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BONE HEALTH, BODY COMPOSITION AND ADIPOKINES IN JUVENILE IDIOPATHIC ARTHRITIS

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ACADEMIC DISSERTATION

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To my family

CONTENTS

ABSTRACT

Background Children with juvenile idiopathic arthritis (JIA) are predisposed to compromised bone health and alterations in body composition because of chronic inflammation, nutritional and hormonal disturbances, limited physical activity and glucocorticoid (GC) therapy. Compromised bone health may present as pathological vertebral compression fractures, but data on their prevalence and risk factors in children are limited. Excess fat, and especially adipose tissue-derived adipokines leptin and adiponectin, may also contribute to impaired bone health. Furthermore, adipokines modulate immunity and inflammation in adults with rheumatic diseases, but their role in JIA has not been explored.

Objectives We evaluated bone health in patients with severe JIA and investigated body composition and adipokines and their contribution to bone health and disease activity in JIA.

Methods We recruited two cohorts of patients for cross-sectional studies. The severe JIA Cohort comprised 50 patients with severe polyarticular or systemic JIA. The GC-treated Cohort included 50 patients with JIA with mostly mild to moderate disease severity and at least three months' exposure to systemic GC. The results were compared with those of sex-and age-matched healthy controls. The study protocol included clinical and laboratory assessments, evaluation of bone mineral density (BMD) and body composition by dual-energy X-ray absorptiometry (DXA), spinal radiography and spinal magnetic resonance imaging (MRI).

Results Spinal radiography showed vertebral compression fractures in 22% of the patients with severe JIA. Patients with fractures had higher weight-adjusted cumulative GC dose, higher disease activity and higher body mass index than those without fractures. Bone age-corrected BMD Z-scores for lumbar spine and whole body were similar between those with and without fractures. On spinal MRI, altogether 28% of patients with severe JIA showed vertebral fractures and several other vertebral changes, including end plate irregularities in 26%, anterior vertebral corner lesions in 16% and disc changes in 46%. Based on concentrations of bone turnover markers, the patients with severe JIA had increased bone resorption, but normal bone formation. Further, patients with severe JIA had increased body adiposity, and their serum leptin was increased even independently of fat mass. Leptin showed an inverse association with bone turnover markers in patients, while in controls the association was dependent on fat mass.
In the GC-treated Cohort, fat mass, lean mass and serum leptin and adiponectin were similar to those of controls, but patients had slightly lower BMD values than controls. Those patients with lumbar spine BMD Z-score <-1.0 tended to have higher serum leptin values than those with higher BMD Z-scores, but in

regression analysis leptin was not associated with BMD. Adipokines did not correlate with current disease activity in either patient cohort.

Conclusions Patients with severe JIA have compromised bone health based on high prevalence of compression fractures. Risk factors include high GC exposure, high disease activity and high body mass index. BMD, as measured by DXA, is unable to differentiate between those with and without compression fractures. According to spinal MRI findings, patients with severe JIA have, besides compression fractures, several other changes involving intervertebral discs and vertebral end plates; the clinical relevance of these remains uncertain. Patients with severe JIA are prone to high adiposity, whereas those with less severe disease have normal body composition despite previous GC exposure. Leptin may negatively contribute to bone metabolism in severe JIA, but larger and longitudinal studies are needed to prove causality and to evaluate whether these preliminary findings are generalizable to other JIA groups. We did not observe a correlation between leptin or adiponectin and disease activity in either JIA cohort. The possible role of adipokines as a modulator of immunity and inflammation in JIA remains to be evaluated.

LYHENNELMÄ

Tausta Lastenreumaan eli juveniiliin idiopaattiseen artriittiin (JIA) voi liittyä kehon koostumuksen muutoksia ja luuston terveyden heikentymistä. Näille altistavia tekijöitä ovat krooninen tulehdustila, ravitsemukselliset ja hormonaaliset tekijät, luustoa kuormittavan liikunnan vähäisyys sekä glukokortikoidihoito. Kroonisia sairauksia kuten liikuntavammaisuutta, syöpäsairauksia tai autoimmuunitauteja sairastavilla lapsilla voi esiintyä selkänikamien murtumia merkkinä patologisesta luuston hauraudesta. Lastenreumaa sairastavien nikamamurtumien esiintyvyydestä, riskitekijöistä ja diagnostiikasta on rajallisesti tutkittua tietoa. Myös lihavuus saattaa olla yksi luuston terveyteen haitallisesti vaikuttava riskitekijä. Rasvakudoksen ja luuston välisen vuorovaikutuksen mekanismeja tunnetaan vielä melko huonosti, mutta rasvakudoksen erittämien adipokiinien, leptiinin ja adiponektiinin, on esitetty olevan osallisina. Adipokiinit myös muokkaavat immuunivastetta. Aikuisiän nivelreumassa korkea leptiini- ja adiponektiinipitoisuus ovat yhteydessä taudin aktiivisuuteen, mutta lastenreumaa sairastavilla yhteyttä ei ole tutkittu.

Tutkimuksen tarkoitus Tutkimuksen tarkoituksena oli selvittää nikamamurtumien ja muiden selkärangan poikkeavuuksien esiintyvyyttä ja riskitekijöitä vaikeaa lastenreumaa sairastavilla potilailla. Lisäksi tutkittiin lastenreumaa sairastavien lasten ja nuorten kehon koostumusta, adipokiinien pitoisuuksia ja adipokiinien yhteyttä luuston aineenvaihduntaan, luun mineraalitiheyteen ja taudin aktiivisuuteen.

Menetelmät Poikkileikkaustutkimukseen rekrytoitiin kaksi potilaskohorttia. Toisessa kohortissa oli 50 vaikeaa lastenreumaa sairastavaa lasta ja nuorta. Toisessa kohortissa oli 50 lastenreumapotilasta, jotka olivat saaneet systeemistä glukokortikoidihoitoa vähintään 3 kuukauden ajan, mutta joiden lastenreuma oli luonteeltaan keskimäärin lievempi. Adipokiinien, luustomarkkereiden ja luun mineraalitiheyden välistä vuorovaikutusta selvittäneissä tutkimuksissa tuloksia verrattiin iän ja sukupuolen suhteen kaltaistettujen terveiden verrokeiden tuloksiin. Tutkittaville tehtiin kliininen tutkimus, laboratoriotutkimuksia ja luuston mineraalitiheyden mittaus. Vaikeaa lastenreumaa sairastaville potilaille tehtiin lisäksi selkärangan röntgenkuvaus ja magneettikuvaus.

Tulokset Tulokset osoittivat, että selän nikamamurtumat ja muut poikkeavuudet ovat yleisiä vaikeaa lastenreumaa sairastavilla. Heistä 22%:lla todettiin nikamamurtumia selän röntgenkuvissa. Kolmen edeltävän vuoden kumulatiivinen painoon suhteutettu kortisoniannos, taudin aktiivisuus ja painoindeksi olivat suuremmat nikamamurtumia saaneilla verrattuna niihin, joilla murtumia ei todettu. Luuston mineraalitiheys ei kuitenkaan eronnut näiden ryhmien välillä. Selkärangan magneettikuvauksessa nikamamurtumia oli 28%:lla ja lisäksi havaittiin runsaasti muita muutoksia: päätelevyn epätasaisuuksia (26%), nika-

man etunurkan muutoksia (16%) sekä välilevymuutoksia (46%). Niillä joilla kuvantamismuutoksia todettiin, oli taipumus olla muita lihavampia. Vaikeaa lastenreumaa sairastavilla luuston hajoamista kuvaavan merkkiaineen pitoisuus oli korkeampi kuin verrokeilla, mutta luuston muodostusmarkkereiden pitoisuudet eivät eronneet ryhmien välillä. Vaikeaa lastenreumaa sairastavat olivat selvästi lihavampia kuin verrokit. Heillä todettiin korkeampi seerumin leptiinipitoisuus sekä käänteinen yhteys leptiinin ja luustomarkkereiden välillä myös silloin, kun rasvan määrä sekoittavana tekijänä otettiin huomioon. Verrokeilla vastaava yhteys näytti olevan rasvakudoksesta riippuvainen.
Glukokortikoidihoitoa saaneiden mutta lievempää lastenreumaa sairastavien lasten kohortissa kehon koostumus tai seerumin leptiini- ja adiponektiinipitoisuudet eivät eronneet verrokeista. Potilailla oli kuitenkin lievästi alentunut luun mineraalitiheys. Potilailla, joilla lannerangan luuntiheys oli ≤-1.0 SD, oli taipumus suurempaan leptiinipitoisuuteen kuin niillä joiden luuntiheys oli >-1.0 SD. Leptiini ei kuitenkaan ollut yhteydessä luun mineraalitiheyteen monimuuttuja-analyysissä. Leptiini ja adiponektiini eivät korreloineet taudin aktiivisuuteen kummassakaan lastenreumaa sairastavien kohortissa.

Johtopäätökset Nikamamurtumat ovat yleisiä vaikeaa lastenreumaa sairastavilla lapsilla ja nuorilla. Altistavia tekijöitä ovat korkea kolmen edeltävän vuoden kumulatiivinen kortisoniannos, taudin aktiivisuus ja lihavuus. Luuston mineraalitiheysmittaus ei kykene erottelemaan nikamamurtumia saaneita muista potilaista. Selkärangan magneettikuvaus löytää enemmän murtumia, mutta myös runsaasti muita muutoksia. Vaikea lastenreuma altistaa lihavuudelle, mutta lievempää tautia sairastavien kehon koostumus ei eroa terveistä ikätovereista. Altistuminen pieniannoksiselle kortisonihoidolle ei näytä vaikuttavan haitallisesti kehon koostumukseen. Leptiinillä saattaa olla negatiivinen vaikutus luuston aineenvaihduntaan vaikeassa lastenreumassa. Tutkimuksessa ei todettu yhteyttä leptiinin tai adiponektiinin ja taudin aktiivisuuden välillä.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals (I-IV):

I Markula-Patjas KP, Valta HL, Kerttula LI, Soini IH, Honkanen VE, Toiviainen-Salo SM, Mäkitie OM. Prevalence of vertebral compression fractures and associated factors in children and adolescents with severe juvenile idiopathic arthritis.
J Rheumatol 2012 Feb;39(2):365-73. Epub 2011 Dec 1.

II Toiviainen-Salo S, Markula-Patjas K, Kerttula L, Soini I, Valta H, Mäkitie O. The thoracic and lumbar spine in severe juvenile idiopathic arthritis: Magnetic resonance imaging analysis in 50 children.
J Pediatr 2012 Jan;160(1):140-6. Epub 2011 Aug 11.

III Markula-Patjas KP, Ivaska KK, Pekkinen M, Andersson S, Moilanen E, Viljakainen HT, Mäkitie O. High adiposity and serum leptin accompanied by altered bone turnover markers in severe juvenile idiopathic arthritis.
J Rheumatol 2014 Dec;41(12):2474-81. Epub 2014 Oct 15.

IV Markula-Patjas K, Valta H, Pekkinen M, Andersson S, Aalto K, Lahdenne P, Viljakainen H, Mäkitie O. Body composition and adipokines in patients with juvenile idiopathic arthritis and systemic glucocorticoids.
Clin Exp Rheumatol, in press.

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ABBREVIATIONS

ALP	Alkaline phosphatase
BP	Bisphosphonate
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
DRI	Daily recommended intake
DXA	Dual-energy X-ray absorptiometry
ESR	Erythrocyte sedimentation rate
FSH	Follicle-stimulating hormone
GC	Glucocorticoid
Hb	Haemoglobin
ICTP	Carboxyterminal telopeptide of type I collagen
IGF-1	Insulin-like growth factor-1
IL	Interleukin
ILAR	International League of Associations for Rheumatology
IQR	Interquartile range
ISCD	International Society for Clinical Densitometry
JIA	Juvenile idiopathic arthritis
LH	Luteinizing hormone
LRP	Low-density lipoprotein receptor-related protein
LS	Lumbar spine
M-CSF	Macrophage colony-stimulating factor
MRI	Magnetic resonance imaging
OC	Osteocalcin
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25-OHD	25-hydroxyvitamin D
OPG	Osteoprotegerin
pQCT	Peripheral quantitative computed tomography
PINP	Aminoterminal propeptide of type I collagen
PTH	Parathyroid hormone
RA	Rheumatoid arthritis
RANK	Receptor activator of nuclear factor-kappa B
RANKL	Receptor activator of nuclear factor-kappa B ligand
TNF-α	Tumour necrosis factor alpha
U-Ca/Cr	Urine calcium-to-creatinine ratio
VAS	Visual analogue scale
WB	Whole body
WHO	World Health Organization
WNT	Wingless type

1. INTRODUCTION

While non-vertebral fractures are common in childhood, vertebral fractures are rare in healthy children and adolescents and, when present, suggest compromised bone health (Mäkitie 2013). According to clinical experience and published reports, vertebral compression fractures appear in certain chronic diseases, including juvenile idiopathic arthritis (JIA). Compression fractures may remain undetected, as they appear even in individuals with normal bone mineral density and are often asymptomatic, especially in children. Impaired bone health in JIA may arise from several disease- and treatment-related factors such as inflammatory cytokines, nutritional and hormonal disturbances, limited physical activity and glucocorticoid (GC) therapy (Burnham 2012).

Because of these risk factors, JIA may also be associated with altered body composition (Bechtold 2009). Inflammatory cytokines, GCs and limited physical activity cause muscle wasting, leading to impaired bone mass accrual. Patients suffering from malnutrition are likely to have decreased fat mass, while GC therapy and limited physical activity predispose to excessive fat mass. Increasing childhood obesity is a worldwide epidemic that also affects patients with JIA (Han et al. 2010). Both low and high fat mass may be associated with adverse skeletal effects (Viljakainen 2011). Recent data suggest that despite increased mechanical loading obesity may be an additional risk factor for impaired bone mass accrual and increased fracture risk in childhood (Dimitri et al. 2012). The underlying mechanisms are largely unknown, but adipose tissue-derived biochemical factors, called adipokines, have been suggested to play a role. Adipokines also modulate immune responses.

Data are limited on the prevalence, risk factors and diagnostics of vertebral compression fractures in JIA. Very little is known about the fat-bone relationship in healthy children, and especially in chronic diseases, including JIA. Our aim, therefore, was to evaluate the prevalence, risk factors and diagnostics of compression fractures in severe JIA. Secondly, we examined body composition and the relationship of adipokines with serum bone turnover markers, bone mineral density and disease activity in JIA.

2. REVIEW OF THE LITERATURE

2.1 Juvenile idiopathic arthritis

2.1.1 Definition and epidemiology

Juvenile idiopathic arthritis (JIA) applies to any arthritis of unknown origin that lasts for more than 6 weeks and begins before the age of 16 years (Petty and Cassidy 2011). For diagnosis, exclusion of other diseases known to cause arthritis is required. JIA is not a single disease entity, but is a group of heterogeneous disease states. In order to characterize homogeneous disease groups, several classification criteria have been published. To standardize the classification, internationally acknowledged criteria for JIA have been proposed since 1993 by the International League of Associations for Rheumatology (ILAR). The current classification (Petty et al. 2004) and the estimated proportion of JIA subtypes are presented in Table 1 (Ravelli and Martini 2007).

TABLE 1. Frequency of disease subtypes according to the ILAR classification for juvenile i diopathic arthritis (JIA).

JIA subtype	Frequency
Systemic arthritis	4-17%
Oligoarthritis; persistent or extended	27-56%
Rheumatoid factor-positive polyarthritis	2-7%
Rheumatoid factor-negative polyarthritis	11-28%
Enthesitis-related arthritis	3-11%
Psoriatic arthritis	2-11%
Undifferentiated arthritis	11-21%

The reported prevalence and incidence of JIA vary considerably throughout the world (Ravelli and Martini 2007). This may reflect diverse classification criteria and heterogeneity of the disease as well as differences in ethnicity, immunogenicity and environmental factors. However, most studies have been conducted on Caucasians in Europe or North America and only a few in developing countries. Data from developed countries indicate a yearly prevalence of 16-150/100 000. Community-based studies on schoolchildren from Belgium and Australia report the highest prevalence, up to 167-400/100 000, implying that chronic arthritis may remain undiagnosed in a substantial proportion of children (Mielants et al. 1993, Manners and Diepeveen 1996). A longitudinal multicentre study estimates an average JIA incidence of about 15/100 000 in Nordic countries and about 21/100 000 in the Helsinki area in Finland (Berntson et al. 2003). A Canadian epidemiologic study of a multi-ethnic cohort shows that JIA is over-represented in children of European origin, who are at the highest risk of developing any of the JIA subtypes, except for rheumatoid factor-positive

polyarthritis, and are especially susceptible to extended oligoarthritis and psoriatic arthritis (Saurenmann et al. 2007). Oligoarthritis is the most common subtype also in the Nordic countries (Nordal et al. 2011). Other autoimmune diseases, such as celiac disease or type I diabetes, are over-represented in patients with JIA and their relatives (Pohjankoski et al. 2012).

2.1.2 Aetiology and pathogenesis

The aetiology and pathogenesis of JIA are poorly understood, but seem to be multifactorial, including both genetic and environmental factors (Prakken et al. 2011). Local tissue damage due to environmental factors, such as viral infections or vaccinations, are assumed to serve as a trigger for an adaptive response towards a self-antigen in a genetically susceptible individual. Once the autoimmune process is ongoing, a range of innate and adaptive immune responses is activated. Synovial inflammation follows as a consequence of an imbalance between proinflammatory effector cells (such as T-helper-17 cells) and anti-inflammatory regulatory cells (such as FOXP3-positive regulatory T cells). The hyperplastic vascularized synovium is infiltrated by several cell types, including T cells, B cells, macrophages, dendritic cells and plasma cells. The inflammatory process may lead to pannus formation, referring to destructive synovial tissue growth towards the cartilage and eventually to erosions of cartilage and bone. Many susceptibility genes have been identified, involving both human leucocyte antigen (HLA) genes and non-HLA-related genes (Ravelli and Martini 2007). Genetic similarity with rheumatoid arthritis (RA) exists only regarding rheumatoid factor-positive polyarthritis, which is associated with HLA-DR4. The genetic and immunological profile of systemic arthritis is clearly different from the other subtypes, characterized by a pronounced activation of the innate immune system and the absence of an association with autoantigens and HLA; systemic arthritis is now characterized as a polygenic autoinflammatory syndrome (Prakken et al. 2011). The target of international collaboration and multinational studies is to characterize genetic and biological markers for better prediction of disease characteristics, prognosis and drug responsiveness, eventually aiming at more individualized therapies (Prakken et al. 2011, Schmeling et al. 2014).

2.1.3 Complications, comorbidities and prognosis

Patients with JIA are at risk for multiple complications arising from several disease- and treatment-related factors. These comorbidities include uveitis and associated complications, such as glaucoma and cataract, although visual impairment is nowadays rare (Kotaniemi et al. 2014). Further, JIA predisposes to overall and local disturbances of growth, pubertal delay, altered body composition, impaired bone mass accrual and increased risk for fractures as well as cardiovascular changes (Smith et al. 2013). More than half of the patients with JIA still have signs of active disease in young adulthood, despite one-fourth using biological drugs (Vidqvist et al. 2013).

2.2 Body composition

2.2.1 Definitions

Body composition comprises fat mass and fat-free mass (2-compartment model) (Wells et al. 1999). In the so-called 4-compartment model, the fat-free mass consists of water, protein and mineral. In the 3-compartment model, fat-free mass includes bone mineral and lean mass, indicating soft tissues other than fat, i.e. mostly muscle (Figure 1). Confusingly, fat-free mass is sometimes called lean body mass or lean mass; in this text, however, lean mass refers to soft tissue without bone mineral. Body composition is prone to constant changes in order to adapt to current requirements. These changes are regulated by multiple factors, including nutrition, physical activity, hormonal factors, immunity and inflammation (Veldhuis et al. 2005, Bechtold and Roth 2009). Especially during puberty, body composition changes considerably and shows sexual dimorphism. Girls gain considerable amounts of fat and less lean mass, while boys mostly gain lean mass (Wells 2007).

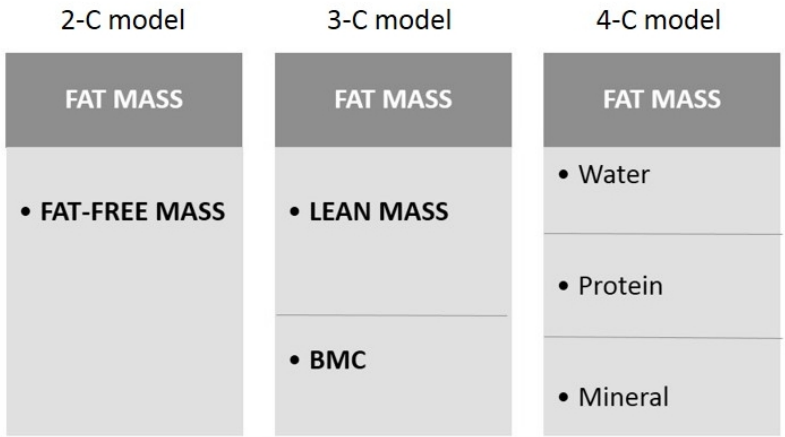


FIGURE 1. Schematic representation of 2-, 3- and 4-compartment models of body composition. BMC, bone mineral content.

2.2.2 Assessment of body composition

Anthropometric methods

Body composition can be estimated by several anthropometric methods, including skinfold measurements, arm muscle circumference or waist-to-hip ratio (Wells and Fewtrell 2006). Body mass index (BMI), weight divided by height squared (kg/m^2), has a limited value when assessing body composition since BMI does not differentiate between fat mass and lean mass. Similar to

BMI, indices for fat mass and fat-free mass relative to body weight have been developed (VanItallie et al. 1990). Fat mass index (FMI) is calculated as fat mass divided by height squared (kg/m^2), fat-free mass index (FFMI) as fat-free mass divided by height squared and lean mass index (LMI) as lean mass divided by height squared.

Dual-energy X-ray absorptiometry

For more precise body composition analysis, indirect technical methods, such as bioelectrical impedance, have been suggested. The reference method for body composition analysis is the 4-compartment (4-C) model (Wells and Fewtrell 2006). It is, however, time-consuming and difficult to perform on young or sick children, as it requires fasting and includes several procedures, e.g. underwater weighing. Therefore, the easily available whole-body dual-energy X-ray absorptiometry (DXA) scanning with low radiation dose is nowadays the most popular method for body composition analysis in children and adolescents. However, different methods may yield variable estimates of body composition. One study evaluated DXA (Lunar DPX/DPX-L) against the 4-C model in 411 healthy children and adolescents aged 6-18 years and showed a strong relationship between the two methods, although DXA underestimated fat percentage (fat%) in those with lower fat% and overestimated fat% in those with higher fat% (Sopher et al. 2004). The researchers concluded that despite its limitations DXA is suitable for body composition analysis in paediatric populations. As measurements vary between devices from different manufacturers, cross-calibration methods have been developed. Shepherd et al. (2012) published paediatric cross-calibration equations to enable comparison of results from the two most commonly used manufacturers, GE Healthcare Lunar and Hologic.

2.2.3 Body composition in juvenile idiopathic arthritis

Rheumatoid cachexia or rheumatoid cachectic obesity is a well-known complication of rheumatoid arthritis (Rall 2004). It manifests as low lean mass and increased fat mass accompanied by whole-body catabolism, even if BMI is normal. Studies specifically evaluating body composition in JIA are scarce, although children also are predisposed to alterations of body composition resulting from several factors (Figure 2). Inflammatory cytokines cause increased protein catabolism and poor appetite (Rall and Roubenoff 2004), which may also result from other factors such as gastrointestinal side-effects of medication, pain or depression. Patients with systemic arthritis have increased resting energy expenditure (Knops et al. 1999). A discrepancy between increased caloric needs and diminished intake may lead to protein/energy malnutrition. Physical inactivity and GCs may further exacerbate decrease in muscle mass (Roth et al. 2007, van Raalte et al. 2009). Several earlier studies have observed evidence of malnutrition based on low BMI Z-score, diminished anthropometric measurements such as arm muscle circumference, reduced

intakes of energy and protein or deficiencies in biochemical nutritional values (Johansson et al. 1986, Henderson and Lovell 1989, Lofthouse et al. 2002, Perfetto et al. 2005, Souza et al. 2006). Nutritional deficits seem to occur especially in patients with systemic or polyarticular JIA, but also those with persistent oligoarticular disease may be affected (Henderson and Lovell 1989, Cleary et al. 2004). By using a bioelectrical impedance method, Lofthouse and colleagues (2002) reported nutritional impairment in those with polyarticular disease, as evidenced by lower fat% and lower total body water, indicating lower lean mass, while those with oligoarthritis only show low body fat%.

Contrary to these findings, some studies report normal (Henderson et al. 2000, Valta et al. 2007) or even high BMI in patients with JIA (Caetano et al. 2012, Pelajo et al. 2012). A very recent Finnish study with 40 prepubertal children with JIA and low disease activity showed increased energy intake and signs of higher central and peripheral adiposity, as evidenced by higher waist circumference and biceps skinfold thickness relative to healthy controls (Grönlund et al. 2014). The tendency for higher BMI may be at least partly related to the global epidemic of increasing obesity associated with sedentary lifestyle and physical inactivity. Further, GCs induce an increase and redistribution of body fat by accelerating fat accumulation in visceral adipose tissue and diminishing peripheral fat stores (van Raalte et al. 2009). However, Souza et al. (2006) report no association between cumulative GC dose and BMI.

In DXA studies, patients with paediatric rheumatic diseases and high-dose GC exposure have shown increased fat mass and diminished lean mass (Mul et al. 2002). In a prospective study by Lien et al. (2005), patients with early JIA had lower gains of lean mass and higher gains of fat% than healthy controls during the 2-year follow-up, indicating that body composition may be altered already early in the disease. A recent peripheral quantitative computed tomography (pQCT) study on children and young adults with a mean disease duration of 5.6 years showed less muscle and more fat in JIA patients than in controls, and these correlated with disease activity and systemic or intra-articular GCs (Stagi et al. 2014). On the other hand, young adults with JIA in remission after a median disease duration of 15.5 years have shown an even better nutritional status with less body fat and more lean mass than healthy controls (Haugen et al. 2002). Especially before the era of biological drugs, also those with GC treatment have shown low BMI. It is likely that severe disease course, physical impairment and malnutrition have earlier been more common than today, contributing to diminished fat mass and muscle mass. The possible effects of biological drugs on appetite or metabolism are largely unknown, but some adult studies suggest a tendency for weight gain (Briot et al. 2008, Engvall et al. 2010). Another important factor affecting body composition in JIA is the tendency for pubertal delay, which postpones gender-specific pubertal changes in body composition. Recombinant human growth hormone therapy has shown not only accelerated growth but also changes in body composition by inducing

an increase in lean mass and, to a lesser extent, a decrease in excessive fat mass (Bechtold and Simon 2014).

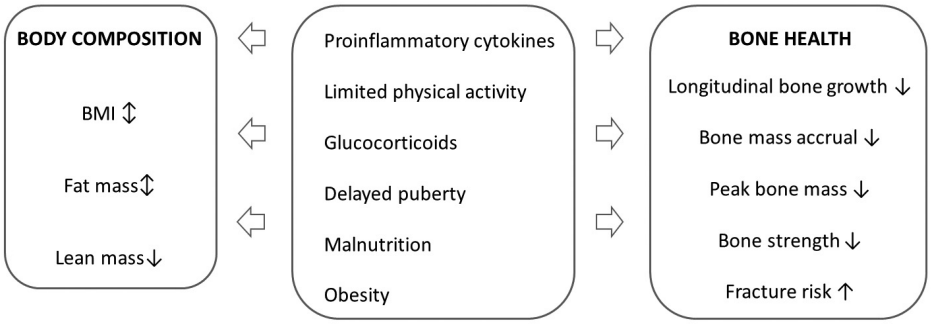


FIGURE 2. Disease- and treatment-related factors with potential effects on body composition and bone health in JIA. BMI, body mass index; BMC, bone mineral content; BMD, bone mineral density.

2.3 Bone

2.3.1 Bone structure and function

The skeleton is part of the locomotor system and provides protection for inner organs and bone marrow (Baron 2003). It also forms a reservoir for storage of calcium, phosphorus and magnesium and participates in the regulation of mineral homeostasis. Bone acts as an endocrine organ by secreting hormones, including fibroblast growth factor 23 and osteocalcin, thereby regulating phosphate metabolism, energy homeostasis and male reproduction (Fukumoto and Martin 2009, Karsenty and Ferron 2012). The unmineralized organic bone matrix, the osteoid, consists mainly (90%) of type I collagen fibres, but includes also non-collagenous proteins, such as glycoproteins and proteoglycans, with bone cells comprising only 2% (Baron 2003). The bone matrix is mineralized by calcium and phosphate-containing hydroxyapatite crystals. This combination enables flexibility and stiffness, both important for resisting fractures. Cortical bone comprises about 85% of total bone and is mostly found in the shafts of long bones, while the metabolically active trabecular (cancellous) bone exists at the end of long bones, in vertebrae and near joint surfaces. The primary structural difference between these two types of bone is that 80-90% of the volume of cortical bone but only 15-25% of trabecular bone is calcified.

2.3.2 Bone cells

The bone-forming osteoblasts arise from mesenchymal stem cells that also give rise to chondrocytes, myocytes, fibroblasts and adipocytes (Baron 2003). Osteoblasts produce type I collagen and other components for the collagenous matrix, and the osteoid is subsequently mineralized. Osteocytes develop from

osteoblasts that have been entrapped in the mineralized matrix. Osteocytes participate in matrix maintenance and mineral homeostasis and communicate with osteoblasts and osteoclast precursors via cytoplasmic processes. Those osteoblasts that stop bone formation turn into bone lining cells that remain on the bone surface. Bone-resorbing osteoclasts are large multinuclear cells that originate from a haematopoietic stem cell similar to macrophages. Osteoclasts resorb bone by releasing acid and proteolytic enzymes to break down the organic matrix.

2.3.3 Bone growth, modelling and remodelling

Longitudinal bone growth is driven by a process called endochondral ossification, which adds cartilage tissue to the growth plates situated at the ends of long bones, then transforming into bone tissue at the adjacent metaphyses (Schoenau et al. 2004, Rauch 2006). Bone growth in both width and shape during childhood occurs by a process called modelling, where osteoblasts and osteoclasts function independently of each other on opposite sides of a piece of bone. Osteoblasts deposit and mineralize bone matrix on the periosteal (outer) surface, thereby increasing the outer circumference of bone (periosteal apposition), while osteoclasts resorb bone on the endocortical (inner) surface of bone, increasing the size of the marrow cavity. Since more bone is formed than resorbed, the net effect of modelling usually leads to thickening of the cortex. Bone remodelling takes place in basic multicellular units and consists of successive cycles of bone resorption, followed by repair due to new bone formation on the same bone surface. "Coupling" refers to the activation of bone forming osteoblasts being tightly linked to the previous action by osteoclasts. The bone resorption cycle lasts about 10 days, followed by bone formation evolving over three months (Singer and Eyre 2008). The main outcome of bone remodelling, in addition to maintenance of calcium homeostasis, is to renew bone. Since the remodelling balance is usually near zero, the process has little effect on the amount of bone (Schoenau et al. 2004). Most bone turnover takes place in trabecular bone due to its high surface area relative to that of cortical bone. Bone remodelling and modelling processes are presented in Figure 3.

In prepuberty, longitudinal bone growth occurs mainly in the lower limbs, while spinal growth predominates in puberty. Almost half of adult peak bone mass, achieved in the third decade, is accrued during the 3-4 years following onset of puberty (Bailey 1997). Bone mass tends to show "tracking", indicating that low bone mass during early childhood predicts low peak bone mass (Wren et al. 2011). Several environmental factors designate whether the genetically determined peak bone mass can be achieved (Arikoski et al. 2002). Due to earlier onset of puberty, girls reach their peak height velocity earlier than boys, at approximately 12 and 14 years in girls and boys, respectively. Obese children reach their peak height velocity earlier than lean children (Marcovecchio and

Chiarelli 2013). Bones grow longer and wider in boys due to an increase in muscle mass and a prolonged period of periosteal apposition.

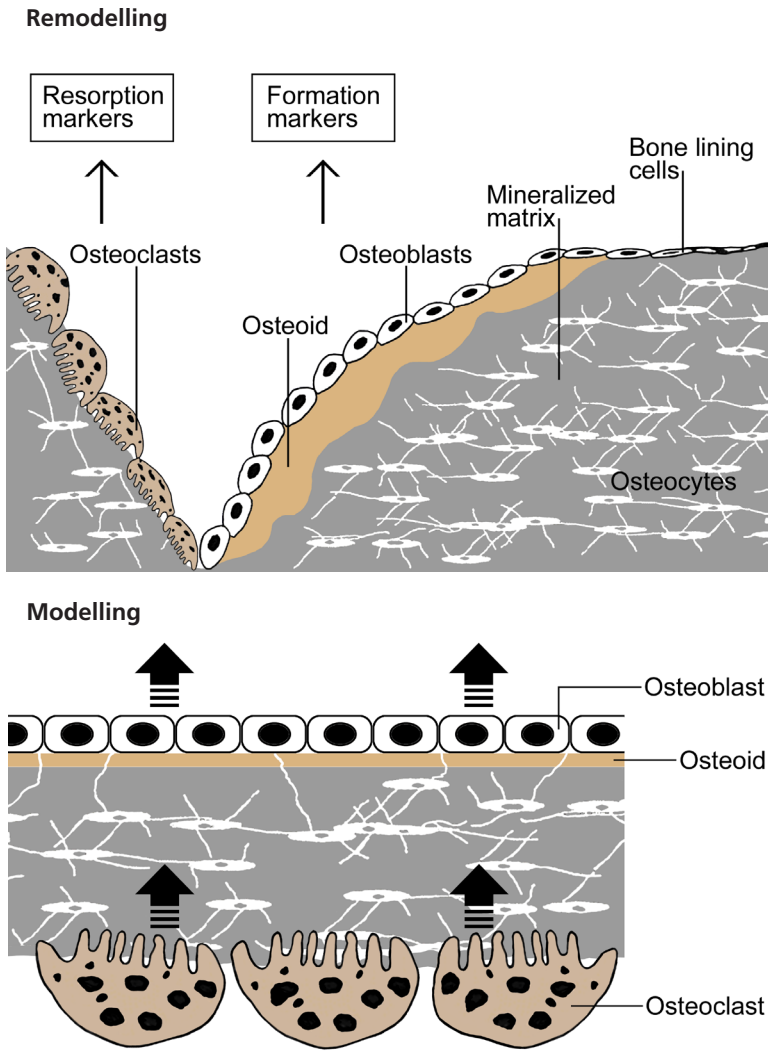


FIGURE 3. Bone remodelling and modelling. Bone repair by remodelling designates a tightly coupled process of bone formation by osteoblasts to form the osteoid for subsequent mineralization, followed by resorption by osteoclasts. Bone turnover markers comprise tissue proteins, their fragments or enzymes, which are released during remodelling and can be measured in blood or urine. Osteocytes act as mechanosensors and communicate via cytoplasmic processes to regulate the balance between bone formation and resorption. In modelling, osteoblasts function on the periosteal and osteoclasts on the endosteal bone surface to enable bone growth in width and shape.

2.3.4 Regulators of bone

Hormonal factors

Hormonal, mechanical and local signals influence bone remodelling. The main hormones regulating plasma calcium and phosphate concentrations include parathyroid hormone (PTH) and vitamin D in its active form, 1,25-dihydroxy vitamin D (1,25$(OH)_2$D) (Misra et al. 2008). Hypocalcaemia due to inadequate dietary intake or intestinal absorption of calcium triggers PTH excretion, resulting in increased bone resorption, enhanced renal calcium reabsorption from the distal tubulus and increased renal synthesis of 1,25$(OH)_2$D. Vitamin D can be acquired through two different routes. By ultraviolet radiation, 7-dehydrocholesterol in the skin is converted to vitamin D3 and then transported to the liver by vitamin D binding protein. Because UV exposure may be limited due to northern or southern latitudes, clothing, etc., intestinal absorption from vitamin D-containing food either as vitamin D2 or D3 is of importance. Via hepatic 25-hydroxylase, vitamin D is converted to 25-hydroxyvitamin D (25-OHD), which is the main circulating vitamin D metabolite and reflects body vitamin D status. Further hydroxylation of 25-OHD to 1,25$(OH)_2$D is accomplished by kidney 1α-hydroxylase (also expressed in several extrarenal tissues), which is activated by low serum calcium or phosphate or by elevated PTH levels. Because of tight feedback regulative mechanisms, synthesis of 1,25$(OH)_2$D declines when serum calcium and phosphate are normal. To remove excess vitamin D, another renal enzyme 24-hydroxylase is activated due to high serum calcium or phosphate and conversion of 25-OHD to 24,25-dihydroxyvitamin D follows (Figure 4).

Several other hormones also regulate bone growth and mineralization (Välimäki and Mäkitie 2009). Growth hormone acts mainly through insulin-like growth factor 1 (IGF-1) on osteoblast function and is the main regulator of bone growth before puberty (Giustina et al. 2008). In addition, the effects of sex steroids are important for bone growth during puberty and for maintenance of bone mass in adulthood (Wells 2007). Oestrogen contributes to endocortical apposition in girls, and through aromatization of testosterone to oestradiol also in boys, whereas testosterone increases muscle mass and periosteal apposition and thereby bone cross-sectional size and strength in boys. Also thyroid hormones, insulin, enteric hormones, cortisol, calcitonin, fibroblast growth factor 23 and adipokines have modifying effects on bone metabolism (Fukumoto and Martin 2009, Misra and Klibanski 2013, Mäkitie 2013, Wojcicka et al. 2013).

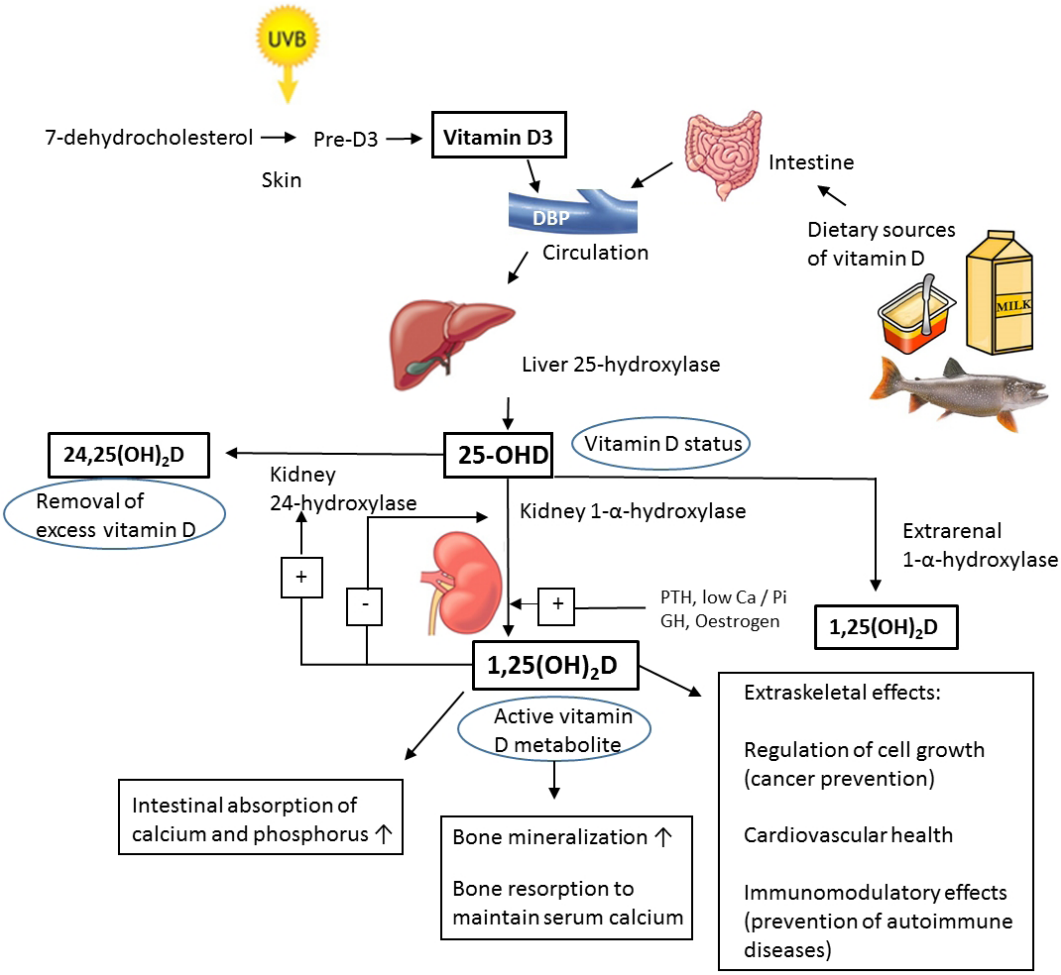


FIGURE 4. Metabolism and effects of vitamin D. Sources of vitamin D include vitamin D-containing food, such as oily fish or fortified margarine or milk products, and production in skin via UV-B radiation. Vitamin D is transported to the liver by vitamin D binding protein (DBP), and converted to 25-hydroxyvitamin D (25-OHD), which reflects body vitamin D status. Further hydroxylation of 25-OHD to 1,25(OH)$_2$D is accomplished by kidney 1α-hydroxylase (also expressed in extrarenal tissues), which is activated by low serum calcium (Ca) or phosphate (Pi) or elevated parathyroid hormone (PTH), growth hormone (GH) or oestrogen levels. Because of tight feedback regulative mechanisms, synthesis of 1,25(OH)$_2$D declines when serum calcium and phosphate are normal. To remove excess vitamin D, renal 24-hydroxylase is activated due to high serum calcium or phosphate, and conversion of 25-OHD to 24,25-dihydroxyvitamin D follows. Vitamin D regulates calcium homeostasis and skeletal health. Vitamin D is also important for muscle health, and several extraskeletal effects have been suggested.

Local factors

A multitude of local hormones, growth factors, cytokines and other factors regulate the differentiation and proliferation of osteoblast and osteoclast lineage cells. The reciprocal relationship between osteoblasts and osteoclasts plays a key role (Schett 2010a). The most important local regulatory pathway of osteoclastic bone resorption is the RANKL/RANK/OPG pathway (Figure 5). Macrophage colony-stimulating factor (M-CSF) is another important factor upregulating osteoclasts. The differentiation of osteoblasts is dependent on, for example, transcription factors. Further, one key regulator in osteoblastogenesis is the wingless-type (WNT) signalling pathway, consisting of molecules that are important regulators of bone formation (Baron 2013). WNT signalling also suppresses osteoclast formation by modulating the production of osteoprotegerin (OPG).

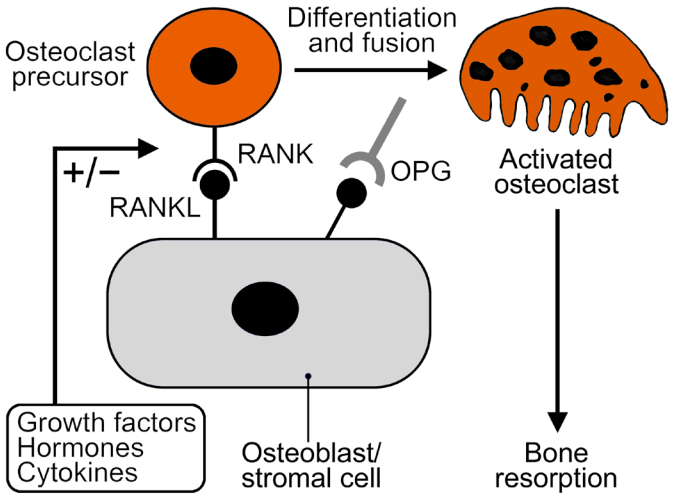


FIGURE 5. Regulation of osteoclasts by the RANKL/RANK/OPG pathway. Osteoblasts express receptor activator of nuclear factor kappa-B ligand (RANKL), a member of the tumour necrosis factor (TNF) superfamily, which can be cleaved to activate osteoclastogenesis by activating its receptor RANK on osteoclasts. At the same time, osteoblasts secrete osteoprotegerin (OPG), a RANKL decoy receptor capable of inhibiting osteoclast formation. The RANKL/RANK/OPG system is regulated by multiple factors.

Mechanical factors

Bone is a dynamic tissue that is able to change its mass and structure by responding to mechanical forces. The "mechanostat" theory described by Frost (1987) reflects the intrinsic control of bone in adapting to mechanical forces by altering its structural characteristics, necessitating bone formation or resorption at any given moment. The mechanosensory role of osteocytes in orchestrating these processes and in maintaining bone quality seems to be crucial (Bonewald 2011). The relationship between muscle and bone is emphasized by the "functional muscle-bone unit" model, which determines whether deficits of bone, muscle or both are the reason for bone loss, indicating a primary, secondary or mixed bone disorder, respectively (Schoenau et al. 2002). Studies on unilateral or generalized physical loading show a significant positive effect on bone mass and structure (Kannus et al. 1995, Baxter-Jones et al. 2008, Nikander et al. 2010).

Genetics

Twin studies suggest that about 80% of the variance in BMD is determined by genetic factors (Pocock et al. 1987). Genetic factors are also important in the regulation of bone size, geometric properties and bone remodelling activity. Regulation of these osteoporosis-related phenotypes is polygenic, and each gene shows rather small effects (Ralston 2010). Rare severe forms of osteoporosis are monogenic and result from a mutation in a single gene (Mäkitie 2013). The most common form of primary osteoporosis is osteogenesis imperfecta, which in types I-IV is caused by a defect in type I collagen resulting from mutations in COL1A1 and COL1A2 genes, but also several other genetic defects in osteogenesis imperfecta types V-XIII have been recognized. Another example of primary osteoporosis relates to mutations in the low-density lipoprotein receptor-related protein (LRP5) gene, which encodes a co-receptor for the WNT signalling pathway and is thus important for osteoblast function. Biallelic loss-of-function mutations in LRP5 cause a disorder called osteoporosis-pseudoglioma syndrome. However, even heterozygotes for LRP5 mutations have low bone mass, and several polymorphisms in LRP5 and LRP6 genes are associated with bone mass accrual and fractures. Similarly biallelic and heterozygous mutations in the gene encoding WNT1, a major ligand for the WNT signalling pathway, result in osteoporosis phenotypes of variable severity (Laine et al. 2013).

2.3.5 Deficiency of calcium and vitamin D

Deficient calcium or phosphate intake results in decreased osteoid mineralization, leading to osteomalacia in adults and rickets in children, as mineral deficiency affects the organization and mineralization of cartilaginous growth plates before epiphyseal fusion. Sufficient calcium intake is especially important during the pubertal growth spurt to enable maximal bone mass accrual, but excess calcium seems not to have much effect on bone mass

accrual in healthy children (Winzenberg et al. 2006). Rickets continues to be a significant problem in Western countries, especially among immigrants with dark skin pigmentation (Shaw and Mughal 2013). Although controversy exists about adequate vitamin D status, recent studies suggest that despite vitamin D-fortified foods and guidelines for supplement use vitamin D insufficiency (25-OHD <50 nmol/l) and deficiency (<37.5 nmol/l) are common throughout the world. In Finland, up to 70% of 7- to 19-year-olds are vitamin D insufficient in spite of median vitamin D intakes of 9-10 ug/day (Pekkinen et al. 2012). Furthermore, low serum 25-OHD concentrations are associated with low BMD, and 25-OHD values explain almost 10% of the variation in lumbar spine or whole-body BMD, even surpassing the effects of physical activity. Finnish data from 2007 to 2010 show that altogether 47% of children with chronic diseases, and especially adolescents, have insufficient serum 25-OHD concentration (Holmlund-Suila et al. 2013). Intrauterine and early postnatal vitamin D status seems to be important for later bone mass accrual. Neonates born to mothers with low vitamin D levels show decreased tibial BMC and smaller cross-sectional area in pQCT (Viljakainen et al. 2010). Further, according to data from the UK, low maternal 25-OHD is associated with lower whole-body and lumbar spine BMC of the child at the age of 9 (Javaid et al. 2006). Not all postnatal vitamin D supplementation studies show positive results on bone mass accrual, but the pre- and peripubertal period may be an especially favourable period to attain positive effects from supplementation (Viljakainen et al. 2006). In 2014, the Finnish Nutrition Council increased the recommended total daily vitamin D intake from 7.5 to 10 ug and suggested daily supplementation of 7.5 ug throughout the year for children aged 2-18 years and 10 µg for those under the age of 2 (National Nutrition Council 2014).

Extraskeletal effects of vitamin D

Several observational studies suggest positive extraskeletal effects of vitamin D (Hossein-Nezhad and Holick 2013). Vitamin D deficiency is associated with an increased risk of developing several diseases, including certain malignancies, infectious diseases, asthma, metabolic syndrome, cardiovascular diseases and cognitive impairment. There is evidence of immunomodulatory effects. Vitamin D supplementation during early life may reduce the risk for type 1 diabetes, and a relationship between vitamin D and other autoimmune diseases, such as multiple sclerosis, Crohn's disease and RA, has also been suggested. Deficiency of vitamin D is also associated with a more active disease course. Children with JIA, especially those with polyarticular or systemic disease, may have a tendency for low levels of vitamin D, but it is unclear whether supplementation has an effect on disease outcome (Nisar et al. 2013). Since results from intervention studies are inconsistent, further evidence is needed to confirm the promising role of vitamin D in extraskeletal health of adults and children (Hossein-Nezhad and Holick 2013, Saggese et al. 2015) (Figure 4).

2.3.6 Fracture incidence and associated factors in childhood

Fractures are common in children; about 50% of boys and 30-40% of girls sustain at least one fracture during childhood (Landin 1983, Jones et al. 2002). Fracture incidence is highest during mid-puberty (Landin 1983, Mäyränpää et al. 2010), which may at least partially result from a decrease in the ratio of cortical to trabecular bone simultaneously with a peak in cortical porosity (Kirmani et al. 2009). Both increase (Landin 1997, Mäyränpää et al. 2010) and, more recently, decrease of overall fracture incidence during childhood have been reported in Sweden and Finland (Tiderius 1999, Mäyränpää et al. 2010). The fracture pattern has changed during recent decades since despite a decrease in hand and foot fractures an increase in clinically more significant forearm fractures has been reported in many studies (Khosla et al. 2003, Helenius et al. 2009, Mäyränpää et al. 2010). Several predisposing factors for the first fracture as well as for subsequent fractures in fracture-prone children have been recognized (Goulding et al. 2000 and 2005, Manias et al. 2006, Mäyränpää et al. 2012). These include low intakes of milk, calcium and vitamin D, low serum 25-OHD, maternal smoking during pregnancy and absence of breast-feeding, higher consumption of carbonated beverages, early age at first fracture and history of fractures in siblings, low levels of physical activity, low BMC or BMD, and higher BMI. In a population-based cohort of almost 1400 children aged 0-15 years, the prevalence of thoraco-lumbar vertebral fractures accounted for only 1% of all acute fractures (Mäyränpää et al. 2010). However, up to 15% of 55 fracture-prone children with a history of at least two to three long bone fractures showed asymptomatic vertebral compression changes on spinal assessment (Mäyränpää et al. 2012).

2.3.7 Osteoporosis and definitions for skeletal fragility in childhood

According to WHO, osteoporosis has been defined as a skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (Kanis 1994). Another definition published by the National Institute of Health (NIH) described osteoporosis as a skeletal disorder characterized by compromised bone strength and increased risk of fracture (NIH 2001). In adults, according to WHO recommendations, osteoporosis can be diagnosed based on a DXA-derived BMD T-score of less than or equal to -2.5. To calculate a T-score, bone densitometry data are compared with results of 20- to 30-year-old healthy persons, i.e. those with peak bone mass. These recommendations are based on epidemiological studies of osteoporotic fracture risk (Kanis 1994). This definition seems limited, however, since about 50% of all non-vertebral fractures occur at a T-score above -2.5 (Schuit et al. 2004).

In children and adolescents, age- and sex-specific BMD reference data are used to calculate a Z-score, designating the magnitude of standard deviation

from the population mean. Low BMD alone is not sufficient for diagnosing osteoporosis in children because no BMD cut-off value predictive of increased fracture risk is known. According to the 2007 International Society for Clinical Densitometry (ISCD) Pediatric Official Positions, the diagnosis of osteoporosis in children requires both low bone mineral content (BMC) or areal bone mineral density (aBMD) (≤ -2.0) and a significant history of fractures (Rauch et al. 2008). These fractures may include either one vertebral fracture or one lower limb long bone fracture or two upper limb long bone fractures. BMC or aBMD results should be adjusted for age, gender and body size, as appropriate. The recent revised ISCD 2013 recommendation (Bishop et al. 2014) includes the following modifications: a) In addition to vertebral fracture, a clinically significant fracture history means either two or more long bone fractures by age 10 years or three or more long bone fractures at any age before 16 years, excluding high-energy trauma (fall from > 3 m or traffic accidents) and b) A vertebral compression fracture alone (crush; loss of vertebral height at any part of >20%), regardless of BMD, is indicative of osteoporosis in the absence of local disease or high-energy trauma. It was also stated that a BMC/aBMD Z-score >-2.0 does not preclude the possibility of skeletal fragility and increased fracture risk. In the absence of a significant fracture history, the terms osteoporosis and osteopenia in a paediatric setting should be avoided and the terms "low BMD for body size" for Z-score ≤-2.0 and "low-normal BMD for body size" for Z-score >-2.0--1.0 preferred (Bianchi et al. 2014).

Primary osteoporosis in otherwise healthy childen is rare, while secondary osteoporosis is common, especially in children with diseases involving chronic systemic inflammation, neuromuscular disabilities, GC treatment or cancer therapies, including chemotherapy and radiotherapy (Kröger and Arikoski 2004, Mäkitie 2013). Signs of compromised bone health may be observed not only after treatment but already at disease onset of childhood malignancies (Arikoski et al. 1999a,b).

2.4 Assessment of bone health

2.4.1 Dual-energy X-ray absorptiometry

DXA measures the relative tissue absorption of X-rays at high and low energies. DXA measurement provides the projectional bone area, bone mineral content and areal bone density as g/cm2. The interpretation of DXA results is not simple in children, and certain pitfalls have been recognized (Gafni and Baron 2004). The most significant limitation of DXA is the analysis of a three-dimensional object with a two-dimensional projection (Crabtree et al. 2014). Because DXA is not able to measure bone depth, results are dependent on bone size. Therefore, DXA underestimates bone density of a short child with smaller bones and overestimates bone density of a tall child with bigger bones. Algorithms

for counting true volumetric BMD have been developed. Based on current recommendations, DXA results should be corrected for body size. However, these suggestions are based on data from healthy children, and correction for bone age may be appropriate in children with chronic disease, considering delay in skeletal maturation. Another limitation of DXA is that it is unable to distinguish between cortical and trabecular bone, but measurements of the whole body and lumbar spine serve as surrogates for cortical and trabecular bone health, respectively.

2.4.2 Biochemistry

Measurements of serum or urine calcium and phosphorus are surrogates for body calcium balance, but do not reflect skeletal reserves of these minerals. Calcium balance is regulated by hormonal factors, such as parathyroid hormone, and the biologically active vitamin D metabolite 1,25-$(OH)_2$D, but body vitamin D status can be estimated by measuring serum 25-OHD concentration (Misra et al. 2008).

Bone turnover markers

The biochemical markers of bone turnover measured from blood or urine reflect the activity of osteoclasts and osteoblasts, but since formation and resorption are usually tightly coupled in time and space, any of the markers reflects the overall rate of bone turnover (Singer and Eyre 2008). In adults, bone turnover markers mainly represent remodelling, while in children these markers are released also during modelling and skeletal longitudinal growth. Bone turnover can be more than 10 times greater in children than in adults, and turnover markers increase especially during the pubertal growth spurt while declining in late puberty (Rauchenzauner et al. 2007). Interpretation of bone turnover marker values is complicated because of several confounding factors, especially in children. In addition to age, pubertal stage and growth velocity, these include hormonal factors, nutritional status, circadian and day-to-day variation and methodological issues (Szulc et al. 2000, Paldanius et al. 2012, Viljakainen et al. 2014).

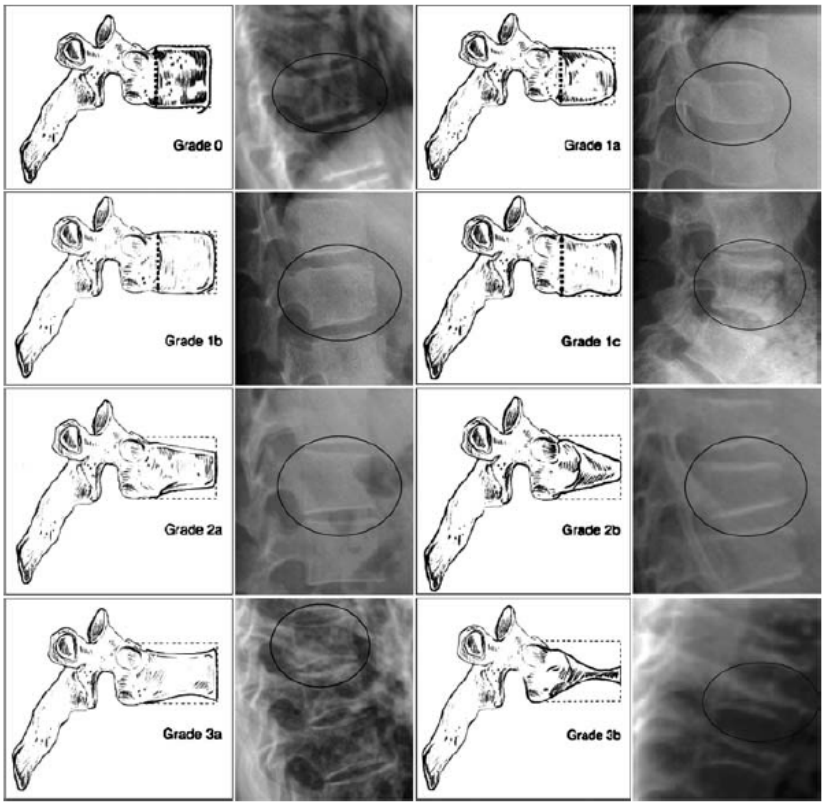
Turnover markers are bone tissue proteins or their fragments or enxymes released from bone cells during bone turnover (Singer and Eyre 2008) (Figure 3). Several formation markers are available. Alkaline phosphatase (S-ALP) is an enzyme found on the osteoblast cell surface that is thought to play a role in osteoid formation and mineralization, thereby reflecting osteoblast activity. Osteocalcin (S-OC) is a protein of the bone matrix with a high affinity to hydroxyapatite. Osteocalcin is considered a specific marker of osteoblast activity and bone formation, but it may be released also during bone resorption, which is reflected by urinary osteocalcin. Procollagen type I amino-terminal and carboxy-terminal propeptides (PINP and PICP) are quantitative measures of newly formed type I collagen. The most used resorption markers include crosslinked telopeptides of type I collagen in their amino-terminal (NTX) and carboxy-terminal (CTX and

ICTP) forms. NTX and CTX are released by cathepsin K cleavage, whereas ICTP is a larger fragment degraded by matrix metalloproteinases. Tartrate-resistant acid phosphatase 5b (S-TRACP5b) is a catalytic enzyme that reflects the number of osteoclasts rather than their activity.

2.4.3 Spinal imaging

Assessment of vertebral morphology by radiography

The classification by Genant et al. (1993) is the most commonly used method for vertebral fracture assessment in adults. Since the Genant method is based on adult data, Mäkitie et al. (2005) have proposed another classification method developed especially for the paediatric population. According to the Mäkitie method, compression fractures are classified as anterior wedge deformities (only anterior part of the vertebra compressed) or compression deformities (also the middle and/or posterior part of the vertebra compressed). Abnormal changes are further classified as mild (Grade 2a) or severe (Grade 2b) wedge deformities or mild (Grade 3a) or severe (Grade 3b) compression deformities. Grades 1a-c represent normal variants of vertebral morphology in children. Figure 6 presents the vertebral morphology assessment by the Mäkitie method.



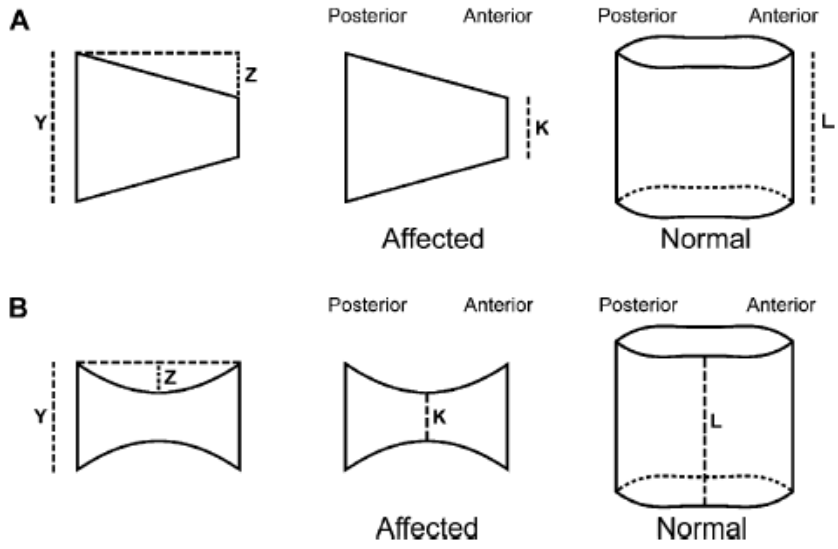


FIGURE 6. Vertebral morphology assessment by the Mäkitie method (Mäkitie et al. 2005). Figures used with the permission of copyright holder. Grades 0 and 1a-c: Normal variants; Grades 2a and 2b: Progressive stages of anterior wedge deformity; Grades 3a and 3b: Progressive stages of compression deformity.

A. In Grade 2a-2b wedge deformities, the intravertebral anterior height reduction (Z=Y-K/2) is >20%; ≥50% anterior height reduction in 2b (severe wedging). On intervertebral assessment, vertebral height reduction (Z-Lx100%) is 5-20% in Grade 2a and >20% in Grade 2b.

B. In compression deformities 3a and 3b, anterior, middle and posterior vertebral heights are reduced. On intravertebral assessment, the loss of middle height compared with posterior height (Z/Yx100%) is >20%. On intervertebral assessment, the middle vertebral height reduction compared with an apparently normal adjacent vertebra (Z/Lx100%) is 5-30% in Grade 3a and >30% in Grade 3b (severe deformity).

Spinal radiographs remain the golden standard for vertebral fracture assessment, but due to suboptimal visibility of the vertebrae, especially in the thoracic area, other methods such as computed tomography (CT) or magnetic resonance imaging (MRI) have been suggested. CT is commonly used in adults for thoracolumbar trauma imaging, but in order to avoid radiation exposure, the use of CT in children is limited.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is based on detecting signals from protons in the tissue hydrogen atoms within a strong magnetic field produced by the MRI scanner (Soimakallio et al. 2005). Hydrogen atoms are excited by a radiofrequency energy pulse to emit a radiofrequency signal, which can be measured. The contrast between different tissues is determined by proton density and the rate at which excited atoms return to the state of equilibrium. Differing magnetization relaxation processes produce tissue-specific values. T1-

weighted images are used especially to visualize anatomical structures, while T2-weighted images can show pathology such as abnormal tissue oedema. Contrast agents such as gadolinium enhance sensitivity and specificity of MRI scans and are used to visualize inflammatory lesions. MRI is increasingly being used in children because it lacks ionizing radiation. In spinal imaging, MRI is superior to conventional radiography in visualization of vertebral changes, including subchondral bone and endplates, and in depiction of soft tissue (Sledge et al. 2001). MRI is the most sensitive imaging modality to detect inflammatory lesions of the axial skeleton, found in adult patients with ankylosing spondylitis (Pedersen et al. 2012). In JIA, MRI is increasingly used for imaging of affected joints, but challenges remain resulting from limited normative data of growth-related changes in healthy children (Lanni et al. 2014). Apart from the cervical spine and sacroiliac joints, involvement of the axial skeleton in JIA remains inadequately studied. Disadvantages of MRI include limited availability, long scanning time, need for sedation in small children and high cost. Contraindications involve metallic implants and cardiac pacemakers.

2.4.4 Other imaging methods and bone histomorphometry

Fracture is related to decreased bone strength, which does not depend only on BMD but also on bone quality (Griffith et al. 2010). Bone quality refers to such factors as bone architecture, turnover, mineralization and cellularity. New high-resolution imaging techniques, such as DXA-based hip structural analysis, pQCT, high-resolution pQCT and MRI, enable more precise evaluation of bone architecture and strength. Histomorphometric analysis of transiliac bone biopsy gives information on bone metabolism, mass and structure (of mainly trabecular bone) and is indicated in children with severe bone fragility of unknown origin (Rauch 2006).

2.5 Bone health in juvenile idiopathic arthritis

2.5.1 Effect of chronic inflammation on bone

Bone is a reservoir for haematopoietic stem cells, from which all immune cells are generated (Schett 2010a). Therefore, bone homeostasis is likely to regulate immune responses and immune memory. Further, osteoclasts originate from the monocyte-macrophage lineage and are actually specialized macrophages with bone-resorbing properties. The relationship between bone and the immune system is bidirectional since immune cells regulate bone remodelling (Schett 2010a,b). These cells are capable of producing both pro-osteoclastogenic and anti-osteoclastogenic cytokines depending on the local environment. In rheumatic diseases, activation of the innate and adaptive immune system leads to the production of molecules with negative effects on bone homeostasis.

Enhancement of osteoclast function plays a key role, and the disturbed balance between bone formation and bone resorption eventually triggers bone loss. Macrophages, T cells and fibroblasts produce several inflammatory mediators, such as TNF, interleukin (IL) 1, IL-6, IL-17 and prostaglandin E2, which stimulate osteoclast formation and activation by inducing expression of RANKL and macrophage colony-stimulating factor (MCSF). Compensatory bone formation is blunted because of the inflammatory process. In fact, TNF downregulates bone formation firstly by inducing WNT antagonist Dickkoph 1, and secondly by inducing the expression of the osteocyte product sclerostin, which is a suppressor of bone formation. As a consequence of these processes, periarticular and generalized osteoporosis follow. (Figure 7)

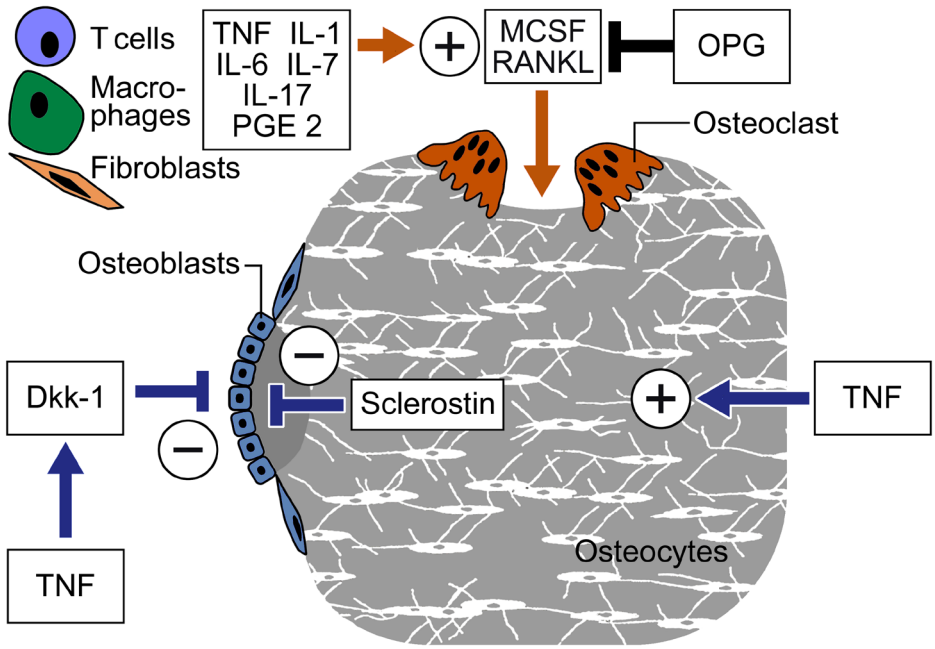


FIGURE 7. Influence of inflammation on bone remodelling. Several inflammatory mediators produced by macrophages, T cells or fibroblasts stimulate osteoclasts and bone resorption by inducing the expression of receptor activator of nuclear factor $_{K}$B ligand (RANKL) and macrophage colony-stimulating factor (MCSF). Bone formation is downregulated by TNF by inducing a WNT antagonist Dickkopf and by inducing the expression of the osteocyte product Sclerostin, a suppressor of bone formation. Modified from Schett et al. (2010b)

2.5.2 Other disease-related factors

Several other disease-related factors predispose children with JIA to impaired bone health. Deficits in muscle mass and function, resulting from effects of inflammatory cytokines, GCs and limited physical activity, are suggested to play a major role (Burnham et al. 2006b and 2008, Roth et al. 2007). Patients with

JIA have been shown to have low body fat content (Lofthouse et al. 2002), but also obesity (Caetano et al. 2012, Pelajo et al. 2012, Grönlund et al. 2014), both of which may predispose to low BMD (Veldhuis et al. 2005, Viljakainen et al. 2011, Misra and Klibanski 2013). In healthy children, pubertal delay is a risk factor for low peak bone mass (Gilsanz 2011). This concept is important in children with JIA, who often have pubertal delay. (Figure 2)

2.5.3 Glucocorticoids

GCs have deleterious effects on bone, mainly trabecular bone. Children using GCs have increased fracture risk (Van Staa 2003). Bone resorption transiently increases, resulting from increased osteoclast survival (Canalis et al. 2007). Decreased bone formation also ensues because of increased apoptosis of mature osteoblasts and osteocytes during long-term GC therapy. Indirect GC effects include decreased intestinal calcium absorption, increased urinary calcium excretion (inducing hyperparathyroidism) and decreased synthesis of gonadotropins, sex steroids and IGF-1. Also myopathy associated with GCs can contribute to bone loss.

2.5.4 Growth

Impaired growth is a well-known complication of JIA (Bechtold 2014), resulting from effects of proinflammatory cytokines, malnutrition and GCs on the growth hormone-IGF-1 axis (Bechtold and Simon 2014). In addition to these systemic effects, inflammatory cytokines such as TNF-α, IL-1ß and IL-6 induce a direct effect on chondrogenesis in the growth plate. Recent advances in antirheumatic medication seem to have changed the growth pattern since normal growth has been reported despite previous GC exposure (Valta et al. 2007). Anti-TNF-α modulators have been a treatment option for severe JIA since the late 1990s and have shown a positive impact on growth, although growth retardation still affects a subgroup of children (Tynjälä et al. 2006, Billiau et al. 2010, Giannini et al. 2010). Catch-up growth is seen also with anti-IL-6 receptor antibody tocilizumab therapy in patients with systemic arthritis (de Benedetti et al. 2014). Positive results have been reported regarding growth hormone therapy in short children with JIA, but close monitoring is required because of increased risk for impaired glucose tolerance due to chronic inflammation and GC therapy (Bechtold and Simon 2014). Disease activity suppresses the effect of growth hormone on growth (Pepmueller et al. 1996).

2.5.5 Bone mineral density

Several DXA studies report decreased BMC or BMD values in patients with JIA, either with or without GC exposure (Kotaniemi et al. 1993 and 1999, Pepmueller et al. 1996, Henderson et al. 1997 and 2000, Lien et al. 2003 and 2005, Valta et al. 2007, Stagi et al. 2014). Patients with systemic or polyarticular disease

are especially vulnerable to reductions in BMD. A pQCT study by Burnham et al. (2008) showed deficits in periosteal circumference, muscle cross-sectional area and cortical section modulus (a measure of bending and torsional bone strength) and low trabecular volumetric BMD in all subtypes, excluding oligoarthritis. The authors concluded that patients with JIA seem to have a mixed bone deficit, indicating that bone mass is low even when considering the reduction in muscle mass. Complementary results were reported in a longitudinal study (Stagi et al. 2014). Some young adults with JIA in remission attain the same BMD as healthy subjects, but those with active disease are at greatest risk for osteoporosis (Zak et al. 1999, Haugen et al. 2000).

2.5.6 Fractures

According to data from a large United Kingdom General Practice Research Database, subjects with childhood-onset arthritis have an increased risk for first fracture compared with healthy controls (Burnham et al. 2006a). The risk is highest during adolescence and after the age of 45 years. A prior fracture increases the risk for a new fracture by 86% in both patients and controls. No threshold for safe GC dosing is known since fractures appear even with relatively low dosages. Although children commonly sustain fractures, vertebral fractures are rare in healthy children and suggest skeletal fragility. Earlier cross-sectional studies report vertebral fractures in children with rheumatic diseases, especially in systemic lupus erythematosus (SLE) and other connective tissue diseases, vasculitis or systemic arthritis, and mostly in association with prolonged GC exposure (Badley and Ansell 1960, Elsasser et al. 1982, Varonos et al. 1987, Mäkitie et al. 2005, Valta et al. 2007, Nakhla et al. 2009). Other risk factors include prolonged bed rest, high disease activity, low BMD, subnormal vitamin D status and male sex.

Huber et al. (2010) observed vertebral fractures in 7% of 134 children with various paediatric rheumatic conditions shortly after diagnosis, at only 30 days after initiation of GC therapy. Those with vertebral fractures included patients with juvenile dermatomyositis, systemic lupus erythematosus, systemic vasculitis and systemic arthritis (2 patients, 9%), but no patients with other JIA subtypes (n=28). In a 3-year prospective follow-up since GC initiation (n=110 at final visit), the incidence of vertebral fractures was 12.4%, and annual incidences in the first, second and third years were 6%, 4.8% and 3.6%, respectively (LeBlanc et al. 2015). During the first year of follow-up those with incident vertebral fractures had received about 50% more GCs (median daily dose 23 vs. 43 mg/m2) than those without and showed more weight gain and greater decline in LS aBMD primarily during the first 6 months, but were mostly asymptomatic, similar to several other reports (Rodd et al. 2012). Every 0.5 mg/kg increase in average daily GC dose was associated with a 2-fold increased fracture risk, which was highest shortly after starting GC therapy and attenuated after cessation of treatment. However, two patients showed fractures more than

one year after cessation of GCs (LeBlanc et al. 2015). A Finnish study on 62 children with JIA and at least 3-months' systemic GC exposure prior to the study showed abnormal findings in 10% of the patients on vertebral fracture assessment based on DXA images, but these changes were not associated with cumulative GC dose, BMD, growth parameters or disease activity (Valta et al. 2007). Also other studies have failed to demonstrate an association between vertebral fractures and lumbar BMD (Mäkitie et al. 2005, Nakhla et al. 2009).

2.5.7 Bone turnover markers

Previous JIA studies evaluating bone turnover markers mostly report reduced bone formation with or without an association with active disease (Pepmueller et al. 1996, Pereira et al. 1999, Lien et al. 2005). Results on bone resorption markers have been conflicting.

2.6 Adipose tissue

2.6.1 Structure and function

Adipose tissue serves as an energy store, but recent research has expanded the understanding of the role of adipose tissue as the largest endocrine organ of the human body. Human adipose tissue comprises two general subtypes, white and brown adipose tissue. Recently, a third adipocyte type has been recognized, called brite ("brown-in-white") or beige adipocyte (Lanthier and Leclercq 2014). Brown adipocytes oxidize fatty acids for thermogenesis and are present mainly in infants, although brown adipose tissue increases also during adolescence (Gilsanz et al. 2012). Beige adipocytes are localized inside white adipose tissue, have thermogenic properties and have a reversible tendency to transform into white adipocytes (Lanthier and Leclercq 2014). White adipocytes store energy as triglycerides. Although the majority of white adipose tissue mass consists of mature adipocytes, they account for less than 20% of the total cells. The other cells include pre-adipocytes, endothelial cells, fibroblasts, macrophages, lymphocytes and stem cells. White adipose tissue is divided into visceral and subcutaneous fat depots. Visceral obesity is also referred to as central or intra-abdominal "apple-type" obesity, while subcutaneous fat excess localizes more peripherally. Positive caloric balance triggers mature adipocyte hypertrophy (increased cell size), and subsequently, adipogenesis, i.e. the differentiation of pre-adipocytes into new adipocytes.

Low-grade inflammation and metabolic syndrome
Instead of controlled apoptosis, hypertrophic obesity is associated with necrotic cell death, which induces recruitment of macrophages and is followed by an inflammatory response (Lanthier and Leclercq 2014). Adipocytes and macrophages secrete various mediators, and this response is characterized

by high serum IL-6, TNF-α and CRP, and low adiponectin levels. The obesity-associated low-grade inflammation is especially important concerning visceral adipose tissue; increased visceral fat is associated with a higher risk for metabolic syndrome than excess subcutaneous fat. Youth obesity is a significant risk factor for metabolic syndrome if obesity continues into adulthood (Mattsson et al. 2008, Juonala et al. 2011).

2.6.2 Adipokines

Adipokines are small pleiotropic messenger proteins secreted mostly but not exclusively from adipose tissue (Tilg and Moschen 2006). These proteins function either as hormones to influence energy homeostasis and regulate neuroendocrine function or as cytokines to affect immune functions and inflammatory processes. Adipokines are widely involved in human physiology, participating in the regulation of appetite, energy expenditure, insulin sensitivity, coagulation, inflammation and bone homeostasis. A multitude of adipokines have been detected. The first to be discovered and the most studied are leptin and adiponectin.

Leptin

Leptin, a product of ob (obese) gene, was the first adipokine discovered in 1994 (Zhang et al. 1994). Leptin is a protein produced mainly by adipocytes, but also by the intestine, placenta, mammary glands, gastric epithelium, skeletal muscle and brain, and even by joint tissues and bone (Scotece et al. 2014). Leptin centrally regulates body weight by inducing reduced food intake and increased energy expenditure. In mouse models, leptin deficiency is associated with morbid obesity. Serum leptin levels are proportional to the overall fat tissue mass, but are 2-3 times higher in females than in males even when adjusted for age and BMI (Tilg and Moschen 2006). In rats, leptin administration induces a reduction of visceral fat, but in humans obese individuals fail to respond to leptin's anorexigenic effects because of hypothalamic leptin resistance (Scotece et al. 2014). However, it is unknown whether these obesity-related central mechanisms similarly regulate leptin's peripheral actions. The effects of leptin are primarily proinflammatory.

Adiponectin

Adiponectin was discovered in 1995-1996 by several groups. Adiponectin is produced mainly by adipocytes, but under certain circumstances also by other cells such as skeletal and cardiac myocytes, endothelial cells, hepatocytes and osteoblasts (Tilg and Moschen 2006). Adiponectin exists as a full-length protein (either as low-, middle- or high-molecular weight forms) and as a globular fragment, and circulates in human serum at significantly higher concentrations than leptin. Adiponectin shows mostly anti-inflammatory effects by suppressing the synthesis of proinflammatory cytokines such as TNF-α and by producing anti-inflammatory cytokines such as IL-10 or IL-1 receptor antagonist. It also suppresses the monocyte-macrophage system. Adiponectin prevents insulin resistance by stimulating glucose uptake and fatty acid oxidation and is

protective against atherosclerosis. Decreased serum adiponectin values are associated with (visceral) obesity, insulin resistance and atherosclerosis. Conversely, in certain situations, including rheumatic diseases, adiponectin also has proinflammatory effects (Gomez et al. 2011).

2.7 Interaction between adipose tissue and bone

2.7.1 Fat and bone

There is growing interest in the relationship between fat and bone, beginning with the fact that adipocytes and osteoblasts both originate from mesenchymal stem cells (Figure 8), and differentiation into the adipocyte or osteoblast lineage has a potential influence on skeletal homeostasis (Misra and Klibanski 2013). Thus far, controversy remains whether fat is beneficial or harmful to bone from childhood into adulthood (Dimitri et al. 2012). Body weight is an important determinant of BMD. Studies on adults show a positive correlation between BMI or fat mass and DXA-derived BMD (Reid et al. 1992). BMI was protective against total fractures, osteoporotic fractures and hip fractures in a meta-analysis comprising 60 000 men and women (De Laet et al. 2005). In contrast, a retrospective study on 1005 postmenopausal women younger than 75 years reported a 28% prevalence of obesity in those with low-trauma fractures, despite mostly normal BMD (Premaor et al. 2010).

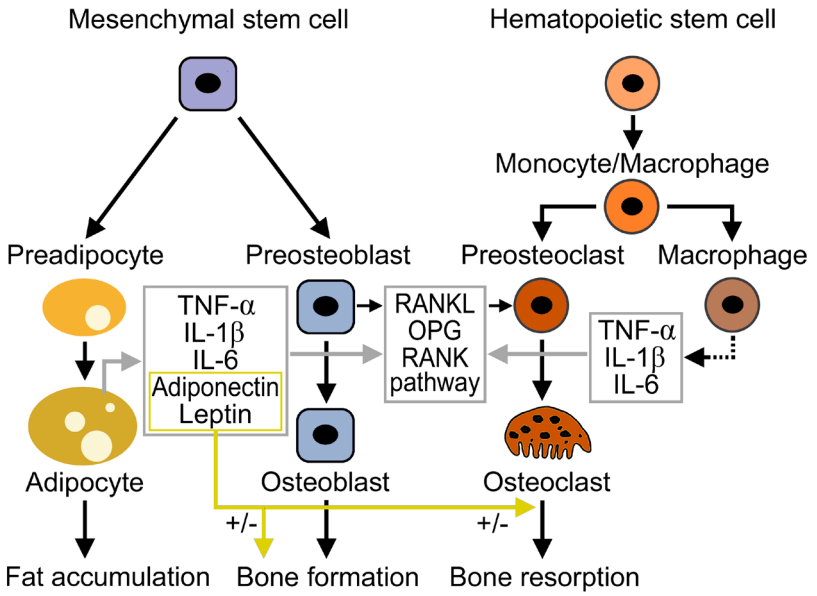


FIGURE 8. Origin of and crosstalk between adipocytes, bone cells and macrophages. Adipocytes and osteoblasts originate from mesenchymal stem cells, whereas osteoclasts and macrophages originate from the haematopoietic stem cell lineage. Adipocytes and macrophages secrete cytokines that affect the RANKL/RANK/OPG pathway, thereby inducing bone resorption. Adipokines regulate bone remodelling in several ways.

The relationship between fat and bone is even more complex during childhood and adolescence, as changes in body composition and bone during growth and especially during puberty complicate the interpretation. Low fat mass is associated with detrimental hormonal changes and low BMD (van Raalte et al. 2009, Viljakainen et al. 2011, Misra and Klibanski 2013). Although some of the obesity-associated hormonal changes, such as high insulin, levels are potentially anabolic for bone, cross-sectional studies suggest that high fat mass may also have a negative impact on bone during childhood and adolescence (Dimitri et al. 2010, Viljakainen et al. 2011, Misra and Klibanski 2013). Obese children appear to have more fractures than their lean peers (Goulding 2005 et al., Manias et al. 2006), and high fat mass was associated with increased risk of subsequent fractures during a 4-year follow-up (Goulding et al. 2000, Jones et al. 2002). This may be partly explained by obesity-associated abnormalities in gait and balance, and subsequently, increased risk for falls (Goulding et al. 2003). However, despite having larger bone area (i.e. bigger bones) and compensatorily increased lean mass, obese children have low bone mass for their body size (Goulding et al. 2000, Dimitri et al. 2010). This effect is emphasized in those with prior fractures (Dimitri et al. 2010). Obesity is also associated with poor bone quality and impaired mechanical properties (Farr et al. 2011).

Nevertheless, the relationship between fat and bone may be age-specific. In prepubertal children aged 5-9 years, DXA studies show a positive relationship of fat mass with bone mass and size (Clark et al. 2006, Goulding et al. 2008, Cole et al. 2012), but this relationship is attenuated during puberty, at least in girls, and becomes negative immediately after puberty (Nagasaki 2004, Clark et al. 2006, Janicka et al. 2007). A pQCT study on 6-year-olds, however, reports an inverse association between fat mass and cortical and trabecular volumetric BMD already in prepubertal children (Cole et al. 2012). Regional fat distribution may be of importance since visceral and subcutaneous fat depots appear to have opposite effects. In females aged 15 to 25 years assessed by computed tomography, subcutaneous fat showed a beneficial effect on bone structure and strength in the femoral midshaft, while the impact of visceral fat was negative (Gilsanz et al. 2009). Consistent findings were observed in DXA assessment of obese adolescent girls (Russell et al. 2010). Furthermore, differences may exist between ethnic groups; the extent of a negative relationship with bone mass may be related more to subcutaneous fat in Caucasian and Latino children, and to visceral fat in Afro-Americans (Afghani and Goran 2006, 2009).

The effect of childhood overweight on bone metabolism seems controversial since either normal or decreased bone formation (Bini et al. 2004, Reinehr and Roth 2010, Dimitri et al. 2011, Flemming et al. 2012,), and either unchanged, decreased or increased bone resorption markers (Bini et al. 2004, Dimitri et al. 2011, Viljakainen et al. 2011) in overweight or obese children have been reported. While most data are not based on longitudinal studies, it is largely unknown whether certain key stages exist when excess fat is detrimental

to bone mass accrual. Furthermore, the mechanisms responsible for these relationships are poorly understood; fat-derived proinflammatory cytokines and adipokines have been suggested to play a role (Figure 8).

2.7.2 Leptin and bone

Most, but not all, in vitro studies report a direct positive effect of leptin on osteoblasts, while leptin also inhibits differentiation of bone marrow cells in the adipogenic direction (Scotece et al. 2014). Many in vivo studies on rodents show opposite results by suggesting an indirect negative effect of leptin on BMD through suppression of the serotonin system in the brain stem, thereby enhancing sympathetic output to the bone. Suppression of bone formation and increase of resorption follows (Ducy et al. 2000, Elefteriou et al. 2005). However, leptin also shows anabolic effects locally in the bone microenvironment (Burguera et al. 2001, Turner et al. 2013). A recent study on obese mice suggested differing effects of leptin on trabecular and cortical bone compartments, as leptin correlated negatively with trabecular BMD and positively with cortical bone cross-sectional area (Fujita et al. 2012). Clinical studies on adults have found either a positive or no association between serum leptin and BMD (Biver et al. 2011), but results from paediatric studies have been even more discordant. Healthy normal-weight (Roemmich 2003, Hong et al. 2010) or obese (Afghani and Goran 2009, Russell et al. 2010) children and adolescents show either a positive (Russell et al. 2010), a negative (Afghani and Goran 2009, Hong et al. 2010) or no relationship (Roemmich et al. 2003) between serum leptin and BMC or BMD. Inconsistent results have been published also regarding effects of leptin on bone turnover markers in children. Leptin has been associated either positively, negatively or not at all with markers of bone formation (Bini et al. 2004, Reinehr and Roth 2010, Dimitri et al. 2011, Flemming 2012, VIljakainen et al. 2014) or bone resorption (Bini et al. 2004, Dimitri et al. 2011, Viljakainen et al. 2014). Weight loss is associated with increased formation markers, but not with ICTP (Bini et al. 2004). In conclusion, the role of leptin in bone metabolism remains poorly understood.

2.7.3 Adiponectin and bone

Adiponectin and its receptors are expressed in osteoblasts and osteoclasts (Shinoda et al. 2006). The in vitro effect of adiponectin on osteoclasts suggests either inhibition (Oshima et al. 2005) or indirect activation through stimulating RANKL and inhibiting OPG (Luo et al. 2006). Adiponectin stimulates the proliferation of osteoblasts in vitro (Luo et al. 2005), but may either increase (Oshima et al. 2005, Shinoda et al. 2006) or decrease bone mass in mice (Williams 2009). Adiponectin affects bone formation by several routes, including local effects, via circulation or indirectly by, for instance, insulin signalling (Shinoda et al. 2006, Williams et al. 2009). Kajimura et al. (2013) suggest that adiponectin regulates bone mass in mice through opposite local and central

mechanisms. Local adiponectin downregulates osteoblast proliferation and favours osteoblast apoptosis, thereby reducing bone formation, bone mass and circulating osteocalcin levels. Conversely, adiponectin signals in the brain, inhibiting the activity of the sympathetic nervous system, and thus, increasing bone formation and bone mass. Suppression of the sympathetic tonus opposes the effects of leptin and leads also to a decrease in energy expenditure (Kajimura et al. 2013). However, clinical studies on adults show an inverse or absent relationship between serum adiponectin and BMD (Biver et al. 2011), and similar results have been reported in children (Hong et al. 2010, Russell et al. 2010, Sayers et al. 2010). In healthy lean or obese children, the scarce data show no correlations between adiponectin and bone turnover markers [Dimitri et al. 2011, Flemming et al. 2012].

2.8 Interaction of adipose tissue with immunity and inflammatory responses in rheumatic diseases

Adipose tissue not only affects bone health but may also contribute to immunity and inflammatory responses in rheumatic diseases. Patients with low BMI have shown the highest scores for radiological damage, and high BMI has been proposed to be protective against these changes in RA (van der Helm-van Mill et al. 2008). Recent studies, by contrast, suggest a link between obesity and increased risk for RA (Crowson et al. 2013), and a positive association between obesity and disease activity (Ajeganova et al. 2013). Obesity also predicts poor response to treatment in early disease (Sandberg et al. 2014). Adipokines are speculated to play a causative role.

2.8.1 Leptin and rheumatic diseases

Conflicting data have emerged regarding whether leptin is harmful or protective for joint structures in RA. Most, but not all, recent data on patients with RA report increased serum leptin levels, which seem to correlate with CRP and higher disease activity. (Otero et al. 2006, Popa et al. 2009, Rho et al. 2009, Gomez 2011, Olama et al. 2011, Yoshino et al. 2011). Leptin stimulates IL-6 production in synovial fibroblasts of RA patients (Muraoka et al. 2013). One study reported that RA patients have higher synovial fluid leptin levels than healthy controls with traumatic knee effusion and that the synovial fluid leptin-to-serum leptin ratio correlates with disease duration, more active disease and erosions (Olama et al. 2012). However, a protective effect of leptin against radiographic joint damage has also been described (Rho et al. 2009). Leptin modulates both innate and adaptive immunity. It activates monocytes and macrophages and regulates actions of natural killer cells and neutrophils. Concerning adaptive immunity, leptin promotes Th1 and downregulates Th2 cell immune responses. It also suppresses the activity of regulatory T cells that act as regulators of autoimmunity (Notley and Ehrenstein 2010).

2.8.2 Adiponectin and rheumatic diseases

Several studies report increased serum adiponectin levels in patients with RA (Otero et al. 2006, Popa et al. 2009, Rho et al. 2009, Yoshino et al. 2011). Serum adiponectin correlates inconsistently with disease activity and CRP (Otero et al. 2006, Popa et al. 2009, Rho et al. 2009, Yoshino et al. 2011), but is associated with progression of radiological joint destruction (Ebina et al. 2009, Klein-Wieringa et al. 2011, Meyer et al. 2013), and lean patients with low levels of visceral fat show the highest adiponectin levels and radiographic damage (Giles et al. 2009). These findings have been suggested to explain the protective effect of obesity against radiological progression (Gomez et al. 2011). Several findings suggest a proinflammatory role of adiponectin in the joint (Scotece et al. 2014). Adiponectin promotes chemotaxis and induces production of inflammatory cytokines, such as IL-6 and IL-8, possibly synergistically with IL-1ß. It may contribute to joint destruction by stimulating matrix metalloproteinases and vascular endothelial growth factor expression in the synovium. Moreover, adiponectin isoforms are able to induce the expression of different genes involved in the pathogenesis of RA.

2.9 Role of fat/adipokines in juvenile idiopathic arthritis

2.9.1 Relationship between adipose tissue and bone in juvenile idiopathic arthritis

Data regarding the relationship between fat mass or adipokines and bone metabolism or bone mass in JIA are lacking.

2.9.2 Relationship of adipose tissue with disease activity in juvenile idiopathic arthritis

Very little data are available on the relationship between adiposity and disease activity in JIA or other paediatric rheumatic diseases. No relationship between fat mass or BMI and disease activity in JIA has been observed (Caetano et al. 2012, Pelajo et al. 2012). Even less is known about the role of adipokines in paediatric rheumatology. Patients had elevated leptin levels, but no relationship existed between leptin or adiponectin and disease activity in paediatric SLE (Al et al. 2009). These relationships have not been previously evaluated in JIA.

3. AIMS OF THE STUDY

Patients with JIA are exposed to complicated interactions between a chronic inflammatory disease and adipose tissue-related factors that may have an effect on bone health. The clinical significance and characteristics of these interactions remain inadequately characterized. These research questions constitute the basis for this thesis. Specific aims for the study were as follows:

1. To assess the prevalence of vertebral compression fractures and contributing risk factors in patients with severe JIA
2. To evaluate spinal MRI findings in children with severe JIA
3. To assess body composition and its relationship with bone mineral density in JIA.
4. To explore interactions between circulating adipokines and bone turnover markers in patients with severe JIA
5. To evaluate interactions between adipokines, bone mineral density and disease activity in JIA.

4. PATIENTS AND METHODS

4.1 Study design and data collection

We recruited two patient cohorts for our cross-sectional studies: One cohort from the Rheumatism Foundation Hospital in Heinola, a tertiary centre for complicated paediatric rheumatology patients in Finland, and another cohort from Children's Hospital, Helsinki University Central Hospital. Both cohorts included consecutive patients with JIA, diagnosed with the revised criteria (Petty et al. 2004) and fulfilling study inclusion criteria (Table 2).

TABLE 2. Inclusion criteria for the two cohorts of patients with JIA.

Severe JIA Cohort (N=50)	GC-treated Cohort (N=50)
1) History of refractory disease with continuous disease activity or recurrent flares requiring permanent antirheumatic medication since JIA diagnosis	1) JIA diagnosis ≥ 2 years prior to recruitment 2) Systemic glucocorticoids ≥ 3 months 3) age 7-<19 years
2) age < 19 years	
3) polyarticular JIA* ≥ 5 years or 4) systemic arthritis ≥ 3 years	

*** Polyarthritis, extended oligoarthritis or psoriatic arthritis with ≥ 5 affected joints**

The **Severe JIA Cohort** was recruited during an 18-month period. Of the 55 consecutive patients fulfilling the inclusion criteria, 5 patients declined due to lack of interest, and thus, the final study cohort comprised 50 patients. The non-participants did not differ from those who consented (with regard to diagnosis, disease duration, duration of GC therapy, height or weight).

The original **GC-treated Cohort** comprised 62 patients (Valta et al. 2007). Because no controls aged below 7 years were available, patients younger than 7 years (8 patients) or with incomplete whole-body DXA data (4 patients) were excluded from the study; the final study cohort thus comprised 50 patients.
For body composition and adipokine studies (III and IV), one to two controls of similar age and gender were chosen for both JIA cohorts from an original cohort of 202 apparently healthy Finnish schoolchildren (aged 7-19 years, 62% girls) from the Helsinki district (Pekkinen et al. 2012). For the Severe JIA Cohort, altogether 89 matching controls (2 for each boy; one for 11 girls and 2 for 30 girls), and for the GC-treated Cohort 88 matching controls (one for 3 boys and 2 for 13 boys; one for 9 girls and 2 for 25 girls) were found. All study participants were Caucasians.

4.2 Methods

The study protocol included clinical assessment, questionnaires, biochemistry, radiograph of the left hand for bone age assessment and bone densitometry. In the Severe JIA Cohort, spinal radiographs and spinal MRI were also obtained. Controls were similarly evaluated for background characteristics, anthropometric data and bone densitometry, but without radiography of the left hand or spinal imaging. Biochemical evaluation also partially differed between patients and controls.

4.2.1 Clinical assessment

The patients' medical records were reviewed for disease and treatment characteristics. The cumulative systemic GC dose, as prednisolone equivalents, was calculated for the preceding three years to determine recent GC exposure for the Severe JIA Cohort and total duration of GC therapy for the GC-treated Cohort. Patients were clinically evaluated by a paediatric rheumatologist. Height, weight, BMI (kg/m^2), number of active joints (including swollen joints and tender joints with limited range of motion), physician's global assessment of disease activity and patient's global assessment of overall well-being on the visual analogue (VAS) scale (0-100 mm) were recorded. Overweight and obesity were defined according to WHO guidelines as BMI Z-score >+1.0 and >+2.0, respectively (www.who.int 2007). Pubertal maturation was clinically assessed according to Tanner (1962) in patients, but was based on assessment of serum gonadotropin and sex steroid concentrations in controls. Inactive disease on medication, i.e. no signs of disease activity during the last six months, was defined according to Wallace et al. (2004); all patients received antirheumatic medication. Juvenile Arthritis Disease Activity Score (JADAS) for 10 or 71 joints was calculated (Consolaro et al. 2009). Childhood Health Assessment Questionnaire (CHAQ; Pelkonen et al. 2001) and questionnaires on the patient's physical activity, back pain and fracture history were filled out by the patients and their parents. Fractures were regarded as high-energy fractures when involving a fall from >3 m or an accident with a motorized vehicle; other fractures were regarded as low-energy fractures. In the Severe JIA Cohort, diet was assessed by a three-day dietary recall, including two work days and one weekend day (completed by 68% of patients). Average daily intakes of calcium, phosphate, vitamin D, energy and protein were calculated by a registered dietician with the computer program DIET32, version 1.4.4.1 (Aivo Finland Corp., Turku, Finland).

Disease and treatment characteristics are presented in Tables 3-5. In comparison with the GC-treated Cohort, patients in the Severe JIA Cohort were very young at disease onset (median 2.3 years), had longer disease duration and higher parameters of disease activity, and a higher proportion of the patients had active disease during the last six months (Wallace et al. 2004). The two patients

with inactive disease on medication had had refractory disease during their disease course of 10 or 14 years. (Table 3) All but three patients in the Severe JIA Cohort had received biological therapies (etanercept 23, infliximab 9, adalimumab 12, anakinra 5 and rituximab 3 patients), and 65% of the patients had a history of two or more biologicals. Multiple drug combinations had been used, and even thalidomide and chlorambucil had been given to 4 patients. In the GC-treated Cohort, 38% of the patients had received TNF-α antagonists. Data on systemic GC exposure and antirheumatic medication at the time of the study are presented in Tables 4 and 5, respectively. All patients in the Severe JIA Cohort had been given several (median 102) intra-articular GC injections during their disease course, and some had received additional injections at their local hospital. In the GC-treated Cohort, 92% of the patients had received intra-articular GC injections (median 7) during their disease course. Six patients with severe JIA (three of them with systemic arthritis) had received bisphosphonates (BPs) (alendronate 1, zoledronate 1 and pamidronate 4 patients) for 7-37 months because of compression fractures (4), low BMD and significant fracture history (1) or low BMD accompanied by constantly active disease and high-dose GC therapy (1). Four patients were still on BP therapy at the time of the study. One patient had received alendronate for one month 4.5 years earlier; this was considered insignificant regarding the bone health parameters.

TABLE 3. Disease characteristics in JIA Cohorts. Values are given as median (range) or N (%).

Disease characteristic	Severe JIA Cohort (N=50)	GC-treated Cohort (N=50)
Age at diagnosis	2.3 (1.1-11.8)	4.7 (1.1-15.3)
Disease duration	10.2 (3.9-16.8)	6.3 (2.0-15.1)
ANA positive, n (%)	13 (26)	11 (22)
Iritis, n (%)	22 (44)	15 (30)
JIA subtype, n (%)		
oligoarthritis, persistent	0	4 (8)
oligoarthritis, extended	14 (28)	14 (28)
polyarthritis, rf-	27 (54)	29 (58)
polyarthritis, rf+	1 (2)	1 (2)
psoriatic arthritis	2 (4)	0
systemic arthritis	6 (12)	2 (4)
Biologicals previously, n (%)	47 (94%)	19 (38%)
Systemic GC previously, n (%)	47 (94%)	50 (100%)
Active disease, n (%) *	48 (96%)	21 (42%)

*** Active disease on medication according to Wallace et al. (2004). GC, glucocorticoid. Two patients from the Severe JIA Cohort had celiac disease and were on a gluten-free diet.**

TABLE 4. Data on systemic (peroral or intravenous) glucocorticoid (GC) therapy; values are given as median (range).

Characteristic	Severe JIA Cohort (N=50)	GC-treated Cohort (N=50)
GC time, years	7.1 (0-15.5)	2.2 (0.25-12.5)
Total cumulative GC dose, g		2.9 (0.5-21.2)
Recent 3-year GC dose, g	3.2 (0-33.3)	
Total cumulative GC dose, mg/kg		102 (11-1095)
Recent 3-year cumulative GC dose, mg/kg	72 (0-911)	
Current GC dose, mg/d	3.1 (1.25-65.0)*	2.5 (1.25-10.0)**
Current GC dose, mg/kg/d	0.07 (0.02-0.7)*	0.05 (0.02-0.3)**

***n=26 and **n=20**

TABLE 5. Antirheumatic medication at the time of the study.

Medication	Number of patients (%)	
	Severe JIA Cohort (N=50)	GC-treated Cohort (N=50)
Methotrexate	28 (56)	38 (76)
Hydroxychloroquine	14 (28)	11 (22)
Sulfasalazine	6 (12)	2 (4)
Azathioprine	9 (18)	0
Cyclosporine A	1 (2)	0
Leflunomide	12 (24)	5 (10)
Sodium aurothiomalate	1 (2)	0
Biologic agents	45 (90)	16 (32)
TNFα antagonists		
Etanercept	23 (46)	4 (8)
Infliximab	9 (18)	7 (14)
Adalimumab	12 (24)	5 (10)
Anakinra	1 (2)	0
Glucocorticoids	26 (52) [a]	20 (40)
Thalidomide	1 (2)	0

[a] Four patients (8%) on daily regimen, others on alternate day regimen. TNFα, tumour necrosis factor alpha.

4.2.2 Imaging studies

Dual-energy X-ray absorptiometry

Bone area (cm^2), BMC (g) and areal BMD (g/cm^2) for lumbar spine (LS; L2-4), total hip and whole body (WB), as well as body composition, were assessed with DXA. In the Severe JIA Cohort, DXA measurements were assessed with Lunar (Lunar Prodigy; GE Lunar, Madison, WI, USA). In the GC-treated Cohort and in controls, Hologic (Hologic Discovery A, Waltham, MA, USA) was used. For Studies I and II, in the absence of a control group, DXA results of the Severe JIA Cohort were compared with published reference values to calculate BMD Z-scores (van der Sluis et al. 2002). For body composition analyses, DXA results of both patient cohorts were compared with those of matched controls. To enable comparison of results between the Severe JIA Cohort and controls, the data for controls were cross-calibrated according to Shepherd et al. (2012). BMD Z-scores for the GC-treated Cohort and controls were calculated according to equipment-specific reference values that have previously been shown to be appropriate for Finnish children (Valta et al. 2009). DXA and bone age radiograph were not obtained for one patient in the Severe JIA Cohort with severe carpal deformities, extreme growth failure and multiple compression fractures. As vertebral fractures are likely to reduce height and because bone age delay is a common complication of JIA, we chose to adjust DXA results not with height but with bone age. Height, age and bone age-adjusted bone mineral content-to-lean tissue mass (BMC/LTM) ratios were calculated according to Högler et al. (2003) for the Severe JIA Cohort.

Spinal radiography

Thoracolumbar radiographs (antero-posterior and lateral projections) were obtained and analysed for compression fractures by two experienced radiologists (paediatric radiologist and orthopaedic radiologist), who were blinded to the patients' clinical diagnosis and status. They analysed the radiographs first individually, and the results were combined for consensus. The less severe alternative was reported as the final conclusion when individual readings differed. Compression fractures were classified according to Mäkitie et al. (2005); a ≥20% vertebral height reduction was regarded as signifying vertebral compression. Compromised visibility partially prevented evaluation of the thoracic spine in 7 patients. Previous spinal radiographs were also retrospectively analysed for vertebral morphology.

Bone age was determined according to Greulich-Pyle (1959) from the patient's left hand radiograph and considered delayed/advanced when it differed from calendar age by more than one year.

Spinal magnetic resonance imaging

MRI examinations of the thoracic and lumbar spine were performed with an open-field 0.23 T MRI unit (Panorama Power, Philips Medical Systems, Vantaa, Finland)

with a spine coil. Sagittal images with 5 mm slice thickness were obtained with T1-weighted turbo spin echo (TR 475.4-500 ms, TE 13 ms, flip angle 90, FOV 360) and T2-weighted turbo spin echo (TR 3987-4118, TE 108, flip angle 90, FOV 360) sequences. In most of the patients, the thoracic spine and the lumbar spine were imaged separately; only in the youngest and smallest patients were they imaged together. One of the patients had undergone MRI in a 1.5 T imager (Philips Intera Achieva, Philips Medical Systems, Best, the Netherlands) in another hospital at the time of the study, and these images were used in the analysis.

All MRI images were analysed by the same radiologists and with similar principles as spinal radiographs. The assessment included the vertebrae, intervertebral discs, spinal canal and neural foramina, back muscles and abdominal subcutaneous adipose tissue, as described below.

Assessment of vertebrae and endplates. Vertebral bodies were evaluated for shape and signal intensity. The vertebrae were classified as normal, wedged or compressed according to Mäkitie et al. (2005). The location of defects in vertebral corners was noted and recorded as i) anterior vertebral corner defects or ii) posterior vertebral corner defects. Endplate irregularities and Schmorl nodes were recorded. Endplates were regarded as irregular when the entire endplate was involved; Schmorl node (herniation of the intervertebral disc into the endplate and the adjacent vertebral body) was characterized by a focal endplate defect.

Assessment of intervertebral discs. Intervertebral disc height and signal intensity were assessed from T2-weighted sagittal image. The discs were classified as grade 0 (normal), grade 1 (mild degeneration) and grade 2 (severe degeneration). Increased disc height with normal signal intensity ("ballooning" of a disc) was noted, if present. Disc herniations were classified as a protrusion (slight bulging beyond the interspace) or a prolapse (extrusion beyond the interspace).

Assessment of spinal canal. Medulla and cauda equina were evaluated for shape and signal intensity. The width of the spinal canal in T2-weighted images was graded as grade 0 (normal), grade 1 (mild narrowing), grade 2 (spinal stenosis) or grade 3 (compression of the medulla). Neural foramina and neural root compression in T1-weighted sagittal images was classified as grade 0 (normal), grade 1 (mild narrowing without impingement of the neural root) or grade 2 (severe narrowing with impingement of the neural root).

Assessment of lumbar back muscle. The lumbar muscular was assessed visually. Apparent size loss on TI-weighted images and high signal intensity streaks on T1- and T2-weighted images representing fat deposits in the muscle were recorded. The findings were classified as grade 0 (normal), grade 1 (mild atrophy) or grade 2 (severe atrophy).

4.2.3 Biochemistry

Blood samples were drawn in the morning between 8:00 and 11:00 and second void urine samples were collected after an overnight fast and stored at -80°C until analysed. Biochemical assessments with abbreviations and applied methods are presented in Table 6. Numerals I-IV refer to the number of the study. Parameters with unspecified methods were analysed by standard methods and compared with reference values. For urine calcium-to-creatinine ratio (U-Ca/Cr), values <0.7 mmol/mmol were considered normal. Vitamin D status was defined based on 25-OHD value as severe deficiency (<12.5 nmol/l), deficiency (<37.5 nmol/l), insufficiency (37.5-<50.0 nmol/l) or sufficiency (≥50 nmol/l) (Misra et al. 2008).

TABLE 6. Biochemical assessment in patient cohorts and controls in Studies I-IV.

	Severe JIA Cohort	Controls I	**GC-treated Cohort**	Controls II	**Method**
Erythrocyte sedimentation rate, ESR	I-III		IV		
C-reactive protein, CRP	I-III				
S-Calcium, Ca; ionized calcium, Ca-ion	I				
P-Inorganic phosphate, Pi	I				
P-Parathyroid hormone, PTH	I				Immunoluminometric method
S-25-hydroxyvitamin D, 25-OHD	I				Radioimmunoassay
S-Insulin-like growth factor-1, IGF-1	I				Immunoluminometric method
FSH, LH (girls >8 years, boys >10 years)	I-III	III	IV	IV	CMIA* / Immunofluorometric method**
Oestradiol (girls >8 years)	I-III	III	IV	IV	Radioimmunoassay
Testosterone (boys >10 years)	I-III	III	IV	IV	CMIA* / Liquid chromatography mass spectrometry**
Bone turnover markers					
S-OC (marker of bone formation)	III	III			Two-site immunoassay based on monoclonal antibodies^
S-ALP (marker of bone formation)	III	III			Standard kinetic method
S-PINP (marker of bone formation)	III	III			Radioimmunoassay
S-ICTP (marker of bone resorption)	III	III			Radioimmunoassay
Adipokines					
S-Leptin	III	III	IV	IV	Human leptin ELISA
S-Adiponectin	III	III	IV	IV	Human adiponectin ELISA

ALP, alkaline phosphatase; OC, osteocalcin; PINP, aminoterminal propeptide of type I collagen; ICTP, carboxyterminal telopeptide of type I collagen; FSH, follicle-stimulating hormone; LH, luteinizing hormone; CMIA, chemiluminescent microparticle immunoassay; ELISA, enzyme-linked immunosorbent assay.
*** Severe JIA Cohort, ** GC-treated Cohort, ^ (Käkönen 2000, Paldanius 2012)**

4.3 Ethical considerations

A written informed consent was obtained from all study participants and their parents. The study protocol was approved by the Research Ethics Committee of Helsinki University Central Hospital. Spinal radiographs were considered clinically appropriate for patients from the Rheumatism Foundation Hospital because they had severe disease and were at risk of vertebral compression fractures. The radiation dose from DXA is very low and absent in MRI. No sedation for MRI studies was required.

4.4 Statistics

Descriptive data are reported as median with range or interquartile range (IQR), or as mean ± standard deviation (SD) or with 95% confidence interval (CI). Simple regression analysis was used for correlations (Pearson or Spearman), the unpaired two-tailed Student's t-test for comparing means and the Mann-Whitney U-test for comparing non-normally distributed variables. The Chi-square test (or Fischer's exact test) was applied when nominal data were compared. Comparison of groups with adjustments was performed with analysis of variance (ANOVA). The association of adipokines with bone turnover markers or bone mass was analysed by linear regression (additive models). Logarithmic transformations for non-normally distributed data were used as appropriate. To avoid errors related to multiple testing, Bonferroni correction was used (Study III); the required significance level depended on the number of predictors in the model. A multivariate logistic regression model was used to identify and determine odds ratios with 95% CI for significant associations with compression fractures. Factors included in logistic regression analysis were dichotomized based on the value's distribution in the cohort or by clinically relevant cut-offs. A P-value of less than 0.05 was considered statistically significant.

Statistical analyses were performed either with Statview® 5.0.1 for Macintosh, 1992-1998; SAS Institute (Studies I and II) or with SPSS for Windows version 17.0 or 21.0 (SPSS Inc., Chicago, IL, USA) (Studies III and IV).

Lumbar DXA data of two patients in the Severe JIA Cohort and one patient in the GC-treated Cohort were excluded from analyses because of compression changes in the lumbar spine. In the Severe JIA Cohort, altogether six patients had received previous bisphosphonate therapy (one of them was not evaluated for DXA or bone turnover markers because of severe skeletal changes and extreme growth failure), and their results were excluded from the analyses evaluating bone mineral density or bone turnover markers.

5. RESULTS

5.1. Clinical and disease characteristics

5.1.1 Anthropometric data and disease activity

The two cohorts with JIA were significantly different from each other. Anthropometric data showed growth delay, higher BMI Z-score and higher percentages of overweight and obesity in the Severe JIA Cohort when compared with healthy controls. In the GC-treated Cohort, Z-scores for height and BMI were similar to controls. Patients in the Severe JIA Cohort were older than in the GC-treated Cohort (median age 14.8 years vs. 12.4 years) (Table 7). Parameters of disease activity at the time of the study are presented in Table 8. As expected based on inclusion criteria, patients in the Severe JIA Cohort had higher disease activity values than their counterparts in the GC-treated Cohort. When patients with systemic disease were excluded, median JADAS-10 scores remained similar (6.0 in Severe JIA Cohort and 2.5 in GC-treated Cohort).

TABLE 7. Anthropometric data in the JIA cohorts and in healthy controls. Data are given as median (range) or proportion (%).

	Severe JIA Cohort N=49	Controls I N=89	p	**GC-treated Cohort** N=50	Controls II N=88	p
Boys / girls, %	18 / 82	20 / 80	0.792	32 / 68	33 / 67	0.908
Age, years	14.8 (7.0-18.7)	14.5 (7.4-18.8)	0.569	12.4 (7.2-17.9)	12.7 (7.4-17.4)	0.702
Pre-/mid-/postpubertal, %	18 / 30 / 52	25 /23 / 52	0.442	36 / 36 / 28	38 / 26 / 36	0.421
Height Z-score	-1.2 (-4.1- +1.9)	-0.1 (-2.3-+2.6)	<0.001	0.1 (-2.9-+1.5)	0.2 (-2.0-+2.8)	0.313
Height-adjusted weight, %	+24 (-15- +123)	+4 (-25-+53)	<0.001	+5 (-17-+49)	+2 (-20-+51)	0.281
BMI, kg/m^2	22.2 (14.6-39.2)	19.2 (13.0-28.5)	<0.001	18.9 (13.2-30.0)	18.4 (13.6-29.0)	0.509
BMI Z-score	+0.8 (-1.6-+4.3)	-0.1 (-3.5-+2.5)	<0.001	0.2 (-1.9-+2.3)	0.0 (-2.2-+2.5)	0.321
Overweight, n (%)*	19 (39)	13 (15)	0.001	10 (20)	14 (16)	0.542
Obese, n (%)*	14 (29)	1 (1)	<0.001	5 (10)	3 (3)	0.113

*** According to World Health Organization. P-values were determined with the Mann-Whitney U-test or the Chi-square test, as appropriate. One 14-year-old girl with extreme growth failure (height Z-score -9.4) and multiple compression fractures was excluded from Study III.**

TABLE 8. Parameters of disease activity as median (range) at the time of the study.

Parameters of disease activity	Severe JIA Cohort (N=50)	GC-treated Cohort (N=50)
Number of active joints	1 (0-38)	0.0 (0-8)
Physician's global assessment	20 (0-70)	5.0 (0-80)
Patient's global assessment	14 (0-67)	3.5 (0-87)
CHAQ	0.125 (0 – 2.375)	0.0 (0-1.625)
ESR, mm/h	12 (3-71)	8.5 (2-65)
CRP	5 (2-129)	
JADAS-10*	6.0 (0-23.6)	2.4 (0-18.3)

*** JADAS, juvenile arthritis disease activity score; values between 0 and 40. Score ≤1.0 indicates inactive disease (Consolaro et al. 2014).**

5.2 Bone health

5.2.1 Dietary data and biochemistry in the Severe JIA Cohort

A three-day dietary recall was completed by 34 patients (68%). The reported median energy intake was 73% of the daily recommended intake (DRI), which is likely to be an underestimate considering the high BMI observed in these patients. A wide variation existed between patients regarding their reported total intakes of calcium and vitamin D, but the intakes on average exceeded minimal recommendations. Calcium and vitamin D supplements were utilized by 47% and 59% of the patients, respectively. Dietary data are presented in Table 9. The median serum 25(OH)D was 53 (range 20-95) nmo/l. Vitamin D concentration was sufficient in 62%, insufficient in 24% and deficient in 14% of patients; only one patient had a value >80 nmol/l. Mild hypercalciuria (U-Ca/Cr >0.7 mmol/mmol) was observed in 5 patients. None of the patients had hypercalcaemia and 2 had mild hypocalcaemia. Occasional patients had hypophosphatemia (n=2), hyperphosphatemia (1) and slightly subnormal (1) or supranormal (1) fP-PTH values. S-IGF-1 was subnormal in 7 patients.

TABLE 9. Dietary data for 34 patients with severe JIA.

	Median	Range
Calcium intake from diet, g/day	1.09	0.19-2.82
Calcium intake including supplements, g/day	1.34	0.23-3.32
Calcium intake including supplements, % of DRI*	155	25-383
Vitamin D intake from diet, ug/day	4.7	1.2-13.7
Vitamin D intake including supplements, ug/day	14	1.2-34
Vitamin D intake including supplements, % of DRI*	187	16-453
% of 2014 DRI**	140	12-340
Phosphate intake, % of recommendation	189	63-540
Dietary energy, % of recommendation	73	36-131
Dietary protein, Energy-%	16.1	12-25.1

*** Finnish National Nutrition Council dietary recommendations from the year 2005**
**** The recommended daily vitamin D intake increased from 7.5 to 10 µg in the year 2014. DRI, daily recommended intake**

5.2.2 Bone mineral density

Patients in the Severe JIA Cohort had decreased median BMD Z-scores for the lumbar spine (-0.8) and whole body (-1.0), but Z-scores for bone age- and height-adjusted BMC-to-lean mass ratios were normal. In the GC-treated Cohort, Z-scores for BMD values and BMC-to-lean mass ratio were lower than in controls (Table 10). DXA results between patient cohorts are not comparable because measurements were performed with different DXA devices. BMD did not correlate with current disease activity or cumulative GC dose in either cohort, but an inverse correlation was observed in the GC-treated Cohort between the current weight-adjusted GC dose and WB BMD (r_s=-0.304, p=0.032); a similar trend was observed regarding LS BMD (r_s=-0.272 and p=0.059). Similar correlations for the current weight-adjusted GC dose with Z-scores for LS BMD (r_s=-0.382, p=0.011) and WB BMD (r_s=-0.330, p=0.029) were seen in the Severe JIA Cohort.

TABLE 10. DXA data in the Severe JIA Cohort as well as in the GC-treated Cohort with controls. Data are presented as median (IQR).

Bone variable	Severe JIA Cohort* N=44	GC-treated Cohort** N=50	Controls** N=88	p-value#
LS aBMD Z-score, for bone age	-0.8 (-1.3, 0.0)	-0.5 (-1.2, +0.6)	0.0 (-0.5, +0.6)	**0.006**
WB aBMD Z-score, for bone age	-1.0 (-1.6, -0.3)	-0.2 (-0.9, +0.8)	0.1 (0.0, +0.8)	**0.009**
BMC/lean mass	0.053 (0.050, 0.060)	0.051(0.048, 0.054)	0.053 (0.050, 0.056)	**0.002**
for height, Z-score	+0.1 (-1.0, +1.0)			
for age, Z-score	-0.3 (-1.0, +0.9)			
for bone age, Z-score	+0.2 (-1.0, +1.1)			

*** Lunar, ** Hologic. # Comparison between GC-treated Cohort and controls (Mann-Whitney U-test). LS, lumbar spine; WB, whole body; BMC, bone mineral content**

5.2.3 Non-vertebral fractures in the Severe JIA Cohort

Altogether 16 patients (32%) had had previous significant (i.e. excluding finger and toe fractures) peripheral low-energy fractures. Nine patients had had only one fracture, 5 patients had 2 previous fractures, one patient had 3 previous fractures and one patient had 4 previous fractures. Two patients with non-vertebral fractures were considered to have significantly compromised bone health based on a significant fracture history and low BMD (Z-score ≤-2.0), thus fulfilling the ISCD criteria for paediatric osteoporosis (Rauch 2008).

5.2.4 Vertebral fractures in the Severe JIA Cohort

Radiographic findings

Five patients had a history of earlier vertebral compressions at some point after JIA diagnosis; all had received bisphosphonates. At the time of the study, altogether 11 patients (22%) had vertebral compression fractures on spinal radiographs. Fractures were mostly (38 of 54 fractures, 70%) located in the thoracic area. Eight patients had more than one (from 2 to 16) fractured vertebra. Visibility of the vertebrae was suboptimal in the thoracic spine of 7 patients. In Figure 9, multiple compressed vertebrae in the thoracic and lumbar spine and densitometry data in a 9-year-old boy with systemic arthritis are shown.

Associations between clinical factors and vertebral fractures

Age and height Z-score were not significantly different between those with and without fractures. Patients with fractures had higher BMI Z-score than those without fractures, but the difference was not significant between the groups regarding fat percentage. Disease duration was shorter, but excluding the active joint count, parameters of disease activity were higher in those with fractures. There was no difference concerning the total duration of GC therapy

between the groups, but the cumulative 3-year and current GC doses adjusted for weight were higher in those with fractures. Z-scores for LS and WB BMD did not differ between the groups. The reported daily intake of vitamin D was higher in patients with fractures, but 25-OHD values were similar. Two patients with compression fractures had never had back pain, and only one patient had back pain daily. No difference was present in the number of non-vertebral fractures between patients with and without vertebral fractures. Factors associated with vertebral fractures are shown in Table 11.

In logistic regression analysis, cumulative weight-adjusted GC dose, CHAQ and BMI Z-score were chosen for further analysis. Cumulative GC dose of >75 mg/kg (48% of patients) was associated with a 7-fold risk (OR 7.2, p=0.016), CHAQ >0.5 (consistent with moderate disease activity; 22% of patients) with a 7-fold risk (OR 7.2, p=0.013) and BMI Z-score >+2.0 (28% of patients) with an almost 5-fold risk (OR 4.7, p=0.052) for compression fractures. Cumulative GC correlated with CHAQ (r=0.29). In stepwise backward analysis, the most significant associations with compression fractures were with CHAQ (OR 16.4, p=0.005) and BMI Z-score (OR 1.89, p=0.029), which together explained 45% of the fracture risk.

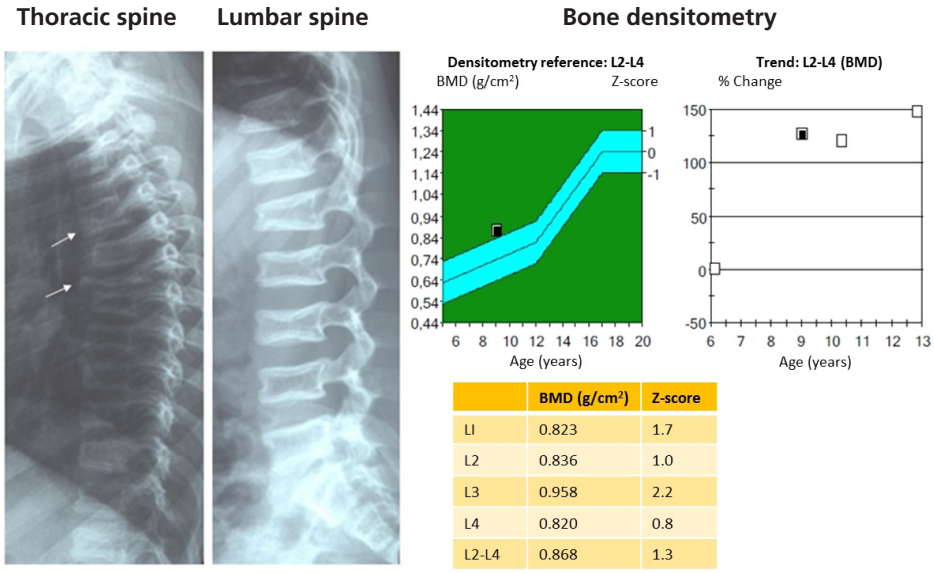


FIGURE 9. Spinal radiographs and bone densitometry in a 9-year-old boy with systemic arthritis. Multiple compressed vertebrae in the thoracic and lumbar spine were observed. Vertebrae Th 4 and 6 were totally collapsed (3b) (arrow), and grade 2b-3a fractures were observed in vertebrae Th5 and Th7-L5. Because of bisphosphonate therapy, vertebrae have sclerotic margins and are therefore more visible. Lumbar spine DXA results of the same patient show rather high BMD values, resulting from bisphosphonate therapy and compression changes in the measurement area.

TABLE 11. Factors associated with vertebral fractures.

Clinical characteristic	**No fractures** (n=39)	**With fractures** (n=11)	p
Demographics and anthropometry			
Sex distribution, boys n (%)	5 (13)	4 (36)	0.073
Age, mean ± SD, years	14.4 ± 3.0	13.6 ± 2.8	0.390
Height Z score, mean ± SD	-1.3 ± 1.2	-1.9 ± 2.8	0.480
BMI Z-score, mean ± SD	+0.7 ± 1.4	+1.9 ± 1.6	**0.029**
Fat %, mean ± SD	35 ± 9.9	41.5 ± 9.6	0.187
Disease characteristics (mean ± SD)			
Disease duration, years	11.1 ± 3.6	8.2 ± 3.5	**0.032**
Number of active joints	2.1 ± 2.3	6.0 ± 11.2	0.670
CHAQ [a]	0.24 ± 0.31	0.80 ± 0.70	**0.004**
ESR, mm/h	14.2 ± 13.8	26.9 ± 22.8	**0.047**
JADAS-10	5.8 ± 4.7	11.4 ± 8.3	**0.024**
GC treatment (mean ± SD)			
Total duration of GC therapy, years	7.1 ± 4.1	7.5 ± 3.5	0.910
Recent 3-year cumulative GC dose, mg/kg	69 ± 67	292 ± 323	**0.012**
Current weight-adjusted GC dose, mg/kg/day	0.04 ± 0.06	0.14 ± 0.22	**0.037**
Skeletal characteristics and nutrition			
LS BMD Z-score, mean ± SD [b]	-0.7 ± 0.9	-0.7 ± 1.6	0.63
WB BMD Z-score, mean ± SD [b]	-1.1 ± 1.0	-0.8 ± 1.1	0.53
BMC/LBM for height Z-score, mean ± SD [b]	+0.1 ± 1.5	-0.6 ± 2.2	0.33
Ca intake, % of DRI	158 (76)	212 (98)	0.154
Vitamin D intake, % of DRI	162 ± 130	282 ± 153	**0.044**
S-25-OHD, nmol/l	53.6 ± 15.8	59.5 ± 10.9	0.170

Statistical significance determined by the Mann-Whitney U-test, Chi-square test or Fischer's exact test as appropriate.
[a] Childhood Health Assessment Questionnaire, 0 = best and 3 = worst; data of 46 patients aged <18 years.
[b] Values are corrected for bone age. Data of 44 patients; five patients with previous bisphosphonate treatment (3 with and 2 without vertebral fractures) were omitted from the analyses, and BMD was not available for one patient.

Magnetic resonance imaging

Altogether 62% of the patients had one or more spinal abnormalities on MRI. Vertebral fractures were observed in 14 patients (28%). The total number of deformed vertebrae was 66 (1-16 affected vertebrae per patient). Altogether 61% of these were located in the lower thoracic spine (vertebrae Th7-Th12), 18% in the upper thoracic spine (Th1-Th6) and 21% in the lumbar spine (L1-L5).

5.2.5 Other skeletal findings on magnetic resonance imaging

Altogether 26% of the patients had endplate irregularities, 16% had anterior vertebral corner lesions and 46% had disc changes (entire or focal). The majority (70%) of endplate changes and disc changes (54%) were located in the lower thoracic spine, but 35% of the disc changes were observed in the two lowest lumbar disc spaces. Three patients with severe vertebral compression fractures had ballooning of the intervertebral discs. Two patients had mild spinal canal narrowing without medullar involvement; none had neural root compression. Figure 10 A shows normal vertebral morphology, and Figures 10 B-D and 11 A-C present abnormal vertebral findings on MRI.

Mostly non-significant differences were present between patients with and without MRI findings. Boys had more vertebral fractures than girls, but no gender difference was noted regarding other changes. Relative to subjects without spinal abnormalities, BMI Z-score tended to be higher in those with various spinal abnormalities such as fractures (p=0.160), endplate changes (entire or focal, p=0.015), anterior corner lesions (p=0.093) and disc changes (p=0.139). Patients with spinal fractures tended to have higher 3-year cumulative GC dose (p=0.086), but similar associations with GC dose were not observed concerning other spinal changes.

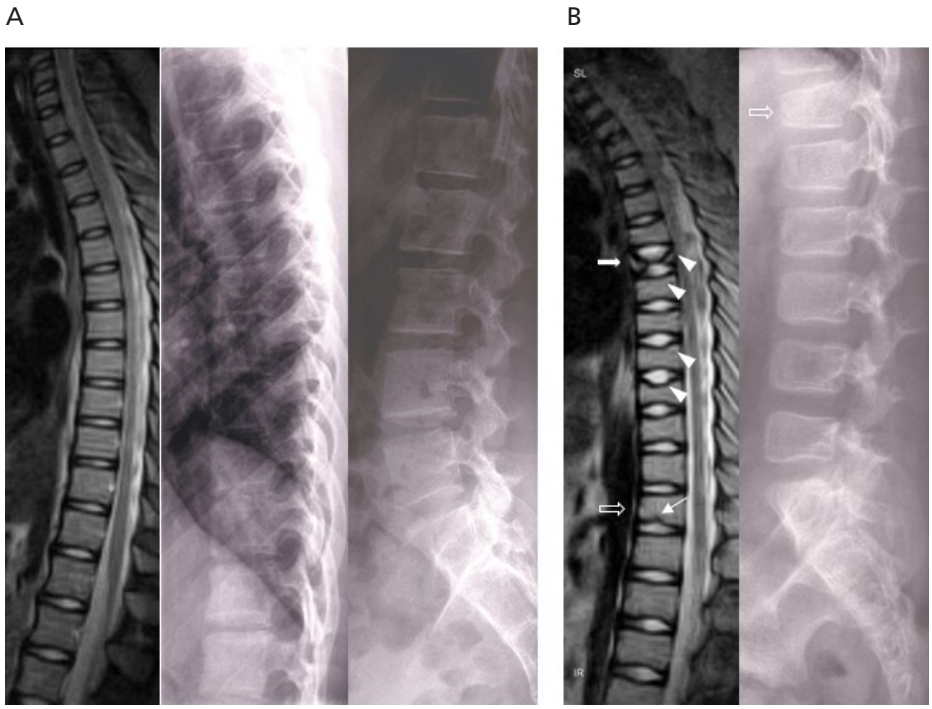


FIGURE 10. A. Normal vertebral morphology in a 15-year-old girl with extended oligoarthritis on MRI and spinal radiography. B. The thoracic spine was poorly visible on radiography in this 13-year-old boy with systemic arthritis. Vertebrae Th1-10 could not be reliably assessed, and only a wedge fracture (Grade 2a) at Th12 (open arrow) was seen on radiography, while multiple fractures in the thoracic spine were visible on MRI. Vertebra Th5 was totally collapsed (arrow), and Grade 3a compressions were observed at Th 4, 6-10. In Th12 vertebra, also a focal irregularity (Schmorl, thin arrow) in the lower endplate was observed. A mild increase in disc height (arrowheads) resulting from endplate depression in adjacent vertebrae was also seen.

C D

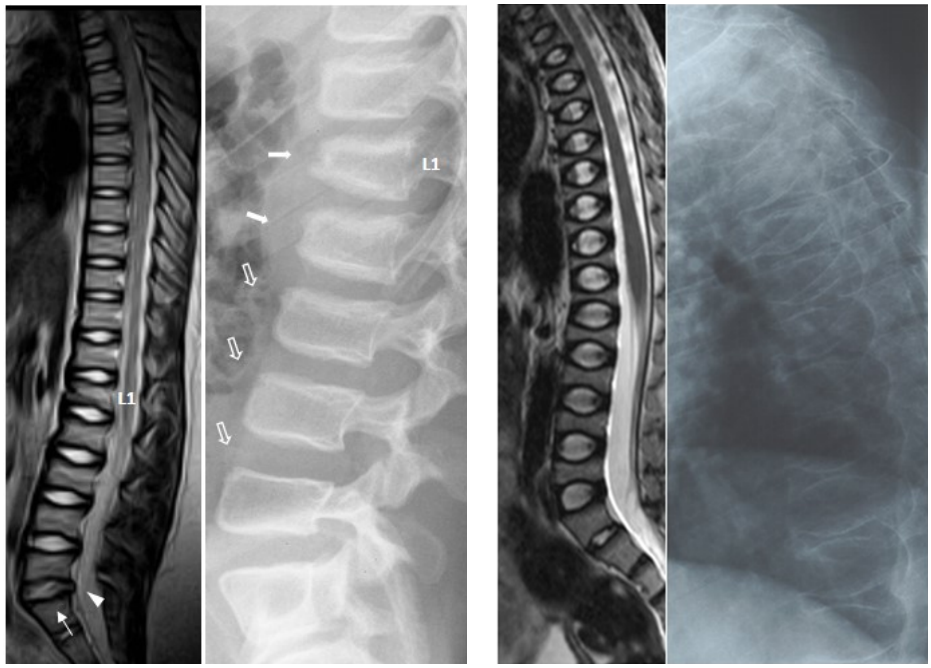


FIGURE 10. C. Multiple vertebral fractures in the thoracic and lumbar spine of a 9-year-old girl with systemic JIA. In Th11-L4 intervertebral disc spaces, a slight increase in height of the discs with normal signal intensity (bright) and in the presacral disc a protrusion (arrowhead) and decreased signal intensity (dark, thin arrow) are seen. Lumbar radiograph also shows wedge deformities (L1 2b, L2 2a, arrow) and compression deformities (L3-5 3a, open arrow). Sclerotic vertebral margins are due to bisphosphonates. D. All subcervical vertebrae except L5 in a 14-year-old girl with treatment-resistant psoriatic arthritis are severely compressed (Grade 3b), and all intervertebral discs are markedly ballooned. Compressions in the thoracic spine are visible also in radiography, although bone seems severely osteoporotic.

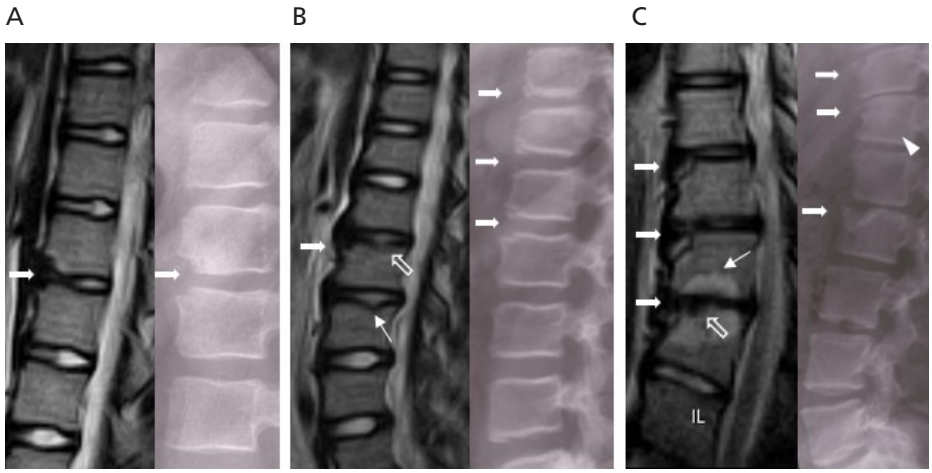


FIGURE 11. Findings on spinal MRI in patients with severe JIA.
A. A corner defect in the lower anterior corner of L1 (arrow) in a 12-year-old girl with seronegative polyarthritis.

B. Anterior longitudinal ligament thickening, disc degeneration (open arrow), several small anterior corner defects (arrow) and Schmorl nodes (thin arrow) in a 16-year-old girl with systemic arthritis.

C. In a 15-year-old boy with systemic arthritis, wedge fracture in Th12 causes kyphosis. On MRI, anterior corner lesions with small bone fragments (arrow) in Th10 and Th11, a large corner defect and subchondral bone oedema (Th11-12, thin arrow) and irregular endplate and disc degeneration (open arrow) are seen. On radiography, Th12 vertebra (arrowhead) was not regarded as fractured, but narrowing of disc spaces Th11-L2 and large anterior corner defects of Th11-12 and L2 (arrow) were observed.

5.3 Body composition and its relationship with bone mineral density

Patients in the Severe JIA Cohort had increased adiposity, as expected based on their increased BMI Z-score, but lean mass was similar to controls. In the GC-treated Cohort, neither fat mass nor lean mass differed from controls. Body composition data for patient cohorts and controls are presented in Table 12. In the GC-treated Cohort, data were further analysed by comparing patients according to lumbar spine BMD Z-scores; a cut-off value at -1.0 was used (Table 13). When comparing the groups according to calendar age-based BMD Z-score, Z-score values below -1.0 ("low BMD") were related to lower Z-scores for height and BMI and to smaller bone size and lower lean mass index. However, when bone age-adjusted BMD Z-scores with similar cut-off values were compared, except for lower BMC-to-lean mass ratio, no differences regarding growth or body composition existed between those with low or "normal" BMD. Altogether 60% and 35% of patients in the low and normal BMD groups, respectively, had active disease during the last six months (p=ns); the median JADAS-10 score reflecting current disease activity was similar between the groups (2.4 vs. 2.3, p=ns).

TABLE 12. Body composition assessment with DXA for a) the Severe JIA Cohort and controls and b) the GC-treated Cohort and controls.

a)	**Severe JIA Cohort** median	(N=49) IQR	**Controls*** median	(N=89) IQR	p-value
Total fat, %	37.1	30.2-46.0	28.8	23.5-33.2	**<0.001**
Fat mass, kg	18.96	11.96-25.33	12.98	9.54-17.82	**<0.001**
FMI, kg/m^2	8.5	5.7-12.2	5.5	4.0-6.9	<**0.001**
Lean mass, kg	29.51	26.16-34.45	31.78	25.51-37.61	0.241
LMI, kg/m^2	13.0	12.3-14.2	12.9	11.8-14.0	0.145

b)	**GC-treated Cohort** median	(N=50) IQR	**Controls** median	(N=88) IQR	p-value
Total fat, %	27.1	23.0-32.3	26.9	21.2-31.4	0.516
Fat mass, kg	11.99	7.73-16.89	11.97	7.97-15.06	0.693
FMI, kg/m^2	5.5	3.7-6.6	5.0	3.7-6.3	0.372
Trunk fat, %	21.4	16.1-26.4	20.9	15.7-25.8	0.830
Trunk fat mass, kg	3.94	2.29-5.82	3.91	2.51-5.43	0.787
Lean mass, kg	31.65	23.09-35.96	30.63	24.91-36.84	0.852
LMI, kg/m^2	13.0	11.6-14.8	13.0	11.9-13.9	0.951

*** Data for the controls of the Severe JIA Cohort were cross-calibrated. FMI, fat mass index; LMI, lean mass index. P-values were calculated with Mann-Whitney U-test.**

TABLE 13. Comparison of data for the GC-treated Cohort according to lumbar spine BMD Z-score for calendar age or bone age; a cut-off value at -1.0 was used.

LS BMD Z-score	For calendar age ≤-1.0 SD (n=16)	>-1.0 SD (n=33)		For bone age ≤-1.0 SD (n=15)	>-1.0 SD (n=34)	
	mean ± SD	mean ± SD	p-value	mean ± SD	mean ± SD	p-value
Gender, boys/girls (%)	12.5/87.5	39/61	0.097	27/73	32/68	0.750
Age, years	12.3 ± 3.0	12.6 ± 3.2	0.782	12.5 ± 2.7	12.5 ± 3.3	0.896
Pre- / postpubertal, n (%)	(44)/(19)	(33)/(30)	0.649	33/13	38/32	0.211
Height Z-score	-0.56 ± 3.0	+0.15 ± 0.9	**0.025**	-0.39 ± 1.0	+0.05 ± 1.0	0.158
BMI Z-score	-0.48 ± 1.0	+0.48 ± 1.0	**0.005**	+0.26 ± 1.3	+0.14 ± 1.0	0.544
Fat, %	26.0 ± 6.2	27.7 ± 7.3	0.238	29.1 ± 7.2	26.2 ± 6.8	0.288
Fat mass index, kg/m^2	4.8 ± 1.7	5.7 ± 2.0	0.162	5.9 ± 2.2	5.2 ± 1.8	0.368
Lean mass index, kg/m^2	12.4 ± 1.9	13.7 ± 1.9	**0.013**	13.3 ± 1.9	13.2 ± 2.0	0.696
Bone area/height, cm^2/m	1223 ± 149	1322 ± 134	**0.039**	1269 ± 166	1299 ± 137	0.649
BMC/lean mass	0.050 ± 0.004	0.052 ± 0.004	0.125	0.048 ± 0.002	0.052 ± 0.004	**<0.001**

BMI, body mass index; BMC, bone mineral content. P-values were calculated with Mann-Whitney U-test.

5.4 Serum bone turnover markers and adipokines

In the Severe JIA Cohort, serum bone formation markers did not differ from controls. However, bone resorption marker ICTP was significantly higher in patients than in controls (mean 15.6 µg/l, 95% CI 13.5-17.4 vs. mean 12.3 µg/l, 95% CI 11.1-13.6 µg/l; p=0.006) suggesting an imbalance in bone turnover. Serum leptin concentration was significantly higher in patients than in controls, even when adjusted for age, gender, pubertal stage and fat mass, as shown in Figure 12. Adiponectin concentrations were similar, but adiponectin-to-fat mass ratio was lower in patients than in controls (median 0.53 vs. 0.77, p=0.004). On the contrary, in the GC-treated Cohort, leptin and adiponectin values were similar to those in controls.

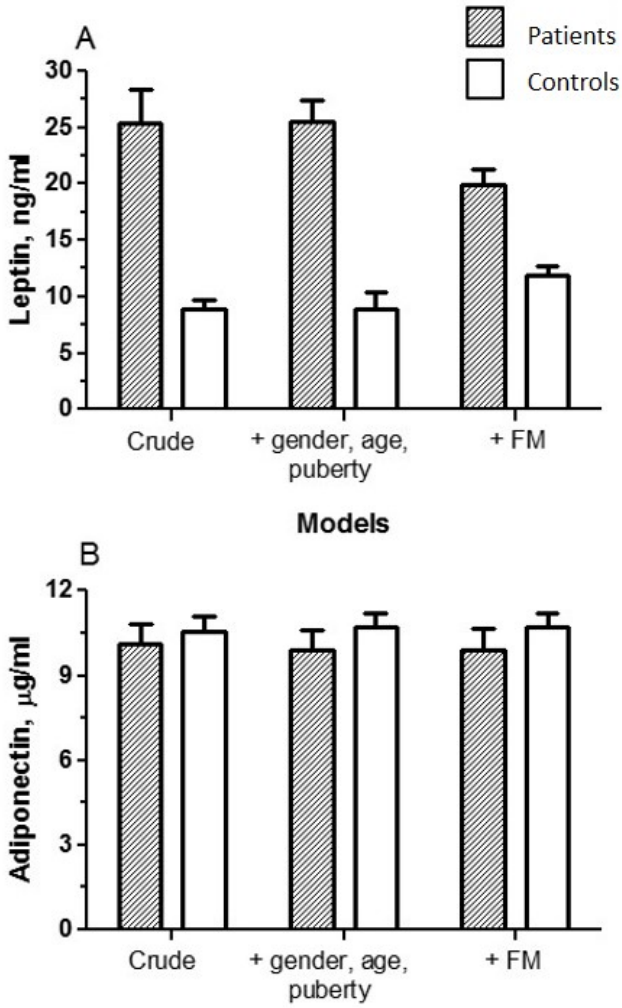


FIGURE 12. Comparison of serum leptin (A) and adiponectin (B) values in patients with severe JIA and healthy controls when 1) unadjusted (crude), 2) adjusted for age, gender and pubertal stage, 3) adjusted additionally for fat mass. Bars designate mean concentrations and whiskers the upper limit of the standard error of the mean.

5.4.1 Association of adipokines with serum bone turnover markers

The association between serum adipokines and bone turnover markers as standardized beta coefficients was assessed for the Severe JIA Cohort and for controls. In controls, inverse associations between leptin and bone turnover markers attenuated after adjustment for confounding factors. In patients, by contrast, associations became stronger, especially concerning PINP (Table 14), but a similar trend was observed concerning other markers when adjusted for fat mass. Although not significant, beta coefficients of the patients remained

different from controls after adjusting for lean mass and current GC exposure. No associations between adiponectin and bone turnover markers were observed in either group.

TABLE 14. Association of serum leptin with serum PINP in the Severe JIA Cohort and in healthy controls (an additive model). Data are given as standardized beta coefficients and p-values.

	Patients (N=44) [P1NP] beta	p	**Controls (N=89)** [P1NP] beta	p
[Leptin]				
Unadjusted	-0.132	0.394	**-0.519**	**<0.001**
Adjusted*	-0.143	0.186	**-0.202**	**0.011**
+ fat mass	**-0.513**	**0.004**	-0.130	0.219
+ lean mass	-0.371	0.048	-0.082	0.466
+ [GC]	-0.349	0.063		

Brackets for ln-transformed (non-normally distributed) variables. * adjusted for age (controls) or bone age (patients), gender and pubertal status. GC; current weight-adjusted GC dose. Statistically significant Bonferroni-adjusted p-values appear in boldface.

5.4.2 Association of adipokines with bone mineral density

The association between serum adipokines and whole-body BMD was similarly tested in an additive model after adjustments for gender, height, pubertal stage and fat mass (lean mass could not be added to the models because of multicollinearity). In the Severe JIA Cohort, an inverse association of leptin with whole-body BMD existed (beta coefficient -0.370, p=0.042; for Bonferroni correction, a required p-value here would be <0.01). The association attenuated (beta -0.298, p=0.117) when correction for bone age instead of pubertal stage was used. These results were not compared with controls because DXA bone data of the controls was not cross-calibrated. Interestingly, in the GC-treated Cohort, serum leptin concentration tended to be higher (median 8.2 vs. 5.3 ng/ml, p=0.064) in those with lumbar spine BMD ≤-1.0 SD than in those with higher BMD Z-scores. The result remained similar when adjusted for gender and fat mass (p=0.061). However, no association between leptin and BMD existed in the GC-treated Cohort or in their controls. Concerning adiponectin, no association with BMD appeared in those with severe JIA. At first sight, no association existed in the GC-treated Cohort either, but an inverse association was observed in their controls (beta -0.127, p=0.047). However, an inverse association between adiponectin and BMD appeared also in the GC-treated Cohort after adjustment for bone age instead of pubertal stage (beta -0.204, p=0.018).

5.4.3 Correlation between adipokines and disease activity

Leptin or adiponectin did not correlate with parameters of disease activity in either cohort.

6. DISCUSSION

JIA predisposes to alterations in body composition and bone health. Several contributing factors have been recognized. These include inflammatory cytokines, nutritional and hormonal factors, limited physical activity and treatment-related effects from GCs. Vertebral compressions indicate pathological skeletal fragility and significantly compromised bone health, but may go undiagnosed as they are often asymptomatic and may be present despite normal BMD. Increased fat mass may also have negative effects on bone health, and furthermore, adipose tissue may modify immunity and inflammatory reactions, and thus, disease activity, in rheumatic diseases. Fat-derived adipokines are suggested to play a key role. We investigated the prevalence, risk factors and diagnostics of spinal compression fractures in severe JIA. In addition, we evaluated body composition and the relationship of adipokines with bone turnover markers, BMD and disease activity in patients with JIA.

6.1 Fractures

Non-vertebral fractures are common in children; approximately every other boy and every third girl sustains one or more fractures during childhood (Landin 1983, Jones et al. 2002, Mäyränpää et al. 2010). Patients with childhood-onset arthritis have even more fractures than their healthy peers (Burnham et al. 2006a). As with healthy children, at a median age of 14.8 years, 30% of patients in our Severe JIA Cohort, with a female predominance, had sustained at least one peripheral fracture. Considering the severe disease course of the patients and the prevalence of fractures overall in children, this was less than expected. However, our findings may at least partly be related to limited opportunities for sports and especially high-risk activities, and it is likely that our patients with severe JIA were less exposed to injuries than healthy children. Fractures were uncommon also in the study by Valta et al. (2007), where only 6% of patients with JIA had a history of peripheral fractures. Since these patients were evaluated at a relatively young age (median 12.4 years), they may not have reached the pubertal peak in fracture incidence. A more reliable estimate of the total risk for childhood fractures in patients with JIA can be derived only from studies extending to young adulthood, with careful assessment of physical activity and trauma mechanisms.

Vertebral fractures are rare in healthy children, and except for those resulting from high-energy trauma, they are regarded as pathological fractures. Compression fractures may remain undetected, however, as they often are asymptomatic (Mäkitie et al. 2005, Valta et al. 2007, Nakhla et al. 2009, Rodd et al. 2012). Compression fractures appear in several paediatric disease states, such as haematological or oncological conditions, solid organ or stem cell transplantation, neurolomuscular disabilities and conditions with chronic systemic inflammation

such as inflammatory bowel disease and rheumatic diseases (Helenius et al. 2006, Kilpinen-Loisa et al. 2010, Laakso et al. 2012, Mäkitie et al. 2005, Taskinen et al. 2007, Valta et al. 2008 and 2009). In JIA, vertebral fractures have been detected, especially in systemic arthritis, but also in other JIA subtypes (Mäkitie et al. 2005, Valta et al. 2007, Nakhla et al. 2009, Huber et al. 2010). The vertebral fracture prevalence in our study, ranging from 22% to 28% depending on the cohort (refering to exclusion of those with poor thoracolumbar visibility on radiography) and imaging method, is the highest reported. This is probably due to the severe disease course in our selected patient population. Therefore, these results cannot be generalized to the overall JIA population. Furthermore, since most of our patients had been diagnosed before the era of biological drugs, which tend to be most effective in early disease, their disease course is likely to differ from that of patients diagnosed nowadays with similar disease. Our findings do, however, indicate the overall high risk for skeletal complications even during childhood in patients with JIA.

In our study, DXA-derived BMD was not a reliable indicator of bone health, as those with vertebral fractures had similar BMD Z-scores for LS and WB as those without fractures. Also others have observed a poor association between BMD and fractures (Goulding et al. 2000, Mäkitie et al. 2005, Valta et al. 2007, Nakhla et al. 2009). Mäyränpää et al. (2012) evaluated a cohort of otherwise healthy, but "fracture-prone" children, defined by their fracture history of two long bone fractures before the age of 10 years, three long bone fractures before the age of 16 years or one or more vertebral fractures at any age. These fracture-prone children had lower BMD values than healthy controls. In those with vertebral fractures, the mean lumbar spine BMD Z-score was also lower than in those without fractures but still within the normal range (-0.8), and whole-body BMD Z-score was even closer to zero (-0.3). Therefore, not only BMD, as determined by DXA, but also other characteristics of bone quality and strength seem to be important for fracture risk. Our study was cross-sectional and could not evaluate causal relationships, but our results support the idea that DXA alone is not an accurate method to assess fracture risk in children with JIA. At the fracture site, BMD may even be increased, and therefore, compressed vertebrae must be excluded from analyses. According to the current recommendations, DXA results should be corrected for body size (Crabtree et al. 2013). However, how these recommendations apply to children and adolescents with a chronic illness is unknown. Since many of our patients had abnormal bone age, and further, because compression fractures reduce height we decided to correct BMD results for bone age. However, it cannot be excluded that hand arthritis may have caused local advancement of growth and subsequently underestimation of BMD Z-scores in some patients. Concerning the severe disease course and high prevalence of compression fractures in our patients, even without correction for height, their DXA results were actually surprisingly good, again indicating that DXA has a limited value in evaluating bone health in this patient group.

Patients with compression fractures had higher cumulative three-year weight-adjusted GC dose than those without vertebral fractures, similar to earlier studies (Nakhla et al. 2009, Rodd et al. 2012). Multiple intra-articular GC injections may also have caused systemic effects in those with chronically active polyarthritis. Despite similar GC exposure, vertebral fractures have been less severe in patients with nephrotic syndrome than in those with paediatric rheumatic diseases (Rodd et al. 2012, Phan et al. 2014), emphasizing the deleterious effects of inflammatory cytokines on bone (Schett et al. 2010a,b). Correspondingly, in our study, patients with compression fractures had higher parameters of current disease activity than patients without fractures. Because GCs are used more frequently in those with active disease and because GCs tend to increase fat mass, it is difficult to conclude whether high GC dose, disease activity and BMI are independent risk factors. In logistic regression analysis, the most significant associations with compression fractures were observed with CHAQ and BMI Z-score. Since CHAQ measures functional disability, those with compression fractures are likely to be the ones with the most severe disease course. Further, our findings are in line with recent observations suggesting that obesity is not beneficial for bone health.

On spinal MRI, 28% of patients had vertebral fractures, which was more than seen by spinal radiography. Visibility on radiographs may be suboptimal, especially in the thoracic area and in obese persons, and MRI is able to show more detailed information about these changes (Sledge et al. 2001). This was observed also in our study. Similar to earlier observations, fractures occurred most often in the thoracic spine (Nakhla et al. 2009, Huber et al. 2010, Rodd et al. 2012). Patients with vertebral fractures on MRI tended to be more obese and to have higher cumulative weight-adjusted GC dose than those without fractures, but these differences were not statistically significant. No difference in disease activity parameters was observed between patients with and without fractures, in contrast to findings on radiography. There are various explanations for this discrepancy. Some patients were deemed to have vertebral fracture on radiographical analysis, but vertebral morphology did not fulfil the criteria for a compression fracture on MRI, and other patients' fractures could only be detected on MRI. The rather small size of our study group and the non-normal distribution with a large variability of disease activity parameters may also account for these differences.

6.2 Vitamin D and calcium

Somewhat surprisingly, those with compression fractures had reported higher daily intakes of vitamin D than those without fractures. This may reflect special attention to vitamin D intake in those with the most severe disease and high GC exposure. We observed a wide variation in reported daily intakes of calcium and vitamin D from diet and supplements, and some of the patients had received over three times more calcium and vitamin D daily

than recommended for healthy children at the time of the study. However, the evaluation of diet was limited since only 68% of patients completed the dietary recall, and it is possible that the reported dietary intakes of calcium and vitamin D are overestimated. Despite these high reported intakes, serum/plasma concentrations of calcium, phosphate and PTH were mainly normal. None of the patients had hypercalcaemia, and five patients had mild hypercalciuria. However, supranormal calcium intakes should be avoided and calcium preferably obtained from the diet, not only because it is more effective, but also because supplement use may be associated with increased cardiovascular risk and other adverse effects (Reid 2014).

Considering the high reported vitamin D intakes, rather high 25-OHD serum concentrations were expected, but vitamin D status was suboptimal (<50 nmol/l) in 38% (insufficient in 24% and deficient in 14%) of patients, and only one patient had a value over 80 nmol/l. High adiposity may contribute to these findings, possibly resulting from a reduced release of vitamin D stored in body fat (Misra and Klibanski 2013). However, our findings are in accordance with observations from the years 2007 to 2010 for 1351 Finnish children with chronic diseases, showing suboptimal 25-OHD values in 47% and deficiency (<37.5 nmol/l) in 29% and 11% during the winter and summer months, respectively (Holmlund-Suila et al. 2013). Especially adolescents show low values. Vitamin D requirements may be higher in chronically ill children than in healthy children, resulting from alterations in absorption and metabolism of vitamin D. There has been controversy and active debate on the optimal serum 25-OHD level and on the recommended daily intake and the extraskeletal effects of vitamin D. In 2011, two different recommendations were published. Similar to the earlier recommendation (Misra et al. 2008), the Institute of Medicine suggests that a 25-OHD concentration of >50 nmol/l is optimal for childhood growth and bone mass accrual (Ross et al. 2011). The Endocrine Society, in turn, recommends a higher concentration (>75 nmol/l) based on potential long-term health benefits (Holick et al. 2011). According to several studies, vitamin D insufficiency and deficiency are common among children and adolescents in Finland (Lehtonen-Veromaa et al. 2002, Pekkinen et al. 2012), and positive effects on bone mass accrual can be achieved with supplementation (Viljakainen et al. 2006). The Finnish National Nutrition Council Recommendations from 2014 have now increased the preferred daily intake of vitamin D for children and adolescents to 10 µg daily, which may not, however, be sufficient for all children (Pekkinen et al. 2012). Especially in children with a chronic disease and an increased risk for suboptimal bone health, higher intakes are probably needed. Obese subjects or those with malabsorption or medications affecting vitamin D metabolism, such as GCs, may need doses two to three times higher (Holick et al. 2011). It may thus be advisable to monitor serum 25-OHD concentration to optimize supplement requirements according to the serum concentrations attained.

6.3 Bone turnover markers

Earlier studies on bone turnover markers in JIA have reported mostly decreased levels of bone formation markers, often associated with active disease. More inconsistent results have been reported regarding resorption markers (Pepmueller et al. 1996, Lien et al. 2005). Contrary to our expectations, concentrations of bone formation markers did not differ between patients with severe JIA and healthy controls. However, the resorption marker ICTP was increased in patients, suggesting an imbalance in bone turnover. Since bone formation and resorption are tightly coupled, it is possible that increased bone resorption in patients leads to increased bone formation, and consequently, higher bone formation marker production. Since bone turnover markers reflect bone metabolism only over the short term, normal formation marker values may be related to relatively low current disease activity. Bone marker values did not correlate with disease activity in our patients, but studies on adults with RA have shown increased ICTP levels in association with disease activity and radiographic progression (Paimela et al. 1994). This marker reflects pathological matrix metalloproteinase-mediated bone resorption (Garnero et al. 2003), indicating that patients with severe disease do have pathological bone resorption.

6.4 Other findings on spinal magnetic resonance imaging

On MRI, we observed a variety of changes, including endplate irregularities (26%), anterior corner lesions (14%) and disc changes (46%) such as disc protrusion or prolapse (18%). The prevalence of these changes in our patients seemed high relative to the limited data in healthy paediatric populations, which mainly focus on the lumbar spine. Tertti et al. (1991) report lumbar disc degeneration in 26% of healthy 15-year-olds and in 38% of those with low back pain. Correspondingly, another study reports lumbar disc degeneration in one-third of 13-year-old children (Kjaer et al. 2005). Only 6% of these healthy children showed endplate changes, but a Finnish study observed endplate injury with disc degeneration in half of the children with previous traumatic vertebral wedge fractures (Kerttula et al. 2000). Probably resulting from recurrent trauma, male elite gymnasts in their thirties show a high prevalence (75%) of disc degeneration and a tendency for several other abnormalities of the thoracolumbar spine, e.g. Schmorl's nodes, apophyseal ring abnormalities and abnormal configuration of the vertebral body (Swärd et al. 1991). Endplate injuries predispose to compromised nutrition and loss of integrity of the avascular intervertebral disc, and thereby, to disc degeneration and possibly to disrupted growth and development of the adjacent vertebrae (Hilton et al. 1976, Swärd et al. 1991). The adolescent spine has growth cartilage areas and immature ossification centres that are susceptible to injury (d'Hemecourt et al. 2000). An area of relative weakness exists at the osteocartilagineous junction, especially before complete fusion of the ring apophysis with the vertebral body (Keller

1974, Swärd et al. 1991). The exact aetiology and significance of the anterior corner defects that we detected remain, however, unknown. Three of our patients with severe compression fractures had disc ballooning, but normative MRI data on the prevalence of ballooning are lacking. Lumbar radiographs have shown disc ballooning in 15% of healthy Japanese adolescents (Tsuji et al. 1984). In our patients, obesity was associated with endplate irregularities, but tended to also be related to other changes on MRI. In adults, obesity is associated with low back pain and disc degeneration, suggested to result from either increased mechanical loads or other mechanisms such as adipokines and inflammatory cytokines (Samartzis et al. 2013).

6.5 Body composition

Body composition has not been systematically evaluated in JIA. Earlier studies have reported a tendency for malnutrition (Lofthouse et al. 2002, Bechtold and Roth 2009). In systemic arthritis, the basal energy expenditure is increased (Knops et al. 1999), but protein catabolism, induced by cytokines, exposes also other subgroups to malnutrition. Although GCs tend to promote fat accumulation and weight gain, they also cause muscle wasting. In earlier DXA studies, systemic GC exposure was related to increased fat mass (Mul et al. 2002, Lien et al. 2005, Caetano et al. 2012) and decreased lean mass (Mul et al. 2002, Lien et al. 2005).

Recent studies report increased BMI or adiposity in JIA (Caetano et al. 2012, Pelajo et al. 2012, Grönlund et al. 2014). Obesity in our patients with severe JIA cannot be explained only by the global obesity epidemic since patients were clearly more obese than healthy controls. Potential contributing factors to the high fat mass include GC exposure and limited physical activity, but possible effects related to biological drugs cannot be excluded. Some adult studies have observed weight gain during anti-TNF therapy, and it is not known whether these drugs influence appetite or metabolism (Briot et al. 2008). However, the lean mass of our patients did not differ from that of controls. These unexpectedly normal values may at least partly result from a compensatory increase of lean mass with respect to high fat mass, but also methodological aspects may have a contributing role.

High adiposity is associated with an increased risk for cardiovascular diseases (Matsson et al. 2008). Obesity may be especially harmful in rheumatic diseases, which per se predispose to cardiovascular complications. In paediatric rheumatic diseases, cardiovascular issues are of concern, especially in juvenile systemic lupus erythematosus. However, signs of a clinically silent atherosclerotic process have been found post-mortem also in patients with JIA (Smith et al. 2013). Obese children with JIA show elevated systolic blood pressure, dyslipidaemia, insulin resistance and subclinical cardiovascular changes such as increased intima-media thickness and left ventricle mass (Glowinska-Olszewska et al. 2013). Therefore, attempts to avoid overweight and obesity in JIA and other chronic inflammatory

diseases should be encouraged to prevent cardiovascular complications. Although malnutrition seems not to generally be a major problem, it may affect a small subgroup of children. In addition to the disease itself, adverse effects of medication, such as methotrexate, sulfasalazine or non-steroidal anti-inflammatory drugs, may cause gastrointestinal symptoms and poor appetite. Despite the lack of data concerning the effects of nutritional supplementation on JIA, nutritional counselling and supplementation should be considered.

Given that increasing obesity is a worldwide phenomenon also in the healthy paediatric population, results from our GC-treated Cohort with mostly relatively low-dose GC exposure (in those with current GC, mean daily dose was 2.9 mg or 0.08 mg/kg) but normal growth, BMI and body composition are encouraging, highlighting the importance of rigorous disease control. Low GC dosages and an alternate-day regimen probably contribute to these favourable findings (Hochberg 2002), although no threshold for safe GC dosing is known. The slight decrease of bone mass may not necessarily have a clinically relevant impact on peak bone mass. Nevertheless, even though the cumulative GC exposure did not correlate with bone mass, an inverse correlation between the current weight-adjusted GC dose and BMD was observed. These findings emphasize the importance of avoiding systemic GC use, which nowadays is possible due to modern treatment modalities.

6.6 Relationship between adipose tissue and bone

Several observations in animal studies and clinical studies suggest a connection between adipose tissue and bone, which may be mediated partly via adipokines. In our study, patients with severe JIA had not only increased fat mass but also significantly higher serum leptin concentration, even independently of fat mass. In controls, the association between leptin and bone turnover markers attenuated when adjusted for fat mass, while in patients the association became stronger. Results were not significant when fully adjusted, possibly because of the rather small sample size, but were clearly different between patients and controls. These findings may be linked to a higher degree of obesity-related leptin resistance in patients, but also to leptin production at sites other than adipose tissue, even in joint tissues (Scotece et al. 2014). Further, our results imply that through other mechanisms in addition to increased fat mass leptin may contribute to suppression of bone turnover in JIA. Similar to studies in healthy children, no association between adiponectin and bone turnover markers emerged (Dimitri et al. 2011, Flemming et al. 2012). Because other studies have not evaluated the relationship between adipokines and bone turnover markers in JIA or other paediatric rheumatic diseases, these preliminary data offer a basis for further research.

The relationship between adipokines and bone mass was primarily evaluated in the GC-treated Cohort in comparison with controls. No association between

leptin and BMD was observed in either group. However, in the Severe JIA Cohort, an inverse association emerged, implying that leptin may have a negative effect on bone mass accrual in severe disease. This finding is also in line with the aforementioned tendency for an inverse association between leptin and bone turnover markers. Results from other studies on healthy lean or obese children regarding the relationship between leptin and bone mass are variable (Roemmich et al. 2003, Afghani and Goran 2009, Hong et al. 2010, Russell et al. 2010). More consistent results have been reported regarding the relationship between adiponectin and bone mass since, similar to adults, observations show either an inverse association or no association (Hong et al. 2010, Russell et al. 2010, Sayers et al. 2010). Consistent with this, we noted an inverse association between adiponectin and whole-body BMD in healthy controls, while in the GC-treated Cohort the association appeared only after correcting for bone age instead of pubertal stage. In those with severe JIA, no association existed. Disease- and treatment-related factors may have affected the results.

6.7 Relationship between adipose tissue and disease activity

Some studies report an association between obesity and disease activity or poor treatment response in adults with RA (Ajeganova et al. 2013, Sandberg et al. 2014). Adipokines are suggested to play a role in immunity and inflammatory reactions in rheumatic diseases. Increased serum concentrations of leptin and adiponectin have been detected in adults with RA, often in association with increased disease activity (Gomez et al. 2011). Data are lacking regarding these issues in JIA. We did not observe a correlation between leptin or adiponectin and disease activity in patients with JIA, but this observation is limited because of low disease activity, especially in the GC-treated Cohort. However, studies in adults suggest that these adipokines may be contributing factors to or markers of pathogenesis and disease activity in rheumatic diseases and are even considered as possible drug targets (Scotece et al. 2014). Further studies on these and other adipokines in children with JIA are therefore warranted.

6.8 Limitations of the study

Some limitations in our studies, related to either patient cohorts or applied methodologies, are noteworthy.

1) Study cohorts: The cross-sectional study design does not allow evaluation of causality, an issue for future research. The rather small study populations may have limited us from observing some significant findings and differences between the groups, did not allow comparison of girls and boys at different stages of pubertal maturation and caused limitations in correcting for confounding factors. As the Severe JIA Cohort represented a selected population, the results are not generalizable to the whole JIA population. However, we evaluated compression fractures, which are more likely to appear in severe disease, and

our results provide valuable data for the evaluation of bone health in patients with a severe disease course.

2) Methodological aspects: DXA may have overestimated fat mass in the Severe JIA Cohort with high adiposity since increasing tissue depth and variation in fat distribution interfere with measurement and result in greater bias (Williams et al. 2006). Other factors, including age, sex, body size and disease state, may have also caused bias in DXA body composition analysis. We were unable to differentiate between visceral and subcutaneous fat; recent data suggest that effects from visceral and subcutaneous fat deposits on bone may be the opposite (Gilsanz 2009); this warrants evaluation in future studies. The exact amount and intensity of physical activity could not be compared between patients and controls.

6.9 Future considerations

Despite advancements in medical therapy of JIA, compromised bone health remains a significant concern, especially in children with active and treatment-resistant disease. Clinicians need to be aware of potential severe skeletal complications, including compression fractures. Guidelines are needed for assessment of bone health in clinical settings. According to a recent suggestion, paediatric patients with high risk for vertebral fractures should be screened annually with thoracolumbar radiography (LeBlanc et al. 2015). Despite limitations, DXA remains the most widely used technique to assess bone mass in clinical practice due to its good availability, low radiation dose and low cost. Modern DXA devices also allow vertebral fracture assessment, but its use is limited in children due to compromised visibility and poor diagnostic accuracy (Mäyränpää et al. 2007). However, peripheral computed tomography and other modern techniques enable the evaluation of bone geometry and bone strength, which are more accurate than DXA in detecting the mechanical competence of bone against fractures (Griffith et al. 2010). Guidelines are needed for effective treatment, including use of bisphosphonates, in secondary osteoporosis. Optimal vitamin D status and characterization of the role of vitamin D in rheumatic diseases are topics for future research.

More accurate methods for body composition analysis in children are needed, especially for children with low or high fat content. To avoid misinterpretations, the terminology should be unambiguous. Longitudinal studies on body composition in JIA would be valuable. Longitudinal studies are also needed to evaluate the mechanisms and determinants of the fat-bone relationship through puberty and into adulthood, both in healthy children and in children with chronic disease. The role of brown adipose tissue is interesting because of suggested anabolic effects for bone (Ponrartana et al. 2012). Leptin and adiponectin affect bone metabolism through several mechanisms, but because of conflicting results their exact roles need to be elucidated. The role

of adipokines in JIA and other paediatric rheumatic diseases also warrants further studies. In addition to leptin and adiponectin, other adipokines, such as resistin and visfatin, are interesting candidates because these proteins have been suggested to have a role in the pathogenesis of RA (Scotece et al. 2014). Similarly, the possible contributing role of obesity and adipokines in vertebral changes should be investigated, as should the prevalence of these changes in the paediatric population and their significance in further morbidity.

As bones and body composition are constantly prone to changes while adapting to current requirements, the maintenance of a healthy life-style, optimal nutrition and continued physical activity should be encouraged in growing children and adolescents in order that they attain maximal peak bone mass and keep bones as strong as possible also in later life. These should also be targets for patients with JIA; adequate information and encouragement and individually tailored exercise programmes should be provided to prevent metabolic and cardiovascular complications and to ensure overall mental and physical well-being.

7. CONCLUSIONS

Several important conclusions can be drawn:

1. Patients with severe JIA have a high prevalence of vertebral fractures, indicating significantly compromised bone health. As vertebral fractures are often asymptomatic, screening with thoracolumbar radiography should be considered for patients with significant risk factors such as high GC exposure, sustained elevated disease activity and possibly obesity.

2. BMD measured with DXA does not reliably separate patients with and without compression fractures.

3. MRI is suitable for detection of compression fractures. MRI also provides information about vertebral endplates and intervertebral discs, which are often affected in patients with severe JIA; high BMI may be one of the predisposing factors.

4. Opposite to healthy controls, leptin tended to be associated inversely with bone turnover markers in patients with severe JIA, suggesting that it may be a contributing factor for or a marker of decreased bone turnover in JIA.

5. Patients with severe JIA have increased serum ICTP, suggesting pathologically increased bone resorption and imbalanced bone turnover.

6. Patients with severe JIA are prone to develop obesity. By contrast, in patients with less severe disease course, medication will usually ensure low disease activity and maintenance of normal BMI and body composition, despite GC exposure.

7. Body composition does not differ between patients with low and normal lumbar spine BMD, when corrected for bone age.

8. Leptin is not associated with BMD in children with JIA, although an inverse association may exist in those with severe disease, suggesting that leptin contributes to bone health in severe JIA.

9. Serum leptin or adiponectin concentrations are not related to disease activity in patients with JIA.

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