonstrated an accurate anatomical fitting at the defect sites. The implanted scaffold constructs enriched with osteoblast-like cells were well tolerated and appeared to support bone formation as revealed by histological and immunohistochemical analyses *in-vivo*. These results indicate that combination of computer modelling and tissue engineering improves bone reconstructive surgery strategies.

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EXPERIMENTAL MANIPULATION OF CALCIFIED TISSUES IN THE RODENT HEMIMANDIBLE

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The tooth organ and surrounding periodontal tissues are extensively used in developmental biology to study organogenesis and cell differentiation, and together represent an advantageous environment for investigating events that regulate the formation of both collagen- and noncollagen-based calcified tissues. In order to study the role(s) and mechanism of action of matrix molecules, as well as the differentiation and physiology of the cells manufacturing them, we have developed an experimental system to access them in an environment that respects the local physiology as well as the whole-animal biology. The system consists of an osmotic minipump (Alzet) hooked to a "window" created in the bone on the buccal aspect of the rat hemimandible, near the apical end of the incisor. We report here three applications of this local targeting system: (a) tracer studies with noncollagenous bone matrix proteins tagged with dinitrophenol, (b) administration of retinoic acid, known for its effect on bone and dental development, and (c) local gene transfer using a lentiviral vector. Immunogold labeling demonstrated that infused, tagged bone sialoprotein and osteopontin integrated sites where their endogenous counterparts were found. Retinoic acid induced morphological noticeable changes in bone remodeling such as an increase in the number of osteoclasts, a result consistent with the agreed effect of retinoic acid on bone metabolism. Infusion of a lentiviral vector encoding for both ameloblastin and the GFP reporter successfully transduced bone, dental and periodontal cells over a distance of about 5 mm. Recently, the system was downscaled to mice hemimandibles. In conclusion, the results demonstrate that the rodent hemimandible window model offers the possibility to study various aspects of calcified tissue formation within the context of the whole-animal biology. It represents a restricted environment that can be exploited for administering locally high concentrations of products that would be toxic systemically, or proteins that are available in limited amounts. The bony window can be used to create local transgenic conditions, and its extension to mice opens the door to experimental manipulations in animals with well-defined physiological and phenotypic alterations. Supported by the Canadian Institutes of Health research.

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TEMPORAL INHIBITION OF CEBPA, CEBPβ AND PPARG2 EXPRESSION AND ADIPOGENESIS IN BONE MARROW STROMAL CELLS BY TRANSFORMING GROWTH FACTOR-β2 IN UNLOADED RATS

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The molecular mechanisms involved in the control of adipogenic stromal cell differentiation *in vivo* are poorly understood. In this paper, we determined the temporal regulatory effect of Transforming Growth Factor beta-2 (TGFβ-2) on adipogenic differentiation induced by skeletal unloading in rat long bone marrow stromal cells *in vivo*. Adult rats were unloading by hind limb suspension for 7 days, and cancellous bone mass and adipogenesis in the rat bone marrow were determined by histomorphometric analysis. The temporal expression of transcription factors involved in osteoblast and adipocyte differentiation were determined in the bone marrow stroma using real-time quantitative RT-PCR analysis. Skeletal unloading induced bone loss, as shown by decreased tibial metaphyseal bone volume, which was associated with decreased Cbfa1/Runx2 expression in the bone marrow stroma at 7 days. In addition, skeletal unloading increased the expression of the adipocyte transcription factors C/EBPa and C/EBPβ at 5 days. This was followed by increased expression of PPARg2 and Lipoprotein Lipase (LPL) and increased adipocyte number and volume in the bone marrow stroma at 7 days. Continuous administration of TGF-β2 prevented bone loss induced by unloading and corrected the expression of Runx2 in the bone marrow stroma at 7 days. TGF-β2 normalised C/EBPa and C/EBPβ expression at 5 days and corrected PPARg2 and LPL expression at 7 days, which resulted in normalisation of adipocyte volume and number at 7 days in unloaded bone marrow. The inhibitory effect of TGFβ on PPARg2 expression was not associated with increased PPARg2 phosphorylation at 4–7 days, a mechanism which is known to inhibit PPARg2 transactivation. These results show that TGFβ2 inhibits bone marrow stromal differentiation into adipocytes *in vivo* through sequential inhibition of C/EBPa, C/EBPβ and PPARg2, which provides a mechanism by which TGFβ2 regulates adipocyte differentiation of rat bone marrow stromal cells.

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TRI-DIMENSIONAL CULTURE OF OSTEOGENIC CELLS INTO A NEW HYDROXY-PROPYL-METHYLCELLULOSE HYDROGEL

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Large bone defect filling remains a major difficulty in orthopedic surgery. Indeed, the available amount of autogenous bone is usually not sufficient and bone allografts often lead to graft necrosis. Over the past decade there has been a significant amount of work on biodegradable scaffolds for the regeneration or repair of skeletal tissues. Besides compact scaffolds such as bioactive glasses, Porites coral, biodegradable polymers and calcium phosphates ceramics, hydrosoluble polymers (hydrogels) are promising approach to cell delivery for tissue engineering. In the present work we have evaluated a newly developed silated hydroxypropylmethylcellulose-based hydrogel as an innovative bone-repair material. The pH and temperature catalyze the reticulation of this cellulosic derivative, allowing the gel to be transformed into a "gelatin" state (Bourges et al., 2002). Self-hardening of the gel depends on silanol condensation from the silane grafted on HPMC. Three human osteosarcoma cell lines (MG-63, SaOS-2 and CAL72) and normal human osteogenic cells (HOST) were cultured in 3D in this hydrogel. We show here that this hydrogel allows the proliferation of osteosarcoma cells into a spheroid conformation as well as the survival of HOST colonies. Using mineralization assay and gene expression analysis of osteoblastic markers we have shown that the osteogenic cells cultured into this hydrogel maintained their osteoblastic phenotype or acquired a more mature differentiation status as compared to 2D culture on plastic. Moreover three-dimensional culture into this hydrogel revealed or enhanced the expression by these cells of cytokines involved in bone remodeling such as Il-1, IL-6, GM-CSF which were not expressed or at very low level when these cells were cultured on plastic. In conclusion, this study assesses the ability of a new hydrogel to maintain the survival, proliferation and differentiation status of human osteogenic cells. We will test this hydrogel associated with calcium phosphate granules and osteogenic cells for its properties as a vehicle for bone formation.

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A NOVEL BIOMATRIX STIMULATES HEALING IN RABBIT ULNA CRITICAL SIZE DEFECTS

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E-Matrix[TM] is a copolymer of denatured collagen and high molecular dextran designed to mimic early fetal mesenchymal connective tissue. A pilot study was carried out to determine if this novel biopolymer would stimulate healing of critical size defects in bone. In this study, a 15 mm section of ulna was bilaterally resected from 7 rabbits taking care to remove all associated periosteum. Periosteum was also removed from the radius underlying the defect. Complete ulnar resection was confirmed by x-ray after surgery. Immediately after removing the section of ulna, the defect was either filled with E-Matrix (7 total) or implanted with a collagen sponge infiltrated with E-Matrix (7 total). After suturing the overlying soft tissue, splints were applied to both forelimbs. The splints were used for approximately 2 weeks. Radiographic examinations were performed at 2, 4, 6, 8 and 10 weeks after surgery. At 10 weeks the animals were euthanized and the forelimbs examined using micro-CT at 35-μM voxel resolution. All X-rays were scored for calcified tissue (presumptive bone) growth within the defect on a 4-point scale (0-none, 1-minimal, 2-mild, 3-moderate, 4-extensive). Location of the new tissue and radius involvement were also noted. New presumptive bone was noted within all defects 2 to 4 weeks after treatment with the amount increasing to 6 weeks in both treatment groups. Moderate or extensive responses were detected in 6 of 7 defects treated with the E-Matrix infiltrated collagen sponge and in 5 of 7 defects treated with E-Matrix without the collagen sponge. Overall there appeared to be more new tissue growth in the defects treated with the E-Matrix infiltrated sponges compared to those treated with E-Matrix alone. In 5 of 7 defects treated with the E-Matrix/collagen sponge and 3 of 7 treated with E-Matrix alone presumed bone ingrowth was noted in the central region of the defect; in the others it was limited to the edges and along the radius. The micro-CT scans confirmed the new bone growth. E-Matrix, either alone or in combination with a collagen sponge, had an osteoinductive effect in this rabbit critical size ulnar defect model.

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NOVEL QTLS FOR BONE BIOMECHANICAL STRENGTH DISCOVERED USING A CHICKEN

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Osteoporosis is the cause of severe skeletal problems in laying hens, and up to 90% of hens in commercial flocks display broken bones during life. Chicken have a short generation time and it is possible to establish large resource pedigrees making it an attractive model for genetic dissection of multifactorial traits. The genome size ($\sim$1200 Mbp) is smaller than for mammals, but has approximately the same number of genes.

This genetic program (Functional Genomics of the Chicken FunChick) is based on a unique resource pedigree, an intercross between two divergent populations: the Red Jungle Fowl (RJF, the wild ancestor of the domestic chicken) and White Leghorn (L13, selected for egg production). The pedigree comprises about 1,000 animals from three generations. The populations differ markedly in growth and body composition. DNA samples from all birds have been collected, and genome scans including 120 microsatellite markers have been performed.

Multiple bone phenotypic traits, including determination of bone mineral density by DXA and determination of volumetric bone density and composition by pQCT have been measured. Biomechanical strength of the bones has been determined by three-point bending and torsion testing. QTL analyses show a number of genetic regions linked to bone biomechanical strength, on chicken chromosomes 1, 11, 7, 21 and 22. LOD scores for these five QTL regions range between 2.82-8.67 for load to failure and stiffness measurements. Also QTLs for bone mineral density have been identified.

A Unigene array using high-quality cDNA libraries from tissue has been produced, using sequences from 30,000 cDNA clones, and expression analysis is underway.

The ongoing chicken genome project will provide the full chicken genomic sequence during the spring 2004. This gives a unique opportunity to find novel candidate genes affecting biomechanical strength of bone and bone metabolism in chicken, where comparative genomics can be used in other model animals and in human genetic studies.

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FTIR IMAGING (FTIRI) ANALYSIS OF FEMORAL NECK BIOPSIES IN HIP FRACTURE CASES

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Osteoporosis has been defined as a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, whereas the mineral and matrix properties have sometimes been assumed to remain unchanged.

The aim of this pilot study was to analyse these properties in the female femoral neck cortex biopsies of hip fracture cases (n = 3, 79–86 years), and post-mortem controls (n = 4, 78–82 years). FTIRI provides quantitative data on crystal size and composition, and on matrix structure and composition at 6–10 μm spatial resolution. The spectra were acquired from 400×400 μm areas (6-8/biopsy). Crystallinity was calculated from the ratio of the peak intensities at 1030 and 1020 cm^{-1}, corresponding to the mineral phosphate vibrations PO43- in a stochiometric and non-stochiometric apatite environment respectively. Collagen maturity ("cross-linking") was calculated using the amide I band from the ratio of the peak intensities at 1660 and 1690 cm^{-1}, corresponding to vibrations in non-reducible and reducible collagen cross-links, respectively. Mineral to matrix ratio was calculated from the integrated area of the phosphate and amide I bands. All calculations were performed using ISYS (Spectral Dimensons, MD).

The bone mineral in the femoral neck fracture cases was more crystalline than that in controls (controls: 1.32 ± 0.01, cases: 1.39 ± 0.02, $P = 0.002$). The mineral to matrix ratio and collagen maturity of fractures cases tended to exceed that in controls (min/matrix: controls: 4.0 ± 0.1, cases: 4.3 ± 0.1, $P = 0.101$). (maturity: controls: 3.37 ± 0.10, cases: 3.58 ± 0.13, $P = 0.205$).

Our results are concordant with previous findings by Kent et al (JBJS 65-B:189 1983) who found that, close to the fracture site, the mineral crystals were enlarged compared those in control specimens. This, according to Gao's recent theoretical study (PNAS 100:5597 2003), would make them more fragile if they (as is likely) contain imperfections. Paschalis et al (CTI 61:487 1997) found that osteoporotic iliac crest biopsies also showed a higher crystallinity than controls.

Our study suggests that bone mass and its distribution might not be the only determinants of fracture risk. The properties of mineralised bone as a composite material that contribute to determining its material toughness appear to show differences between fracture cases and controls. These warrant more detailed and systematic investigation.

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GENE EXPRESSION PROFILE IDENTIFIES DIFFERENT CLASSES OF BONE THERAPIES PTH, ALENDRONATE AND SERMS

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Parathyroid hormone (PTH) given intermittently has been shown to have an effect on bone mass by stimulating osteoblast differentiation to increase bone formation. On the other hand, the antiresorptive agents estrogen, selective estrogen receptor modulators (SERMs) and bisphosphonates suppress bone turnover by inhibiting bone resorption. The molecular mechanisms that mediate these markedly different biological responses in bone remains largely unknown. The present investigation was designed to determine whether gene expression profiles of drug efficacy could be used to classify the different classes and subclasses of therapies or treatment outcomes. Gene expression profiles for each class was generated by microarrays at times and doses previously established for bone efficacy. Female, 6 month old rats were administered rhPTH (1–34) as a once daily injection (8μg/100 g) for 1 week. For antiresorptive agents, 6 month old ovariectomized rats were treated with 17α ethinyl estradiol (E), at 0.1mg/kg, Raloxifene (Ral) at 3.0 mg/kg, EM652 at 0.1 mg/kg and Alendronate (Ald) at 8 μg/kg for 5 weeks. RNA isolated from metaphyseal femoral bone was labeled by in vitro transcription and hybridized to an Affymetrix microarray containing 8800 rat genes. Gene expression analysis ($P < 0.05$) demonstrated that 316 genes were regulated by intermittent PTH, whereas a total of 3484 genes were regulated by different antiresorptive treatments. Pattern recognition/classification algorithms revealed common and unique subsets of genes that were regulated by PTH or antiresorptive treatment. Interestingly, the expression profile of these genes was sufficient to distinguish PTH from antiresorptives. Furthermore, the gene expression profile separated E and Ald from EM652 and Ral. Overall, PTH increased the expression levels of formation genes and turnover markers while the antiresorptive agents Ald and E had a strong suppressive effect. By contrast, Ral showed mild to no suppressive effect on the expression levels. Taken together, these results suggest the potential utility of this profile of prognostic markers that may be used for monitoring and differentiating the actions of these and perhaps future bone therapies.

Genetics

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ASSOCIATIONS OF POLYMORPHISMS IN THE PROMOTER REGION OF THE OSTEOPROTEGERIN GENE AND LOW BONE MINERAL DENSITY IN MALTESE POSTMENOPAUSAL WOMEN

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Osteoprotegerin (OPG) is a decoy receptor that controls osteoclastogenesis by binding to RANKL, preventing its interaction with RANK present on osteoclast precursors, and thus affecting the differentiation and maturation of osteoclasts. A number of polymorphisms in the OPG gene have been previously described and associated with BMD and fracture risk. In this study we examined two polymorphisms, A163-G (AseI) and a T950-C (HpaI), in the promoter region of this gene in a group of postmenopausal women in Malta.

111 postmenopausal women (55.6 $\pm$ 7.3 years) were recruited for this study. Polymorphisms in the OPG gene were analysed by PCR-RFLP while BMD at the lumbar spine and femur was measured by DEXA. Serum concentrations of procollagen type I and urinary DpD crosslinks were used as biochemical markers of bone turnover.

Genotype frequencies for the A163-G polymorphism were: AA 78.9%, AG 20.2%, GG 0.9%; while for the T950-C polymorphism were: TT 18.2%, TC 59.1%, CC 22.7%; and all were in Hardy-Weinberg equilibrium ($P > 0.05$). No evidence of linkage disequilibrium was observed between the two polymorphisms (chi = 3.93; p = 0.41; df = 4). For the T950-C polymorphism, a statistical significant difference was observed for trochanter BMD when comparing TT and CC homozygotes (t-test: $P = 0.04$), with TT homozygotes having a lower BMD. Homozygotes TT were also observed to have the lowest BMD at both lumbar and femoral sites, although the difference between the three genotypes was not statistically significant (ANOVA: LS $P = 0.73$; FN $P = 0.43$). Both biochemical markers of bone turnover were observed to be highest in TT homozygotes (ANOVA: Procollagen P = 0.02; DpD P = 0.16), showing an overall increase in turnover in this group. A statistical significant difference was observed in the distribution of genotype frequencies between normal individuals (LS and FN t-score > -1.0) and those that were either osteopenic or osteoporotic at one or both anatomical sites (chi = 8.78; $P = 0.01$; df = 2), with the TT genotype found more frequently in the abnormal group. The A163-G polymorphism does not seem to have an effect on lumbar and femoral BMD (t-test: LS $P = 0.28$; FN $P = 0.27$)

or bone turnover (t-test: Procollagen $P = 0.47$; DpD $P = 0.16$), when comparing AA homozygotes with AG heterozygotes.

In conclusion, a weak association was observed between the T950-C polymorphism and trochanter BMD indicating the possible role of osteoprotegerin gene variants on postmenopausal bone loss in women in Malta.

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FAILED TOTAL HIP REPLACEMENT MEMBRANE GENE EXPRESSION ANALYSIS

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Purpose: In attempting to unravel the complex cellular responses leading to prosthetic loosening investigators have been limited to studying gene expression of extracellular molecules about which most is known whereas new microarray technology allows simultaneous expression profiling of thousands of genes from a complex sample such as the membrane formed around loosened hip prostheses.

Methods: Two groups of 8 patients were recruited who have undergone primary total hip arthroplasty for osteoarthritis and subsequently developed either septic or aseptic loosening ± osteolysis. The control group consisted of one group of 5 patients with the same initial diagnosis who had undergone identical procedures, developed no clinical or radiological signs of aseptic or septic loosening, but had come to revision surgery for other complications as defined by the Swedish Hip register: fracture without previous osteolysis, dislocation, technical error, implant fracture, polyethylene wear or pain. Periprosthetic membrane was harvested at the time of revision surgery and subjected to RNA extraction. cDNA was then synthesized and hybridised to a Human Genome u95 Genechip ®array which contains a complete set of known human genes. Data normalisation, data filtering and pattern identification was performed using Genechip®3.1 software (Affymetrix, Santa Clara, CA).

Results: This has revealed the involvement of a large number of genes coding for transcriptional regulators upstream from the extracellular and cell-cell signalling molecules already known to be involved in osteolysis and deep infection and which may ultimately control the responses to wear particles and bacterial challenge. Differential expression of genes involved in cell survival and death, cell growth regulation, cell metabolism, inflammation and immune response was found. Most interestingly pathways for control of local bone resorption and inflammatory response have been shown to be highly activated.

Conclusions: The identification of these new pathogenetic mechanisms of total hip replacement failure make new indicators of disease susceptibility and prognosis plus new drug targets direct possibilities.

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THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA C161T POLYMORPHISM AND BMD IN POSTMENOPAUSAL WOMEN. THE DANISH OSTEPOROSIS PREVENTION STUDY

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Differentiation of mesenchymal precursor cells involves activation of peroxisome proliferator-activated receptor gamma (PPARgamma) when switching from osteoblastic lineage to adipocytic lineage. PPARgamma is expressed in adipocytes and primary osteoblasts. Induction of PPARgamma inhibits osteoblast differentiation and expression of osteoblast maturation markers. PPARgamma is inhibited by estradiol, directing the precursor cells towards osteoblastogenesis. Polymorphisms in the PPARgamma gene may be associated with development of osteoporosis by affecting these pathways. A previous study has suggested association between bone mineral density (BMD) and a C161T silent single nucleotide polymorphism (SNP) in exon 6 of the PPARgamma gene in postmenopausal Japanese women. In this study, we genotyped 425 Danish postmenopausal women (mean age (SD) 50.7 (2.9) years) for the C161T SNP. The women participated in a trial investigating the effect of hormone replacement therapy (HRT) on development of osteoporosis. In total, 150 of the women were assigned to HRT at baseline either by randomization or by personal choice. We investigated association with BMD at baseline and change in BMD over 5 years. BMD was measured by dual energy X-ray absorptiometry at the lumbar spine, femoral neck, total hip, and ultradistal forearm using Hologic scanners. A fragment containing the C161T SNP was amplified using PCR. After cleavage with BsaA1, restriction fragments were separated by electrophoresis. Genotype frequencies were CC 75.1% CT 23.3% and TT1.6%. Because of the low frequency of women homozygous for the variant allele, genotypes were dichotomized for analysis (CC versus CT + TT). Body mass index (BMI) did not differ by genotype. At baseline there was no association between genotype and BMD (age- and BMI adjusted t-test) in any region. During follow-up 92 women were treated with HRT continuously over

5 years, 224 women remained HRT-free during the study. No association was seen between genotype and BMD change at any region irrespective of treatment group. These results are not indicative of the PPARgamma C161T polymorphism as a marker for low BMD, increased bone loss, or effect of HRT on BMD in recent postmenopausal Danish women.

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ESTROGEN RECEPTOR ALPHA AND AROMATASE GENE POLYMORPHISMS: ROLE IN THE RESPONSE TO HRT IN POST-MENOPAUSAL WOMEN

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Genetic factors regulate BMD and possibly development of osteoporosis. Estrogens play a pivotal role in maintaining bone. The formation of estrogens from C19 steroids is catalyzed by aromatase in women and men. It is known that polymorphism at the human ERalpha and at the aromatase genes are associated with low BMD in postmenopausal women. We evaluated the possibility of interaction between aromatase and ERalpha genotypes with bone mass and we assessed the response in BMD to HRT in postmenopausal women. Subjects consisted of 209 Italian postmenopausal women with a range of age 36–76 years (mean 61.3 ± 8.6). Subjects under HRT received TTS 50micrg/day and nomegestrol acetate 5mg/day (12 days/month). LS-BMD was measured at the baseline and after 1 year. Pvu II and Xba I polymorphism of the ERalpha was determined by PCR and TTTA repeats by sequence analysis. The capital P and X and the lower-case p and x represent respectively the absence and the presence of the restriction site. For the TTTA repeats polymorphism the subjects were divided on the basis of the mean TTTA repeats: low (<8) medium (8–10) and high (>10). The genotype distribution for ERalpha was as follow: XX 42.3%; Xx 43.9%; xx: 13.76% (x2 analysis: $P = 0.3$) and PP: 33.3%; Pp:47.09%; pp: 19.53% (x2 analysis: $P = 0.07$) and for the aromatase TTTA repeats was: low: 41%; medium 40% and high:19%. The genotype with a low number TTTA repeats was more frequent in osteoporotic and osteopenic subjects in comparison with normal (42.8 and 39.2% vs.17.86%). Ancova analysis did not show any statistical differences in the LS-BMD of various ER alpha genotypes ($P = 0.6$), although LS-BMD tended to be lower in subjects with pp and xx genotypes. During HRT an increase of the LS-BMD was present in all the genotypes suggesting a feeble influence of the polymorphism on the hormone response. The same results were observed for the aromatase gene polymorphism. The absence of difference in the LS-BMD in subjects with or without HRT suggests a low influence of the aromatase gene on the HRT response. In conclusion, ER-alpha and aromatase gene polymorphism do not seem to influence the response to HRT.

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LOCALIZATION OF THE GENE CAUSING THE OSTEOPETROTIC PHENOTYPE IN THE INCISORS ABSENT (IA) RAT ON CHROMOSOME 10Q32.1

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Many of the insights into the factors that regulate the differentiation and activation of osteoclasts are gained from different spontaneous and genetically induced osteopetrotic animal models. The osteopetrotic *incisors absent (ia)*rat exhibits a generalized skeletal sclerosis and a delay of tooth eruption. Although the *ia* rat has well been studied phenotypically, the genetic defect still remains unknown.

To map the *ia* locus, we outcrossed the inbred *ia* strain with the inbred strain Brown Norway. Intercrossing F1 animals produced the F2 generation. Segregation analysis in 31 mutant F2 animals and 6 mutant F4 animals enabled us to assign the disease causing gene to rat chromosome 10q32.1. Homozygosity for the *ia* allele was obtained for two of the markers analyzed (D10Rat18 and D10Rat84). Key recombinations delineate a candidate region of 4.7 cM flanked by the markers D10Rat99 and D10Rat17. Although the sequence of this chromosomal region is not complete, over 140 known or putative genes have already been assigned to this region. Among these, several candidate genes with a putative role in osteoclast functioning can be identified, including Atp6n1a, Itgβ3 and Nsf. However, at this point it can not be excluded that one of the genes with a currently unknown function is involved in the pathogenesis of the *ia* rat. Narrowing of the candidate region combined with mutation analysis of the genes from this region will finally provide us more insight into the pathogenesis of this osteopetrotic animal model.

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TRUI POLYMORPHISM IN THE VITAMIN D RECEPTOR GENE AND BONE METABOLISM

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Genetic epidemiology has shown various bone characteristics to be under strong genetic control. Vitamin D receptor (VDR) gene represents a key candidate gene for both association and linkage approaches dissecting the genetic basis of osteoporosis. 3′ end of the VDR gene is rich in polymorphic loci (BsmI, ApaI and TaqI). As we have published recently, a novel polymorphism, recognised by the TruI restriction enzyme, had compromised identification of the BsmI genotypes. A mismatched base at the BsmI primer binding region led to a drop out of b allele and thus to a false prevalece of BB genotypes (BB 50, Bb 61, bb 54,χ^2 = 11.17; P < 0.01; reanalysed as following BB 31, Bb 80, bb 54;χ^2 = 0.0203; P < 0.90). This experience showed that in spite of methodically simple assessment of single nucleotide polymorphisms by a restriction analysis, the results should be interpreted with a caution.

To the author's best knowledge the TruI polymorphism has not been investigated in relation to bone metabolism in postmenopausal women in contrast to the remaining VDR polymorphisms. In the current study we are analyzing the distribution of the TruI genotypes in a cohort of 165 peri- and postmenopausal women of caucasian origin, as well as an association of the TruI polymorphism to diverse parameters of bone metabolism (denzitometric, biochemical and hormonal). The data are under investigation in our laboratory and will be displayed on a poster.

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CLINICAL AND MOLECULAR ANALYSIS IN PATIENTS PRESENTING WITH AUTOSOMAL DOMINANT OSTEOPETROSIS, TYPE II

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Autosomal dominant osteopetrosis, type II (ADO II) is an inherited bone disease characterized by skeletal sclerosis, commonly noted in pelvis, vertebrae and skull base, due to reduced osteoclast bone resorption. Patients present with an outcome ranging from very mild, with minor symptoms, to severe forms, with multiple spontaneous fractures, haematological deficiency and neural failure. Genetically, patients harbour mutations in the chloride channel type 7 (CLCN7) gene, encoding for a protein that provides chloride conductance for efficient proton pumping in the resorbing lacuna. To better understand the pathogenesis of the disease, we performed genotype-phenotype correlations in six patients. CLCN7 gene sequencing showed three novel (Y99C, K691E, A788D) and a known (R286 W) missense mutations in five subjects, while the sixth patient harboured no mutations in the coding region of the gene. Radiographically, all patients showed diffuse sclerosis, with "bone in bone" and "rugger-jersey spine" appearance. Probands had normal or increased serum level of bone alkaline phosphatase isoenzyme (ALP) and osteocalcin (OC), except two of them, one with unknown gene mutation and one with R286 W, both characterised by recurrent fractures and low serum OC. The R286 W mutation case also showed high calcitonin (CT) and low IGF-I levels. This 35 years old male patient had an initial mild course in childhood, later presenting with severe haematological and neural (blindness/deafness) deficiencies, recurrent epilepsy crisis and a fatal outcome. Identical mutation was shared by a 16 years old young woman which had a significantly different phenotype, with mild sclerosis and minor symptoms. Notably, at variance with the other R286 W case, this subject had high serum OC and IGF-I levels. The patient with unknown gene mutation also showed mild anaemia. Where families could be analysed, penetrance was 50%. In conclusion, we discovered novel CLCN7 gene mutations, with the R286 W associated in different families to significantly different clinical manifestations. Monitoring the milder form will clarify whether it will become severe later in life as occurred in the other patient. We also observed that radiologically diagnosed ADO II patients may harbour mutations either in different genes or in non-coding region of the CLCN7 gene. Finally, it would be interesting to evaluate whether low OC serum levels correlate with propensity of bones to fracture.

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AN ASSOCIATION BETWEEN POLYMORPHISMS IN THE CARTILAGE OLIGOMERIC MATRIX GENE AND FRACTURE IN THE THOROUGHBRED HORSE

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Fractures are common in both human and equine athletes and represent a failure of bone to withstand the forces placed upon it during exercise. In the horse, bone loading is influenced by the properties of its flexor tendons and recent studies have shown a correlation between equine bone and tendon strength. Cartilage oligomeric matrix protein (COMP) is an important mediator in the development of tendon matrix and low levels of COMP in equine flexor tendons predispose them to injury. This led us to hypothesise that polymorphism(s) in the COMP gene could be associated with fracture risk in horses. A dinucleotide $(CA)_{14}$ microsatellite was identified in the equine COMP gene, genotyped in 50 Thoroughbred horses and two alleles of 244 and 250 base pairs identified. Genotyping of a population of 86 fracture cases and 172 controls was then undertaken. A case was defined as non-monotonic fracture confirmed by radiography, ultrasound or scintigraphic imaging. Controls were matched based on age, gender and training history. Data were analysed using conditional logistic regression which showed that fracture cases were 2.3 times more likely to have the 244/244 genotype compared with the 244/250 genotype (95% CI 1.3–4.3, $P = 0.006$). In conclusion, these results show an association between COMP genotype and risk of fracture in the Thoroughbred horse; further evidence that equine bone's ability to resist injury may be influenced by the properties of its associated soft tissues. This is the first study to demonstrate that a gene coding for a protein that is not expressed in adult bone may potentially influence a skeletal phenotype.

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G1294C POLYMORPHISM OF THE CYP1B1 GENE IS ASSOCIATED WITH ALTERETED ESTROGEN METABOLISM AND BONE DENSITY PHENOTYPES

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We have previously shown that estrogen metabolism to either inactive 2-hydroxylated (2-OH) or active 16a-hydroxylated (16-OH) metabolites is a determinant of bone mass in postmenopausal women; increased urine 2-OH metabolites and a high 2-OH/16-OH ratio are negatively correlated with bone mineral density (BMD). Cytochrome P 4501B1 (CYP1B1) is one of the major enzymes that metabolize estrogen. In this study, we investigated the effect of G1294C polymorphism of the CYP1B1 gene (Val432Leu) which has been shown to be associated with breast cancer, on the pattern of estrogen metabolism and bone mass. Genotyping was performed by restriction fragment length polymorphism (the C variant produces a PstI restriction site) in a multiethnic cohort of 173 postmenopausal women and 60 elderly men. While the 3 genotypes are fairly well-distributed among Caucasians, the GG genotype is prevalent in African-Americans, but it is absent in Asians (Table). In the whole population, subjects with the GG genotype had a lower 2-OH/16-OH ratio (1.74 ± 0.17) compared to those with the GC and CC variants combined (2.13 ± 0.12), although the difference barely missed statistical significance ($P = 0.07$). Accordingly, urine 2-hydroxy- and 2-methoxy-estrogen levels tended to be higher in GC and CC individuals combined relative to GG subjects (9.07 ± 0.54 vs 7.3 ± 0.77 ng/mg Cr., $P = 0.065$ and 1.01 ± 0.04 vs 0.93 ± 0.05 ng/mg Cr., $P = 0.067$, respectively). More importantly, BMD in females with the GC and CC genotype was significantly lower than in GG women at both lumbar spine (0.91 ± 0.01 vs 0.95 ± 0.02 mg/cm^2, $P = 0.04$) and femoral neck (0.69 ± 0.01 vs 0.74 ± 0.01 mg/cm^2, $P = 0.003$), but there was no difference in serum E2 between GG versus GC and CC women combined (13.3 ± 1.5 vs 14.5 ± 1.4 pg/ml, P > 0.10). There were no significant differences in either urinary estrogen metabolites or BMD among male subjects of different genotypes. Mean BMD at all sites evaluated were highest among African-Americans and lowest among Asians. In conclusion, our data demonstrate that polymorphism of the CYP1B1 gene conditions specific patterns of estrogen metabolism, and this in turn may affect bone mass in postmenopausal women.

Table:

Genotype	Total	Whites	Blacks	Asians
N	233	215	8	10
G/G	69 (30%)	63 (29.3%)	6 (75%)	0 (0%)
G/C	105 (45.0%)	98 (45.6%)	1 (12.5%)	6 (60%)
C/C	59 (25.0%)	54 (25.1%)	1 (12.5%)	4 (40%)

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A POLYMORPHISM IN THE FOS RELATED ANTIGEN-1 (FRA-1) IS NOT ASSOCIATED WITH RISK OF OSTEOPOROTIC FRACTURES

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Bone mass is under strong genetic influence. Linkage studies in families have demonstrated a locus for regulation of bone mass on chromosome 11q. One of the candidate genes in this region is the Fos Related Antigen-1 (FRA-1) gene. The effect of Fra-1 has been examined in transgenic mice, where over expression leads to an osteosclerotic phenotype. We have previously demonstrated that the C101T polymorphism located in the first exon of the FRA-1 gene is associated with bone mass. Perimenopausal women with the TT genotype had lower lumbar spine BMD. We therefore wanted to examine the influence of this polymorphism on bone mass and risk of osteoporotic fractures in 409 patients with osteoporosis and 340 normal controls.

In patients with vertebral fractures the TT genotype was found in 21.1%, the TC genotype in 53.1% and the CC genotype in 25.8%. The corresponding numbers in the normal controls were 21.3%, 47.4% and 31.3%, respectively, chi-sq = 2.29, ns. BMD of the lumbar spine in individuals with the TT, TC and CC genotypes were: 0.908 ± 0.183 g/cm^2, 0.899 ± 0.181 g/cm2 and 0.898 ± 0.160 g/cm^2, respectively, ns. Similarly, BMD of the different hip regions were not different between individuals with the different genotypes. Multiple linear regression analyses demonstrated that BMD of the lumbar spine and the femoral neck was determined by age, sex and weight, but not influenced by the FRA-1 C101T polymorphism.

In conclusion: In this study we found no evidence, that the C101T polymorphism in the first exon of the FRA-1 gene has an effect on bone mass or fracture risk. The population examined in this study is older than the previously examined perimenopausal women and it is therefore possible that the effect seen in the perimenopausal women is lost with increasing age.

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C4887A POLYMORPHISM OF THE CYP1A1 GENE CONDITIONS ESTROGEN METABOLISM AND BONE DENSITY

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The ratio of urinary 2-hydroxyestrogen (inactive) / 16a-hydroxyestrogen (agonist) products of estrogen metabolism is an important determinant of postmenopausal bone density. We tested the hypothesis that polymorphisms of CYP1A1, a gene that codes one of the estrogen metabolizing enzymes, affects the balance between inactive and active metabolites, and in turn bone density in postmenopausal women. In a cohort of 143 untreated women, we detected 3 restriction fragment length polymorphic (RFLP) variants. Of these, the C to A transversion at position 4887 revealed significant interracial differences in allele frequencies (table). Biochemical analysis of urine estrogen metabolites revealed that, relative to individuals with the most prevalent CC haplotype, subjects with the CA and AA variants combined have significantly higher urine levels (mcg/gm creatinine) of 16aOHE-hydroxyestrone (16aOHE1: 5.67 ± 0.51 vs. 4.22 ± 0.23, $P = 0.01$) and urinary estriol (E3) (8.00 ± 0.74 vs. 6.17 ± 0.34, $P = 0.03$). The inactive 2-hydroxyestrone (2OHE1) and 2-methoxyestrone (2MeOE1) were not significantly different among the groups, resulting in significantly lower ratios of inactive to active metabolites in the variants compared to the wild type (1.69 ± 0.22 vs 2.08 ± 0.11, $P = 0.04$, for 2OHE1/16aOHE1; and 1.05 ± 0.09 vs 1.22 ± 0.05, $P = 0.02$, for 2OHE1 + 2MeOE1/16aOHE1 + E3). Ratios were lower in the two variant haplotypes regardless of race. Surprisingly, proximal femur bone mineral density was lower in the CA and AA variants compared to the CC genotype (0.72 ± 0.01 vs 0.67 ± 0.01, $P = <0.01$ in the femoral neck, ; 0.65 ± 0.01 vs 0.60 ± 0.01 $P = <0.01$ in the trochanter; and 0.86 ± 0.01 vs

0.81 ± 0.02, $P = 0.03$ in the total hip). The other 2 CYP1A1 polymorphsisms (A4889G, T6235C) were not associated with differences in either urinary metabolites or bone density. In conclusion, we identified 3 polymorphic variants of the CYP1A1 gene, whose prevalence varies among ethnic groups. Of these, only the C4887A variant (Thr461Asn) is associated with biologic differences in estrogen metabolism, which translate into differences in bone density.

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ADULT-TYPE HYPOLACTASIA (C/T-13910 POLYMORPHISM) AND BONE MINERAL DENSITY IN FINNISH ADULTS

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Adult-type hypolactasia (lactase non-persistence) is a heritable condition caused by a decline in the activity of lactase- phlorizin hydrolase (LPH) in small intestine during maturation. The prevalence of adult-type hypolactasia and the age of onset of hypolactasia vary significantly between different populations. In Asian and African populations hypolactasia is developed by the age of 2 years and most adults are lactase non-persistence. On the other hand the hereditary persistence of lactase in adulthood is common in people of northern European descent. In Finns the age of onset of hypolactasia is between 10 and 20 years, and the prevalence of lactase non-persistence in adults is about 17 %. Lactase persistence has shown to correlate strongly with the C/T- 13910 polymorphism located 13.9 kb upstream from the lactase structural gene. Hypolactasia may limit the intake of milk products resulting in lower calcium intake and thus increased risk for low bone mineral density (BMD). We examined how C/T-13910 polymorphism is associated with calcium intake and BMD in Finnish adults. Our subjects (n = 264) are a subset of a multicentre follow-up study, Cardiovascular Risk in Young Finns Study, that was started in 1980 to evaluate the cardiovascular risk factors and their determinants in children and adolescents. A subset of four age cohorts (20, 23, 26 and 29 years of age) participated in BMD measurements in 1991-1992, and we invited the same subjects for a follow-up measurement in 2002- 2003. BMD was measured at the lumbar spine and femoral neck and in the statistical analysis T-scores were used instead of density values. We used the same DXA device for both measurements in order to compare the change in bone mass over time. Data on weight change, smoking and drinking habits, physical activity and habitual calcium and vitamin D intake were collected by questionnaires. In women calcium intake was highest for the TT- genotype and lowest for the CC-genotype (hypolactasia) at the beginning of the follow-up (ANOVA $P = 0.09$), but the difference declined over time. In men the difference in calcium intake between the genotypes was smaller but increased over time. BMD T-score was lowest for the hypolactasia genotype at both measurement sites and times in both genders, but the difference between the genotypes was significant only in women at the lumbar spine follow-up measurement (ANCOVA $P = 0.05$).

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LINKAGE ANALYSIS IN A LARGE PEDIGREE WITH HYPEROSTOSIS CRANIALIS INTERNA EXCLUDES X-LINKED INHERITANCE

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Hyperostosis Cranialis Interna is a rare bone dysplasia belonging to the group of hyperostoses. Radiologically, the disorder is characterised by intra-cranial hyperostosis and sclerosis of the calvaria and skull base, while the remainder of the skeleton is spared. CT-scans show bony overgrowth of the temporal bone and narrowing of the internal auditory canals, the optic canals and the orbital fissures. Clinical symptoms are caused by compression of the cranial nerves and include recurrent facial palsy, impairment of the senses of

Table:

Genotype	Total	Whites	Asians	Blacks
N	226	208	8	10
C/C (%)	188	83.6	62.5	90
C/A (%)	36	15.4	37.5	10
A/A (%)	2	1	0	0

smell and taste, auditory and visual impairment, vertigo and nystagmus. Markers of bone turnover, like serum alkaline phosphatase and urinary hydroxyproline, are normal. So far, only one Dutch three-generation family with nine affected members has been described. In this family, radiological symptoms are observed from the second decade onwards and progress throughout life. The inheritance pattern is dominant, but since there is no male-to-male transmission, X-linked inheritance cannot be excluded. Since X-linked inheritance can be associated with skewed X-inactivation, we assessed the inactivation pattern of the X-chromosomes in four affected and two healthy women of the pedigree. However, no significant difference could be detected between the two groups. Given that the result of this test is not conclusive for exclusion of X-linked inheritance, we next performed a genetic screen of the X-chromosome. Polymorphic markers were chosen from the Généthon map and analysed in 21 family members. In total, 58 markers were analysed, excluding 70% of the X-chromosome. Furthermore, haplotype analysis of the residual regions excluded linkage in the remaining 30% as well. We conclude that Hyperostosis Cranialis Interna is not inherited in an X-linked, but in an autosomal dominant fashion. A genome-wide search will have to be performed to localise the causative gene in this disorder.

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OSTEOPROTEGERIN GENE EXPRESSION IN BONE IS NOT INFLUENCED BY OSTEOPROTEGERIN GENE SEQUENCE VARIATIONS

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Osteoprotegerin (OPG) plays an important role in the regulation of bone remodeling and OPG gene is considered as one of the candidate genes for genetic control of bone mass. Several polymorphisms were identified in the OPG gene and some of them were found to correlate with bone mineral density (BMD). However, published studies yielded conflicting results. Therefore, the aim of our present study was to evaluate the influence of this polymorphisms on OPG gene expression in bone tissue.

Eighty-four patients undergoing total hip arthroplasty due to osteoarthrosis (n = 56; 63.3 ± 11.8 years old) or osteoporotic femur fracture (n = 28; 74.3 ± 8.4 years old), were included in the study. RNA was isolated from bone samples obtained during surgery. OPG and RANKL mRNA were measured by real-time PCR. Levels of OPG and RANKL mRNA were normalised to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. OPG gene polymorphisms were analysed by PCR-RFLP methods. Restiction endonucleases XbaI, AseI, HincII, SmlI and BclI were used for genotyping of polymorphisms 149T > C, 163 A > G, 950 T > C, 1181 G > C and 6950 A > C, respectively.

Our results showed no significant influence of either promoter or coding region polymorphisms on mRNA levels of OPG or OPG/RANKL ratio in bone tissue.

This is the first study in which the function of polymorphisms in the OPG gene was studied on the level of gene expression *in vivo*. By this approach we can evaluate the influence of polymorphisms more precisely than through association with BMD. The drawbacks of *in vivo* studies are the differences in interfering factors such as systemic or local regulators of gene expression. These can only be avoided in *in vitro* studies, which could give the final answer to influence of OPG gene sequence variations on OPG gene expression.

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GENETIC DISPOSITION FOR ADULT LACTOSE INTOLERANCE AND RELATION TO BONE PROPERTIES AND FRACTURES DURING LIFETIME

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Background: Lactose intolerance is the inability of the intestine to hydrolyze lactose into galactose and glucose and may result in calcium malabsorption. We recently showed a close association of a −13910 T/C polymorphism (LCT) nearby the lactase-phlorizin hydrolase gene to bone density and fractures (Obermayer-Pietsch et al., JBMR 19, 2004). In the present study, we investigated the relation of this polymorphism to bone properties (density, ultrasound, lab) and bone fractures in women at different ages during lifetime.

Methods: We determined LCT genotypes TT, TC and CC in 318 premenopausal, 258 early postmenopausal women and 989 elderly women (36 ± 9, 62 ± 9 and 84 ± 6 years, resp.) using a polymerase-chain-reaction-based assay.

Genotypes were related to nutritional calcium intake, bone mineral density, ultrasound and laboratory parameters and the occurrence of bone fractures.

Results: All ages investigated showed equal genotype frequencies for lactose intolerant CC genotypes (24.3%, 24.8% and 24.3% resp.). Women with CC genotype differed for bone properties and fractures as compared to the other genotypes, increasing with age. In elderly women, hip fracture incidence was significantly associated with LCT genotypes by OR 4.6 [CI 1.1–20.7] (P = 0.001).

Conclusions: CC genotypes of the LCT(T/C-13910) polymorphism are associated with individual bone properties and predispose to bone fractures presumably resulting from differences in intestinal calcium absorption by lactase deficient individuals. Genetic testing for lactase deficiency may therefore be important to test an individual risk for calcium malabsorption and osteoporosis.

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A TWO-COMPONENT CONDITIONAL GENETIC ABLATION SYSTEM USING PUDTK AND THE CRE/LOXP TECHNOLOGY

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Genetic ablation experiments are used to resolve problems regarding cell lineages and the *in vivo* function of a certain group of cells. Here a general strategy that combines the power of puDtk and the Cre/loxP technology to accomplish time- and tissue-specific ablation is described. A transgenic mouse, puDtk selector, carrying PGK-loxP-neo-bpA-loxP-puDtk-bpA construct on its X chromosome was generated. When puDtk selector was crossed to a conditional cre line, puDtk expression should be activated as a result of the DNA recombination between the loxP sites in the Cre-expressing tissue of the bigenic progeny. Conditional ablation of the Cre-expressing tissue can therefore be achieved at the time that ganciclovir (GCV) is administrated. To demonstrate the feasibility of this strategy, puDtk selector mice were crossed to Col2Cre mice carrying transgenic cre expressed in differentiating chondrocytes. Upon GCV administration, differentiating chondrocytes were successfully ablated in bigenic animals at different developmental stages. Phenotypic analysis of Col2-ablated mouse reveals a completely disorganized growth plate without proliferating chondrocytes and osteochondrodysplasia. Dwarfism, macrocephaly, macroglossia and umbilical hernia were also observed in ablated 18.5 dpc embryos. Thus, we have generated a transgenic line which will enhance the utility of the existing conditional cre lines so that various kinds of time- and tissue-specific ablation experiments can be performed by initiating mouse crosses.

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CHARACTERIZATION OF NOVEL GENES OF A HUMAN FETAL CARTILAGE CDNA LIBRARY

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The following study describes one part of an EST-sequencing project. Initiated to find so far not described genes in poorly understood pathways, we generated and sequenced 5000 EST clones from a human cDNA library of pooled fetal cartilage tissue. All generated sequence data were compared to the non-redundant Genbank/ EMBL/ DDBJ and dbEST databases using the Ensembl Genome Browser and the EST identification task ESTsweep provided by the HUSAR bioinformatics group in Heidelberg/ Germany as well as the UCSC Genome Bioinformatics browser.

An extensive examination of the produced data pointed out that about 77% match to known genes/ mRNAs, 10% to genomic sequences, whereas 9.5% still match to anonymous ESTs only (3.5% were contaminated). We scanned these anonymous ESTs for domains by using the SMART browser of EMBL in Heidelberg/ Germany and started to characterize the most interesting clones by Northern Blot analysis with RNA from human, murin and rat cell lines. Additional RT-PCR and *in-situ* hybridizations on whole mouse embryos and sections helped us to understand the expression pattern of these genes during development. To determine the full-length cDNA sequence of all interesting clones we are planning to generate a high quality full-length cDNA library following the RIKEN protocol. This way we should be able to characterize every gene that might be important in ossification, postnatal growth and development of the skeleton and present candidate genes for inherited monogenic (skeletal dysplasias) as well as complex disorders (osteoarthritis / osteoarthrosis and osteoporosis).

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IDENTIFICATION AND CHARACTERIZATION OF POTENTIAL MOLECULAR TARGETS IN INHIBITING BONE RESORBING HUMAN OSTEOCLASTS

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For the research on osteoclast genetics, we have used HG-U95A and HG-U133A high capacity oligonucleotide microarrays by Affymetrix Inc. We have established human osteoclast assay, where high numbers of resorbing osteoclasts were induced from peripheral blood precursors and grown on bovine bone slices. From these cultures we have RNA in a time series manner. The extraction days were 0, 5, 7, 9, 12, 15, 18 and 21. The data obtained were analyzed with the Silicon Genetics' program GeneSpring 5.1. The major tasks for analyzing the chips after normalization of the data were principal component analysis (PCA) and hierarchial, k-means and SOM-clustering. From these clusters we ensured the behaviour of already known ''osteoclast biomarker genes'', such as cathepsin-K, CAII, TRAFs, c-Src and TRACP. Further filtered gene clusters included approximately 200 genes, in which there was also some novel ESTs. Some interesting genes analyzed were the disintegrin family genes, ADAMs among galectins and genes from RAB-gene family. The over 4-fold expression peak from day 5 to 7 for a cluster of 42 genes, including cell cycle-dependent genes like CDC2 and 20 arised some special interest. Confirmation of the microarray data was done by 1) qPCR studies of selected RAB-genes (RAB 13, RAB 20 and RAB 27A and B), 2) osteoclast-specific immunoassay studies of TRACP and 3) custom cDNA-microarray studies of known osteoclast related genes expressed on bone-grown human osteoclasts vs. plastic-grown osteoclasts from a 9-day culture. This last experiment gave us the opportunity to screen more detailed the genes affecting the attachment of human osteoclasts. From this experiment over two fold higher gene expression levels between growth matrixes were recorded for genes like integrin alpha V and MMP-7 (bone grown osteoclasts) or ATPase 6 N1A and cathepsin D (plastic grown osteoclasts). Our culture methods together with DNA-microarray generated expression data gives us the power to redefine the genetical milestones in the differentiation and function of resorbing human osteoclasts in a novel way. This level of understanding will benefit the development of new drugs that can inhibit the formation and/or function of this monocyte-derived highly specialized cell.
Keywords: human osteoclast, DNA-microarray, gene expression profiles

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TRANSCRIPTION REGULATION IN HUMAN OSTEOCLASTS: COMBINATION OF DNA MICROARRAY AND IN SILICO TRANSCRIPTION FACTOR STUDIES

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We have used the novel transcription factor (TF) database and binding site screening methods to study the possible common factors behind the human osteoclast gene expression data. Our expression data comes from a human osteoclast microarray experiment from Affymetrix HG-U133A oligo chips and the gene panels gained from the analyzed microarray data. We selected three different gene set profiles from our k-means clusters. Affymetrix oligoarrays includes also vast amount of unknown genes, which we studied separately from known genes. We used the EnsMart program for getting the 1 000 bp of the 5' upstream sequences for every gene in our data sets. TF binding sites from TRANSFAC database were obtained with Match program and we programmed also some own helper tools for handling the huge data amount coming from those database programs. The result showed us that both known genes and unknown genes in our gene profiles had common transcription factor binding sites in their 5' upstream sequences. These sites included factors like AP-1, USF, AREB6, c-Ets-1, Elk-1, Lmo2, GATA-1 and GATA-X. However there were only some slight differences in TF data between different gene panel profiles. We took another approach for studying specific marker genes of osteoclasts, namely RANK, TRACP, CA-II, calcitonin receptor and cathepsin-K. Vista programs were used for conservative binding site studies for human and mouse, in which 5 kbp upstream sequences were analyzed. We found some TF clusters for these marker genes: for cathepsin K, calcitonin receptor and CA-II the most common TFs were from CAP_01 and OCT_03, GEN_INI_B and STAT-family. For RANK the common factors were from GATA-family. These novel algorithms and methods for transcription factor studies are in their primary stages and critical evaluation of results and methods have to be possessed. However, the results from microarray data combined with in silico transcription factor studies are a powerful method to study the genetics and regulation of differentiating

human osteoclasts. Keywords: human osteoclast, transcription regulation, DNA-microarray, TRANSFAC

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THE GENERATION OF ANTIBODIES AGAINST ADULT HUMAN MESENCHYMAL STEM CELLS

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Adult marrow contains rare mesenchymal stem cells (MSC) that form colonies in vitro and differentiate into multiple stromal cell types in vivo. MSC and their immediate progeny are not identifiable morphologically and there are no definitive markers. We have used antibody phage display to generate monoclonal antibodies against MSC enriched fractions of bone marrow mononuclear cells (BMMNC), to facilitate the identification and isolation of MSC within adult marrow.

In order to obtain our MSC enriched target population, we have previously compared colony formation following immunoselection of different, antibody-defined BMMNC sub-populations. Of the antibodies tested, CD49a was shown to give the best enrichment of colony forming cells.

We have used a diverse synthetic phagemid library to generate phage antibodies against MSC enriched BMMNC. To reduce the selection of non specific clones, we used a simultaneous positive/negative selection strategy whereby library phage were incubated with unseparated BMMNC, and bound phage subsequently recovered from magnetically separated CD49a+ subpopulations. The selected library was amplified in E.coli, rescued with helper phage and phages were concentrated from the bacterial culture supernatant by polyethylene glycol precipitation. After each round of selection, enrichment and diversity of library phage clones were assessed by titration and PCR/BstN-1 digestion respectifully. When the diversity reduced, a dominant clone was analysed for binding to BMMNC, CD49a+BMMNC and to cultured bone marrow stromal cells (BMSC) by FACS.

There was a substantial enrichment of library phage after 2 and 3 rounds of selection, and after 3 rounds of selection, sequence analysis confirmed that 1 clone had dominated the population (c15). Binding of c15 to unseparated BMMNC was negligible but slightly increased in CD49a+ sub-populations (1.67% above naïve library). Binding was increased to 65% in BMSC cultured in the presence of dexamethasone, suggesting that this antibody may recognise stromal cells of the osteogenic phenotype.

In summary, we have generated phage antibodies against MSC enriched BMMNC. Tissue specificity and biochemical characterisation of 1 clone is now in progress and other unique clones (from selection 2) will shortly be assayed for binding to target cells. It is hoped that phage antibodies generated in this way will provide a much-needed resource for the study of mesenchymal stem cell differentiation.

Late Abstract

Bone Development and Tissue Engineering

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BONE TISSUE ENGINEERING BY CO-CULTIVATION AND STIMULATION OF OSTEOBLASTS AND OSTEOCLASTS IN POROUS SCAFFOLDS COMPOSED OF MINERALIZED COLLAGEN

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Introduction: In vitro engineered implants for replacement and repair of bone should meet these conditions: mechanical stability, variable shape and size, capacity of remodelling. To achieve this aim we developed porous constructs of mineralized collagen type I and introduced both osteoblasts and osteoclasts on it and stimulated them by mechanical strain.

Materials and methods: The starting composit material - mineralized collagen I - was produced in a biomimetic process in which the collagen fibril assembly and mineralization with calcium phosphate occurs simultaneously. Two- and three-dimensional, porous scaffolds were generated by freeze drying and crosslinking of the collagen with EDC, a carbodiimide derivative. The process led to pores with

diameters up to 200 μm. Whereas mouse osteoblasts were directly seeded onto the constructs, osteoclasts had to differentiate from human monocytes. The cells were co-cultured under the presence of 1,25-dihydroxy-vitamin D3, dexamethasone, M-CSF and RANKL. The mechanical stimulation was carried out by compression strain using piezoelectric actuator. C-LSM, SEM, TEM and quantitative PCR were used to study osteoblast and osteoclast growth and differentiation as well as remodelling of the scaffold.

Results: The use of mineralized collagen led to porous scaffolds with an increased mechanical stability compared to native collagen I. Osteoblasts showed good adherence and proliferation on the porous material. Newly synthezised collagen could be detected using SEM. Immunostaining and quantitative PCR revealed the presence of typical osteoblastic markers like ALP and bone matrix proteins as well as osteoclastic markers like TRAP and vitronectin receptor in co-cultures. TEM revealed degradation and resorption of the scaffolds by osteoclasts.

Discussion: Mineralized collagen I is a completely biocompatible material and its composition is close to that of natural bone tissue. The co-culture and stimulation of osteoblasts and osteoclasts on scaffolds of mineralized collagen might be a very useful strategy for the therapy of large bone defects by *in vitro* engineered bone tissue.

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