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Author: Moray J. Campbell

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Author: Marie B. Demay

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Highlights from the 21th Workshop on Vitamin D in Barcelona, May 2018

James C. Fleet¹, Moray J. Campbell², Carsten Carlberg³, Marie B. Demay⁴, Peter R. Ebeling⁵, Inez Schoenmakers⁶, John White⁷, JoEllen Welsh⁸, *Martin Hewison⁹

¹Department of Nutrition Science, College of Health and Human Sciences, Purdue University, West Lafayette, IN, 47907-205, USA. Electronic address: fleet@purdue.edu

²Pharmaceutics and Pharmaceutical Chemistry, College Of Pharmacy, Ohio State University, 536 Parks Hall, Columbus, OH, 43210, USA. Electronic Address: campbell.1933@osu.edu

³School of Medicine, Institute of Biomedicine, University of Eastern Finland, POB 1627, FI-70211 Kuopio, Finland. Electronic address: carsten.carlberg@uef.fi

⁴Endocrine Unit, Massachusetts General Hospital and Harvard Medical School. 50 Blossom St, Thier 11, Boston, MA 02114, USA. Electronic address: demay@helix.mgh.harvard.edu

⁵Peter Ebeling, Department of Medicine, Monash University, Melbourne, VIC 3800, Australia. Electronic address: Peter.Ebeling@monash.edu

⁶Department of Medicine, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich NR4 7TJ, UK. Electronic address: I.Schoenmakers@uea.ac.uk

⁷Departments of Physiology and Department of Medicine, McGill University, 3655 Drummond Street, Room 1112, Montreal, QC H3G 1Y6, Canada. Electronic address: john.white@mcgill.ca

⁸University at Albany Cancer Research Center, 1 Discovery Drive Suite 304D, Rensselaer, NY 12144, USA. Electronic address: j.welsh@albany.edu

⁹Institute of Metabolism & Systems Research, Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Level 2, IBR, Rm 225, The University of Birmingham, Birmingham, B15 2TT, UK. Electronic address: m.hewison@bham.ac.uk

*Author for correspondence:

The 21st Workshop on Vitamin D was held at the Centre de Convencions Internacional de Barcelona (CCIB) from May 16-19, 2018. The Workshop was organized by **Dr Martin Hewison** (Chair, University of Birmingham, Birmingham, UK) with the invaluable support of **Dr JoEllen Welsh** (CFO, SUNY Albany, USA) and **Roxanne Hall** (Meetings Plus). Invited presentations and topical sessions were chosen by the Workshop Executive Committee, with

input from the Program Advisory committee (<http://www.vitamindworkshop.org>). The Workshop, which attracted 252 attendees from 41 countries, began the evening of May 16 with an opening reception and 24 Plenary Poster presentations. The following three days were filled with exciting science, divided into 12 sessions of symposium and promoted abstract oral presentations covering topics in basic science, clinical research and epidemiology, 158 poster presentations. Delegates who presented at the Workshop were invited to submit manuscripts for peer reviewed publication in this special edition of the Journal of Steroid Biochemistry and Molecular Biology. Each submitted manuscript was peer reviewed according to Journal standards. Guest editors were **Dr Martin Hewison** (University of Birmingham, UK) and **Dr James Fleet** (Purdue University, USA). Topical summaries of all oral sessions are presented below, and include links to manuscripts accepted for publication in the special edition of the Journal of Steroid Biochemistry and Molecular Biology.

The first day started with opening remarks by the Chair, **Dr Martin Hewison**, followed by **Session I** entitled the **Year in Vitamin D: Basic and Translational** chaired by **Dr Carsten Carlberg** (University of Eastern Finland) and presented by **Dr Marie Demay** (Harvard Medical School, and Massachusetts General Hospital). Dr Demay provided an excellent overview of some of the key publications within the vitamin D field in the years 2017 and 2018, focusing primarily on basic science research. She started with a report that vitamin D receptor (VDR) regulates microbiota-dependent inflammation of gut mucosa by suppressing the apoptosis of intestinal epithelial cells, i.e. VDR plays a significant role in attenuating the inflammatory response in the intestine [1]. Another paper showed that the cytokines IL12 and IL18, which synergize to promote T_H1 cell differentiation, trigger anti-microbial response against tuberculosis up-regulate VDR gene expression and induce in this way the anti-microbial VDR target genes *CAMP* and *DEFB4A* in macrophages [2]. In contrast, schistosomiasis-induced liver fibrosis is promoted, when the miRNA miR351 down-regulates VDR expression [3]. Thus, interferon γ -mediated down-regulation of miR351 and/or vitamin D analog-stimulated VDR activation may be an effective treatment of the worm-induced liver fibrosis. miR675, which co-locates with the *H19* gene encoding for a long ncRNA, is another miRNA that decreases VDR expression. However, in colon cancer cells VDR is not repressed, when the biologically active form of vitamin D, $1\alpha,25$ -dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), down-regulates the *MYC* gene, which encodes for a transcription factor prominently up-regulating the *H19* gene [4]. In renal cell carcinoma cell lines VDR suppresses proliferation and metastasis via affecting the expression of the *TRPV5* gene encoding for a calcium-selective channel [5]. In multiple myeloma cells $1,25(OH)_2D_3$ can induce an osteoclast-like phenotype via the induction of cytoskeleton remodeling and up-regulation myeloid and osteoclast markers [6]. This reinforces the bone erosive activity of multiple myeloma cells. Dr Demay also discussed a paper

describing that VDR associates in a ligand-dependent manner with nuclear matrix proteins via the hinge region between its DNA-binding and ligand-binding domain [7]. Therefore, during interphase VDR locates in the nucleus but during mitosis in the cytoplasm. A large pharmacore docking screen identified a novel non-steroidal VDR agonist showing potent cardio-protective effects while being devoid of hypercalcemia [8]. Another non-steroidal VDR agonist showed to significantly inhibit chronic pancreatitis *in vivo* without increasing serum calcium levels [9]. Dr Demay showed special interest in an article reporting the knockout of the 25-hydroxylase *Cyp2r1* in zebrafish [10], which resulted in decreased somatic growth and obesity. The results of this study suggested that vitamin D signaling promote fatty acid oxidation by inducing the expression of the co-activator Pgc1a. The topic “metabolism” was covered by a paper indicating that the vitamin D compounds, 1 α -hydroxyvitamin D₃, 1 α -hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ (25(OH)D₃) lower cholesterol *in vivo* in hypercholesterolemic mice via the activation of 1,25(OH)₂D₃ [11]. Finally, Dr Demay discussed a report on the effects of early life vitamin D exposure on later skeletal health [12]. A mouse model system was used to demonstrate that early life vitamin D depletion results in abrogation of the response to mechanical loading, with consequent reduction in bone size, mass and strength during both childhood and adulthood.

The subject of **Session II** was **Vitamin D and Bone Disease** and was chaired by **Dr Roger Bouillon** (Katholieke Universiteit Leuven). **Dr Wolfgang Högler** (University of Birmingham) gave an excellent overview of the increasing problem of nutritional rickets. He discussed how many governments fail to adjust prevention programs for rickets in relation to the changes in population demographics. Indeed, people with dark skin are immigrating into countries with high latitude, resulting in low production of vitamin D. This vitamin D deficiency results in rickets and hypocalcemic complications with even cardiac deaths in infants. Infantile vitamin D supplementation programs are thus essential and monitoring of supplementation is needed [13]. **Dr Paul Anderson** (University of South Australia) discussed several novel aspects of vitamin D metabolism in mature osteoblasts. The focus was especially on the role of local synthesis of vitamin D in these cells. Two complementary conditional transgenic mouse models were used, one with inactivation of *Cyp27b1*, the other with overexpression of the gene. Both models came to the same conclusion, indicating that *Cyp27b1* activity in osteoblasts promotes bone formation. Adequate serum 25(OH)D₃ levels are thus required for bone health [14]. **Dr Jeffery Roizen** (Children’s Hospital of Philadelphia) described a novel genetic form of vitamin D deficiency in two patients with early-onset rickets, with a mutation in *CYP3A4* resulting in a 10-fold greater enzyme activity. The enzyme activity of *CYP3A4* was even higher activity than of *CYP24A1* (24-hydroxylase) in catabolizing vitamin D metabolites, leading to enhanced elimination of vitamin D. **Dr Lieve Verlinden** (Katholieke Universiteit

Leuven) discussed the role of VDR signaling in osteocytes during lactation. Mice with inactivation of VDR in osteocytes have normal bone and calcium homeostasis. During lactation however, bone loss was smaller in the mutant lactating mothers compared to wild-type. This decreased calcium release from bone in the mother had no effect on bone growth or mass in the offspring. These findings suggest compensation by other organs, although no major effects were observed in the intestine or kidney. Thus, VDR signaling in osteocytes contributes to the bone loss observed during lactation. **Dr Arnaud Molin** (Caen University Hospital) presented that in a cohort of patients with hypercalcemia and suspected *CYP24A1* deficiency, they tried to find differences in the biochemical data that would reflect mutations in *CYP24A1* rather than in renal sodium-phosphate co-transporters *SCL34A1* and *SCL34A3*, which can give a similar clinical phenotype. Patients with *CYP24A1* and *SLC34A1* mutations had similar biochemical phenotypes, but patients with *SLC23A3* mutations showed lower serum calcium and phosphate levels.

Session III focused on **Bioinformatics** and was chaired by **Dr John White** (McGill University Montreal) and **Dr Martin Tenniswood** (Albany University New York). As with all fields, genomic technologies and approaches are applied frequently to understand the actions of the VDR) in terms of where it binds, what genes are regulated and how this changes across cell types and disease states. This session illustrated where progress is being made, and challenges remain. Firstly, **Dr André G. Uitterlinden** (Erasmus Medical Center, Rotterdam) gave an overview of what Genome Wide Association Studies (GWAS) can reveal about how genetic variation impacting vitamin D signaling is detectable in large cohorts. For example, Dr Uitterlinden is the PI of a large cohort of 20,000 elderly patients (the Rotterdam Cohort) in which GWAS approaches have been applied to test associations between aspects of vitamin D signaling, for example serum levels of 25(OH)D₃ and the pre-disposition of aging-related syndromes including those related to bone health. Genomic approaches are also being applied to cell-base studies with the goal to understand the major or most significant pathways identified. Next, **Dr Susan Coort** (Maastricht University) gave an overview of the challenges in pathway analyses and illustrated how software her group has developed can be used to illustrate and identify the significant pathways. For example, her group and colleagues have developed tools including WikiPathways and PathVisio to help identify the important components of activated pathways in response to vitamin D treatments [15].

Bioinformatic and analytical approaches to better understand different aspects of VDR signalling were then presented. **Dr Carsten Carlberg** (University of Eastern Finland) addressed the time-dependent nature of vitamin D signaling and has used so-called machine learning to integrate multiple high-dimensional data sets (e.g. receptor binding, changes in

histone modifications and changes in gene expression) to identify sub-sets of relationships that support a choreographed aspect to VDR signaling that is integrated with other transcription factors to bring about complex phenotypes such as cell differentiation [16]. Finally, **Dr Moray Campbell** (Ohio State University) examined differences between African American and European American prostate cancer patients in terms of which patients had stable disease, compared to those whose disease progressed. These studies revealed key roles for several microRNAs, uniquely in African American patients, and integration with publicly available data strongly supported roles for VDR to be involved in the regulation of these genes.

In **Session IV** the workshop addressed recent developments in **Vitamin D and Cancer** chaired by **Dr George Studzinski** (Rutgers, New Jersey Medical School) and **Dr JoEllen Welsh** (Albany University New York). **Dr Alberto Muñoz** (Instituto de Investigaciones Biomédicas, CSIC-UAM and CIBERONC Madrid) provided a state-of-the-art overview of the actions of vitamin D in colon cancer [17]. Dr Muñoz began by highlighting the epidemiology linking vitamin D-deficiency with the incidence and mortality of colon cancer. A role for vitamin D in colon cancer is further supported by the fact that VDR is abundantly expressed in cells from the gastrointestinal tract. However, it is important to recognise that levels of VDR (and also the vitamin D-activating enzyme CYP27B1) may be down-regulated in colon cancer tissue, whilst catabolic enzymes such as CYP24A1 are up-regulated. The over-arching effect is to promote resistance to $1,25(\text{OH})_2\text{D}_3$ in colon cancer. Despite this, studies *in vitro* have shown that $1,25(\text{OH})_2\text{D}_3$ inhibits the proliferation of colon cancer cells and the role of the Wnt/ β -catenin pathway in this process was highlighted, with $1,25(\text{OH})_2\text{D}_3$ acting to inhibit elevated Wnt/ β -catenin signalling in colon cancer. Dr Muñoz also described recent studies in which organoids using cells from colon cancer patients have been used to demonstrate the anti-neoplastic effects of $1,25(\text{OH})_2\text{D}_3$. Likewise, a key new facet of vitamin D action in colon cancer is its role in mediating anti-inflammatory responses against infiltrating immune cells in colon cancer. Finally, Dr Muñoz highlighted the growing interest in a role for vitamin D in mediating gastrointestinal responses to vitamin D and the role that this may play in the initiation and progression of colon cancer.

Dr Gilles Laverny (IGBMC-CERBM GIE) described the role of vitamin D in controlling prostatic pre-cancerous lesions. The presentation focused on phosphatase and TENsin homolog (PTEN) as the most frequently mutated or deleted tumor-suppressor in prostate cancer. Mice with prostatic deletion of the *PTEN* gene in adults show develop prostatic intraepithelial neoplasia (PIN) within a month following PTEN knockout. When crossed with VDR knockout mice the *PTEN* knockout mouse showed enhanced prostate size and PIN,

indicating that VDR signalling impacts PIN progression. Conversely, treatment of *PTEN* knockout mice with a $1,25(\text{OH})_2\text{D}_3$ analog reduced proliferation and increase apoptosis of PIN cells. **Dr John White** (McGill University Montreal) described studies of vitamin D and the tumor-suppressor and E3 ligase FBW7. $1,25(\text{OH})_2\text{D}_3$ rapidly induces VDR-FBW7 interaction with c-MYC, promoting proteasomal degradation of c-MYC on DNA. Similar observations were also made for other FBW7 targets such as cyclin E, c-Jun and MCL1. FBW7 depletion diminished the anti-proliferative effects of $1,25(\text{OH})_2\text{D}_3$ via an apparent inhibition of DNA-binding of liganded VDR, demonstrating a novel mechanism by which vitamin D can exert anti-cancer responses.

New data on the epidemiology of vitamin D and cancer were presented by **Dr Fiona O'Sullivan** (Trinity College Dublin) who described studies of a cohort of samples from the UK Biobank ($n=500,000$). Internet based emissions monitoring was used to calculate the UVB exposure of each participant. When comparing highest to lowest tertile of UVB exposure, there was decreased risk of colorectal, breast and lung cancer, indicating that there was an inverse correlation between UVB radiation exposure and risk of common cancers, with vitamin D being likely factor in this association. The final presentation in Session IV explored the potential use of a $1,25(\text{OH})_2\text{D}_3$ analog, (Inecalictol), to treat aggressive, triple-negative breast cancer (TNBC). **Dr Justine Vanhevel** (Katholieke Universiteit Leuven) described how in TNBC cells *in vitro*, Inecalictol potently suppressed cell proliferation and this effect was enhanced in combination with a CDK4/6 inhibitor Palbociclib. Thus combination therapy with Palbociclib and Inecalictol may provide a novel treatment strategy for TNBC.

The first full day of the meeting ended with **Session V** focused on **Vitamin D, Immunology, and Respiratory Disease** chaired by **Dr Margherita Cantorna** (Pennsylvania State University) and **Dr Carlos Bernal-Mizrachi** (Washington University). **Dr Adrian Martineau** (Queen Mary University London) provided an introduction to the antimicrobial properties of vitamin D, including the induction of antibacterial proteins, autophagy and intracellular iron-regulatory pathways by innate immune cells such as macrophages. The importance of these mechanisms in mediating anti-microbial responses to vitamin D was further underlined by localised conversion expression of the enzyme 1α -hydroxylase in monocytes and macrophages, thereby enabling antimicrobial responses to $25(\text{OH})\text{D}_3$ with this effect being compromised under conditions of vitamin D-deficiency. Dr Martineau described recent meta-analyses for the effect of vitamin D on acute upper respiratory infection. By using disaggregated individual participant data from multiple vitamin D supplementation studies from around the world, it was possible to show that vitamin D supplementation decreased acute respiratory infections. Notably this anti-infection response to vitamin D supplementation was

most pronounced in individuals who were vitamin D-deficient (< 25 nmol/L serum 25(OH) D_3) before treatment, and in those who did not receive large bolus doses ($>30,000$ IU) of vitamin D supplementation [18]. At a mechanistic level, Dr Martineau pointed out that while vitamin D was an effective anti-bacterial agent for respiratory pathogens such as *Mycobacterium tuberculosis* (*M. tb.*), the causative organism for tuberculosis. However, it appears that *M. tb* itself can corrupt antibacterial responses to vitamin D by stimulating dysregulation of vitamin D metabolism in target cells such as macrophages.

Dr Wim Janssens (Katholieke Universiteit Leuven) described the link between vitamin D and chronic obstructive pulmonary disease (COPD). Although COPD is strongly associated with cigarette smoking, the initiation and development of COPD has also been linked to vitamin D-deficiency. This is due to the fact that morbidity and mortality from COPD involve repeated airways infection, inflammation and skeletal muscle dysfunction, all of which may be influenced by vitamin D. In particular, antibacterial and anti-inflammatory responses to serum 25(OH) D_3 by cells from the immune system provide a mechanistic basis for vitamin D supplementation in COPD patients. Epidemiology has linked low serum 25(OH) D_3 status, with impaired lung function, risk of COPD and accelerated lung function decline. Recent vitamin D supplementation trials have demonstrated clinically important risk reductions for acute COPD exacerbations, particularly for those patients who were vitamin D-deficient prior to supplementation. The connection between COPD and other diseases such as lung cancer and cardiovascular disease means that new strategies to manage vitamin D-deficiency in COPD patients are needed.

Dr Carsten Carlberg (University of Eastern Finland) detailed the importance of epigenetics in mediating immune responses to vitamin D. In a study carried out *in vivo* using healthy adults supplemented with a single bolus dose of vitamin D (80,000 IU) showed that that rise in serum 25(OH) D_3 by in average 15 nmol/L was sufficient for statistically significant alterations in chromatin accessibility at some 850 genomic loci after only 1 day of supplementation [19]. Notable clusters for vitamin D supplementation-induced chromatin modifications were in the human leukocyte antigen (HLA) region of chromosome 6. **Dr Richard Mellanby** (University of Edinburgh) described studies in which his team had explored the link between vitamin D-deficiency and autoimmune diseases such as multiple sclerosis. They showed that a key role of vitamin D is to modulate the priming of naïve T cells via actions on the dendritic cells that present antigen to T cells. Using bone marrow-derived dendritic cells they identified 7 genes that were regulated by 1,25(OH) $_2D_3$ in the presence of absence of the immunogen lipopolysaccharide. This groups of genes included CD31, a member of the immunoglobulin superfamily. CD31 gene knockdown of increased the ability of 1,25(OH) $_2D_3$ -treated dendritic

cells to prime T cell activation, indicating that CD31 was a crucial component of the mechanism by which $1,25(\text{OH})_2\text{D}_3$ is able to regulate antigen presentation and T cell priming via dendritic cells.

Dr Jef Serré (Katholieke Universiteit Leuven) further expanded the earlier discussion of vitamin D and COPD by describing studies of the impact of vitamin D-deficiency and cigarette smoke exposure on lung inflammation and bacterial clearance after acute infection with Non-typeable *Haemophilus influenzae* (NTHi) using a mouse model. The interesting observation was that, independent of cigarette smoke, bacterial clearance in NTHi mice was improved with vitamin D deficiency. The proposed mechanism for this effect was through enhanced inflammation and resulting stimulation of antibacterial proteins, or via increased breakdown of extracellular matrix [20]. In the final presentation of this session, **Dr David Jolliffe** (Queen Mary University London) completed the series of presentations on vitamin D and COPD by describing a systematic review and meta-analysis using individual participant data. Vitamin D supplementation did not affect the overall rate of moderate/severe exacerbations of COPD. However, pre-specified sub-group analysis showed that significant protective effects of vitamin D supplementation were observed in COPD patients with baseline serum $25(\text{OH})\text{D}_3$ of less than 25 nmol/L, but not those above 25 nmol/L.

The second full day of the meeting began with **Session VI on Vitamin D and Nutrition**, chaired by **Dr Inez Schoenmakers** (University of East Anglia) and **Dr Kevin Cashman** (University of Cork, Ireland). **Dr Mairead Kiely** (University College Cork) represented the ODIN consortium (Food-based solutions for optimal vitamin D nutrition and health throughout life). Her presentation focused on integration of ODIN results using its specialized food composition dataset for vitamin D, dose-response data from RCTs with vitamin D and vitamin D fortified and bio-fortified foods and data from 10 nationally representative dietary surveys from 4 European countries. These data were used to conduct modelling experiments to model and demonstrate the feasibility of achieving vitamin D intakes according to recommendations without increasing the risk of excessive intakes. Further modelling with different thresholds of 25OHD , considered country-specific UVB availability and the plasma $25(\text{OH})\text{D}_3$ response to vitamin D intake, showed how (bio) food fortification strategies may be applied to increase vitamin D status across the population and prevent vitamin D deficiency without reaching toxic levels of $25(\text{OH})\text{D}_3$. **Dr Jakob Linseisen** (Ludwig-Maximilians-Universität, München and IARC-WHO, Lyon) presented data from the European Prospective Investigation into Cancer and Nutrition (EPIC) consortium. He addressed the complexity of the dietary assessment across different countries. He reported a North (high) – South (low) gradient in vitamin D intake from both food and supplements. Dr Linseisen critically reviewed the apparent conflicting

associations between disease outcomes and dietary vitamin D intake versus plasma 25(OH)D₃ concentrations found in observational studies. He acknowledged that this may be due to the limited influence of dietary intake of vitamin D on vitamin D status. He called for meta-analyses of observational studies assessing the relationship between vitamin D intake and status and disease outcomes, particularly for those for which a RCT design is unsuitable.

Dr Nick Shaw (University of Birmingham) presented on behalf of the British Paediatric Surveillance Unit Nutritional Rickets Surveillance Group (BPSU). He described the results of an in-depth investigation standardized according to BPSU criteria of cases of nutritional rickets (n=125) identified through surveillance amongst 3500 pediatricians in the UK and Ireland. The national estimated annual incidence was 0.48 cases per 100,000 children under 16 years, with the majority presenting under the age of 5 years (annual incidence 1.39 per 100,000). The incidence was higher in boys than girls and the majority were of Black and South Asian ethnicity. The majority of affected children did not meet the recommended intake of vitamin D or receive supplementation. Also, a number of non-vitamin D deficient cases were identified, in which calcium deficiency was suspected. Dr Shaw called for a better UK health policy to increase the uptake of vitamin D supplement use and adequate calcium intake to prevent nutritional rickets. **Dr Javeria Saleem** (University of the Punjab, Lahore) on behalf of an international consortium from London, UK and Auckland, New Zealand) presented the results of a 8-week randomized intervention study with adjunct administration of vitamin D₃ (200,000 IU at 2 and 4 weeks) or placebo with standard treatment of children aged 6-58 months with uncomplicated severe acute malnutrition in Pakistan. Although no effect on the primary outcome was found (the proportion of participants gaining >15% of baseline weight after 8 weeks of treatment), co-administration of vitamin D increased weight-for-height gain and reduced the proportion of children with indices of delayed development. **Dr Jette Jakobsen** (National Food Institute, Technical University of Denmark) presented results from a randomized cross-over intervention study with 10 µg/day vitamin D₃, 25(OH)D₃ or vitamin D₂ on 25(OH)D levels in serum. The design allowed the elimination of inter-individual differences on the dose-response and showed significant differences in the 6-week increase in serum 25(OH)D between different forms (compared to vitamin D₃, vitamin D₂: 0.44 and 25(OH)D₃: 1.5).

Dr Hajar Mazahery (Massey University, New Zealand, on behalf of an international consortium from Australia and Harvard University) presented data from the VIDOMA trial evaluating the effects of 1-year supplementation with Vitamin D, Omega-3 or a combination of Vitamin D and Omega-3 compared to placebo on symptoms of Autism Spectrum Disorder (measured as irritability and hyperactivity scores). The irritability scores significantly

decreased in all treatment groups whereas the hyperactivity score only significantly decreased in the group given vitamin D. Analyses of post-supplementation plasma concentrations reflecting the response to the intervention (the plasma Omega-3 index and 25(OH)D₃) suggested a relationship between the achieved concentrations and irritability and hyperactivity scores.

Session VII on Non-Calcemic Target Tissues for Vitamin D was chaired by **Dr Glenville Jones** (Queen's University Ontario) and **Dr Marie Demay** (Massachusetts General Hospital) and was opened by **Dr Darryl Eyles** from the University of Queensland in Brisbane, who provided an excellent overview of our current understanding of vitamin D signaling in the developing brain. Dr Eyles has studied vitamin D metabolism in the brain and the effects of hormonal vitamin D on several aspects of neuronal function using cell and animal models. Recent work has focused attention on how vitamin D signaling vitamin D regulates the synthesis, elimination and signaling of dopamine in developing and adult brains. This was followed by a talk by **Dr Maria Tamayo** (Universidad Autonoma de Madrid, Spain) on the beneficial effects of the vitamin D analogue paricalcitol in a setting of cardiac dysfunction induced aortic constriction in mice. Evidence was provided that paricalcitol had beneficial effects in this model by improving cardiac function and by reversing left ventricular remodeling that occurs during heart failure. Normalization of K⁺ currents was also observed in paricalcitol-treated animals. **Dr Visalin Nair-Shalliker** (University of Sydney) presented a talk on a clinical study examining the association between post-diagnosis plasma levels of both 25(OH)D₃ and 1,25(OH)₂D₃ and overall survival of prostate cancer patients in a large Australian cohort, PCOSun. The study found that plasma 1,25(OH)₂D₃ levels interacted significantly with disease aggressiveness. Risk of death in men with aggressive prostate cancer was lowest in those with 1,25(OH)₂D₃ levels >106 pmol/L (HR= 0.26; 95%CI: 0.11-0.59). The study concluded that high plasma post-diagnostic vitamin D levels is associated with longer overall survival in men diagnosed with aggressive prostate cancer. **Dr Natalia Carillo-Lopez** (Hospital Universitario Central de Asturias, Oviedo) then described a study of the potential contribution of defective induction by vitamin D of microRNA-145 on the onset and progression of vascular calcification in an experimental model of chronic kidney disease. MiRNA-145 is strongly expressed in vascular smooth muscle cells, and vascular calcification in chronic kidney disease markedly aggravates the risk of cardiovascular mortality. The study found that silencing miR-145 in vascular smooth muscle cells exposed to calcifying conditions enhanced expression of marker of osteogenic differentiation and increased calcium deposition. Notably, miR-145 ablation also compromised the vascular phenotype and increased calcium content under non-calcifying conditions. The session closed with a talk from **Dr Joseph Bass** and colleagues (University of Nottingham) on a clinical study of the role of the VDR in regulating skeletal muscle mass.

37 individuals underwent 20 weeks of whole-body resistant exercise training. Neither serum 1,25(OH)₂D nor total 25(OH)D₃ were associated with VDR or thigh muscle hypertrophy responses. However, VDR gene expression positively corresponded with resistance exercise training-induced hypertrophy, with the greatest upregulation being observed in high responders. Moreover, in animal studies, VDR overexpression in rat hind limbs stimulated myofibre hypertrophy, whereas VDR ablation led to muscle atrophy, and evidence was provided that autophagy was enhanced in VDR-deficient muscles.

Session VIII on New Functions for vitamin D was chaired by **Dr Geert Carmeliet** (Katholieke Universiteit Leuven) and **Dr Rebecca Mason** (University of Sydney). The session was opened by **Dr Motonari Uesugi** (Kyoto University) who presented the recently published study of his group on 25(OH)D₃ acting as an endogenous inhibitor of the cholesterol-sensing membrane receptor SREBP [21]. 25(OH)D₃ induces proteolytic processing and ubiquitin-mediated degradation of the SREBP partner protein SCAP. This observation provides the opportunity for a pharmacological control of SREBP/SCAP and may lead to a new therapeutic approach for lipid disorders. Next a joint talk of **Drs Chantal Mathieu** and **An-Sofie Vanherwegen** (Katholieke Universiteit Leuven) summarized their findings how vitamin D controls the capacity of human dendritic cells to induce functional regulatory T cells by regulation of glucose metabolism [22, 23]. They showed that 1,25(OH)₂D₃ imprints human monocyte-derived dendritic cells with tolerogenic properties through the reprogramming of their glucose metabolism. The *PFKFB4* gene, encoding for a glycolytic enzyme, was identified as a primary vitamin D target gene. PFKFB4 inhibition in 1,25(OH)₂D₃-treated dendritic cells blocks their capacity to induce suppressive regulatory T cells, i.e. alterations in the bioenergetic metabolism of immune cells are central to the immune-modulatory effects of vitamin D. In the same line **Amadeo Muñoz García** (joint PhD program of the universities of Maastricht, Netherlands, and Birmingham, UK) presented his pathway analysis of existing transcriptomic datasets for multiple myeloid cell types indicating that innate immune responses to 1,25(OH)₂D₃ in dendritic cells are strongly associated with mitochondrial energetic pathways. Results of these bioinformatic predictions were experimentally validated by metabolite analysis. **Dr Stella Davies** (Cincinnati Children's Hospital, OH, USA) reported on vitamin D binding protein (DBP) kinetics in the first 3 months after bone marrow transplantation in 131 children. She suggested that DBP modifies survival by i) serving as an actin scavenger that removes endothelium-damaging F-actin released into the circulation during chemotherapy and ii) by functioning as a macrophage-activating factor. These mechanisms may also be relevant in other clinical settings, such as major trauma, sepsis and burns. **Dr Kay Dube** (Ulster University) spoke on the *UGT1A* gene locus, which encodes for a family of phase II metabolism enzymes facilitating the glucuronidation of many endogenous

and exogenous substances, as a VDR target. VDR and its ligands markedly enhance the expression of several members of the *UGT1A* gene family and a VDR binding site within the regulatory region of the *UGT1A1* gene was identified. The capacity for VDR to modulate glucuronidation in extra-hepatic tissues suggests a clinical importance of vitamin D in hyperbilirubinemia and various cancers. Finally, **Dr Sharon Huish** (University Hospital of Coventry) reported on a study, in which vitamin D₃ supplementation corrected vitamin D deficiency and decreased erythropoietin needs of 350 hemodialysis patients. This indicates that even in end stage renal disease the ability to synthesize 1,25(OH)₂D₃ is maintained when in the presence of adequate substrate is provided.

Session IX, Vitamin D Chemistry and Analogs, was opened by **Dr Alexander (Leggy) Arnold** (University of Wisconsin, Milwaukee) who showed that calcitroic acid, a natural 1,25(OH)₂D₃ catabolite, is an inhibitor of P450 enzyme activity. Metabolism of 1,25(OH)₂D₃ in liver microsomes is slowed in the presence of calcitroic acid. Because of its acid functionality, calcitroic acid is distributed very differently in the body than 1,25(OH)₂D₃. The group developed a novel LC/MSMS to quantify calcitroic acid in different tissues with low limits of detection. This was followed by a talk by **Dr Martin Kaufmann** (Queen's University Ontario) on detection and quantification *in vivo* of the C24-oxidation product of 25(OH)D₃ in serum of mice treated with 24,25(OH)₂D₃. 25OHD₃ is catabolized by CYP24A1 to calcitroic acid and excreted. The group used followed the multi-step catabolism of vitamin D₃ metabolites *in vivo* using LC-MS/MS methods based on liquid-liquid- and immuno-extraction approaches. Amplification of 24,25-(OH)₂D₃ catabolism by exogenous enabled detection of the complete downstream C24-oxidation pathway products *in vivo*, including calcitroic acid [24]. The results provide a basis for studying alternative routes of vitamin D catabolism that may occur in pathological states. **Dr Robert Tuckey** (University of Western Australia) focused on the catalytic properties of the 25(OH)D₃ 3-epimerase in human and rat liver microsomes. The product, 3-epi-25(OH)D₃, is generally the third most abundant form of vitamin D present in the serum after 25(OH)D₃ and vitamin D₃. 3-epi-1 α ,25-dihydroxyvitamin D₃ has lower biological activity than 1,25(OH)₂D₃. Thus, 3-epimerization causes at least a partial inactivation of the hormone. The gene encoding the epimerase has not been identified. The group found that NADH was the preferred co-factor for both the human and rat enzymes and that the reverse reaction was catalyzed by both rat and human enzymes, but at lower rates than the forward reaction.

The final day of the meeting began with **Session X**, a plenary talk on **A Year in Vitamin D – Clinical** presented by **Dr Peter Ebeling** (Monash University). Dr Ebeling began by discussing the studies that have led to the description of Hereditary Vitamin D-dependent Rickets Type 3 (VDDRIII) [25]. As outlined earlier, this is due to mutations in the gene for the enzyme *CYP3A4*

that potentially enhance the ability of CYP3A4 to catabolize 25(OH)D₃ and 1,25(OH)₂D₃ to less active metabolites, similar to that normally mediated by CYP24A1. The focus of the remainder of the talk was the dramatic expansion of observational studies of vitamin D status and randomized control trials (RCT) for vitamin D supplementation. A literature search (January 1, 2013 to March 31, 2018 (Scopus)) revealed 632 publications relating to vitamin D RCT, and about 3000 vitamin D RCTs were registered with the ClinicalTrials.gov registry. Several health outcomes were included as the primary end-points of these published RCTs: falls, frailty, cardiovascular disease, maternal and infant health, musculoskeletal disease, type 2 diabetes mellitus, cancer, critical illness, osteoarthritis, respiratory diseases, infection and immunity. Dr Ebeling then presented examples of findings from some major trials (selected on the basis of >100 participants and numbers of citations).

Falls/frailty: A 12-month, double blind trial of 200 ambulatory men and women (mean age 78 years) given monthly 24,000 IU vitamin D₃ vs. 60,000 IU vitamin D₃, or 24,000 IU vitamin D₃ and 300µg calcifediol [26]. The primary end point [change in Short Physical Performance Battery scores (SPPB)] was no different across groups. In fact, repeated chair stands showed less improvement in the 60,000 IU and 24,000 IU+ 25(OH)D₃ groups. There was an increased percentage of fallers in the 60,000 IU and 24,000 IU + 25(OH)D₃ groups (67% and 66%, respectively, versus 24,000 IU group (48%; p=0.048). Despite the lack of a control group, these data suggest monthly high-doses of vitamin D should be avoided in the elderly. A large, double blind, placebo-controlled trial (ViDA) enrolled 5108 participants (58% male; mean age 65.9 years) treated with a 200,000 IU loading dose of cholecalciferol followed by monthly 100,000 IU doses for a median of 3.3 years. Only approximately 25% of participants were vitamin D deficient at baseline. Mean baseline 25(OH)D₃ was 63 nmol/L, with only 2% <25 nmol/L. There was no difference in falls between vitamin D and placebo over up to 4 years of follow up. Reported falls were 1312 (52%) vitamin D vs. 1326 (53%) placebo; HR=0.99 (0.92, 1.07) p=0.82 [27, 28].

Cardiovascular Disease: In ViDA there was no difference in incidence of cardiovascular disease (CVD) between groups 303 (11.8%) vs. 293 (11.5%); HR=1.02 (95% CI; 0.87, 1.20). The primary study outcome was negative, but this outcome focused on a large number of different CVD outcomes and it is unlikely that vitamin D deficiency could have benefits for all of these [29]. Interestingly, in the same study cohort, analysis of blood pressure showed that vitamin D supplementation lowered central BP parameters among adults with vitamin D deficiency but not in the cohort as a whole [30]. In the ViDA cohort vitamin D supplementation also improved persistence with statins use in older adults on long-term statin therapy, suggesting a possible role for vitamin D supplementation as an adjunct therapy for those on long-term statins [31].

Musculoskeletal: Amongst the secondary outcomes recorded in the ViDA study, non-vertebral fractures were similar; 156 (6%) in vitamin D-supplemented vs. 136 (5%) in placebo groups; HR 1.19 (95% CI 0.94–1.50) $p=0.15$. Interpretation of these outcomes has limitations: 1) these were secondary trial outcomes; 2) the trial was underpowered to detect differences in fractures (and falls); 3) bolus D dosing was used, which based on recent RCT data in the elderly would not now be recommended in that group.

Cancer: In a large double blind, placebo-controlled trial of 2303 postmenopausal women (mean age 65 years), subjects were treated with 2,000 IU cholecalciferol per day and 1500 mg per day of calcium or placebo for 4 years. Incident cancer was confirmed in 45 (3.9%) in the vitamin D group and 64 (5.6%) in placebo [difference, 1.69% (–0.06%, 3.46%); $p=0.06$]. Kaplan-Meier incidence over 4 years tended to be less in vitamin D vs. placebo; $p=0.06$. Although the study was negative, it does suggest a trend for vitamin D to reduce cancer, which needs to be confirmed in larger RCTs [32]. In a Japanese cohort of 33,736 participants followed for an average of 15.9 years, with a randomly selected sub-cohort of 4,044 plasma 25(OH)D was inversely correlated with the risk of total cancer, with multivariable adjusted HR, for the highest to lowest quartile of 0.78 (0.67 to 0.91), (P for trend=0.001) [33]. An inverse association was found for liver cancer and plasma 25(OH)D, adjusted HR for the highest to lowest quartile of 0.45 (0.26 to 0.79), (P for trend=0.006) and for breast cancer in premenopausal women (P for trend=0.03). This study suggested that higher vitamin D concentrations were associated with a lower risk of total cancer, liver cancer and breast cancer in premenopausal women [33].

Acute respiratory infections: A large meta-analysis of 25 eligible randomised controlled trials showed a 12% decrease with vitamin D (total 11,321 participants, with individual patient data obtained for 10,933 (96.6%). Vitamin D reduced the risk of acute respiratory tract infections (RTIs) among all participants (OR=0.88, 95% CI 0.81-0.96; $p<0.001$). Protective effects were seen in those receiving daily or weekly vitamin D without additional bolus doses (OR=0.81, CI 0.72-0.91), but not in those receiving bolus doses. Protective effects were greatest in those with baseline serum 25(OH)D₃ <25 nmol/L (OR=0.30, CI 0.17-0.53); p interaction=0.006). These data show vitamin D supplementation protected against acute RTI, and patients not receiving bolus doses and those with severe vitamin D deficiency experienced the most benefit [18].

Knee osteoarthritis: Two recent systematic reviews and meta-analyses of the effects of vitamin D supplementation on knee osteoarthritis, in four RCTs involving 1136 patients, showed vitamin D supplementation of more than 2000 IU vitamin D₃ per day resulted in small decreases in the WOMAC pain score and function in patients with knee OA. However, there was no beneficial effect on the prevention of tibial cartilage loss. Therefore, there is currently

a lack of evidence to support the use of vitamin D supplementation in preventing the progression of knee OA [34].

Dr Ebeling concluded that there is discordance between the largely negative findings of RCTs compared with the outcomes of observational studies associating vitamin D deficiency with adverse disease outcomes. Reverse causality with the illness itself contributing to low vitamin D levels may explain this. The results of many RCTs have also been inconsistent. However, overall evidence from RCTs shows vitamin D reduces fractures (when administered with calcium), in the institutionalised elderly, but not in community-dwelling adults. Vitamin D is also likely to reduce acute respiratory tract infections (if not given as a bolus dose), and may reduce falls in those with the lowest serum 25(OH)D levels. Currently, there is no consistent or conclusive evidence for a positive vitamin D impact on other diseases, including cancer, or on all-cause mortality.

Session XI chaired by **Dr Martin Hewison** (University of Birmingham) and **Dr Mairead Kiely** (University College Cork) focused on **Vitamin D and Reproduction**. **Dr Carol Wagner** (Medical University of South Carolina) discussed the role of vitamin D in preventing health disparities during pregnancy, specifically the elevated rates of adverse events of pregnancy in African-Americans in the USA. Dr Wagner described three randomized control vitamin D supplementation trials. In the first two of these, funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (n=350), and Thrasher Research Fund (n=160), the level of circulating 25(OH)D₃ was linked to improved pregnancy outcomes. However, poor adherence meant that vitamin D supplementation (versus placebo) did not have significant effect on pregnancy outcome. In the third trial, (Funded by WK Kellogg Foundation, n=257), 4400 IU/day vitamin D was compared to 400 IU in women with otherwise healthy pregnancies (at 8-12 weeks of gestation). The higher dose of vitamin D was not associated with improvement in individual pregnancy morbidities, but was associated with lower risk (OR=0.26, p=0.034) of combined morbidities (hypertension, diabetes, preeclampsia, preterm delivery, chorioamnionitis, miscarriage, intrauterine fetal death, bacterial vaginosis) in African-American women. Dr Wagner concluded that higher dose supplementation with vitamin D should inform future global health policy surrounding pregnancy interventions.

Dr Catherine Hawrylowicz (Kings College London) discussed the impact of vitamin D supplementation during pregnancy on pregnancy offspring and, specifically, the neonatal immune system. Her studies have focused on how maternal vitamin D status during pregnancy can affect asthma-related risk factors and outcomes in later life. In the Vitamin D Antenatal Asthma Reduction Trial maternal supplementation with 4,400 IU vitamin D per day (n = 26)

was compared to a control arm of treatment with 400 IU vitamin D per day ($n = 25$). Flow cytometric analysis of resulting cord blood immune cells showed enhanced responses to mitogen stimulus in cells from women supplemented with the higher level of vitamin D, and a corresponding elevation of pathogenic recognition receptors such as toll-like receptors (TLR). The conclusion from these studies was that enhanced maternal vitamin D status during pregnancy plays a key role in modifying the baby's immune responsiveness.

Dr Inez Schoenmakers (University of East Anglia) described studies in which her group had investigated the relationship between maternal and fetal $25(\text{OH})\text{D}_3$ following maternal vitamin D supplementation. In a study of a subset of women (34 weeks gestation) from the MAVIDOS trial receiving either placebo or supplementation with vitamin D (1,000 IU/day), maternal and cord blood were assessed for various vitamin D metabolites. Maternal and cord concentrations of all vitamin D metabolites studied (total and free $25(\text{OH})\text{D}_3$, 3-epi- $25(\text{OH})\text{D}_3$ and $24,25(\text{OH})_2\text{D}_3$) were significantly higher with supplementation. Compared to maternal concentrations, cord total $25(\text{OH})\text{D}_3$ and $24,25(\text{OH})_2\text{D}_3$ were lower, 3-epi- $25(\text{OH})\text{D}_3$ comparable and free $25(\text{OH})\text{D}_3$ higher. These observations indicated that maternal concentrations of vitamin D metabolites were not necessarily a direct reflection of the vitamin D available to the fetus. **Dr Ankana Ganguly** (University of Birmingham) described more mechanistic studies in which she had investigated the effects of vitamin D on placental development, specifically the ability of vitamin D to promote the invasive properties of fetal trophoblastic cells. Using cell models of the extravillous trophoblastic cells that invade maternal decidua during placental formation, studies showed that $1,25(\text{OH})_2\text{D}_3$ potently stimulated matrix invasion. This effect was associated with negligible expression of VDR or CYP24A1 in trophoblastic cells, consistent with non-genomic response to $1,25(\text{OH})_2\text{D}_3$. By contrast, $1,25(\text{OH})_2\text{D}_3$ inhibited matrix invasion by non-trophoblastic cells in a VDR-specific manner, suggesting placenta-specific effects of vitamin D during early pregnancy. In the final presentation of this session, **Dr Richard Mellanby** (University of Edinburgh) described a unique model for studying the effects of vitamin D on reproductive health. Wild, unmanaged, populations of Soay sheep on the uninhabited Scottish Island of St Kilda were studied between 2011 and 2016. Total plasma $25(\text{OH})\text{D}_3$ levels in these animals were related to age and color of the sheep (with $25(\text{OH})\text{D}_3$ being lower in dark colored sheep). Plasma levels of $25(\text{OH})\text{D}_3$ predicted fecundity in some years but not in others. The underlying basis for this is unclear but may explain the relatively stable distribution of light and dark colored sheep in this wild population of animals.

The final section of the meeting, **Session XII**, was focused on **Optimizing Vitamin D** and was chaired by **Dr Klaus Badenhop** (Goethe University Frankfurt am Main) and **Dr Dan Bikle**

(University of California San Francisco). **Dr Kevin Cashman** (University College Cork) provided a comprehensive overview of his work considering individual dose-response data compared to aggregated data from intervention trials on the assessment of dietary reference values (DRVs) for the population. He showed how considering between-person variability influences estimates of DRV using different target values of plasma 25(OH)D₃. He also considered the potential effects of the frequency, form and mode of vitamin D intake on bioavailability and the response in plasma 25(OH)D₃ and how this may influence the assessment of dietary requirements of the population [35].

Dr Martin Kaufmann (Queen's University, Kingston) showed a novel approach for the differential diagnosis of genetic and non-genetic causes of hypercalcemia by profiling of 6 vitamin D metabolites (25(OH)D₃, 24,25(OH)₂D₃, 25(OH)D₃-26,23-lactone, 23,25,26(OH)₃D₃, 1,25(OH)₂D₃, and 1,24,25(OH)₃D₃) by LC-MS/MS. Vitamin D metabolite concentrations and their ratios were evaluated in groups of patients with known causes of hypercalcemia and also after interventions to normalize plasma calcium. This 6-metabolite profiling assists in the identification of patients requiring genetic testing and to decide on intervention strategies to control hypercalcemia. **Dr Rachida Rafic** (VU Amsterdam, LUMC, Leiden) showed gender-dependent relationships between plasma 25(OH)D₃ concentrations and different body fat deposits (total body, abdominal subcutaneous, visceral and hepatic) in a population-based cohort study of adults. Overall, the amount of visceral fat tissue was most strongly negatively associated with plasma 25(OH)D₃ concentrations. In women, an inverse association with plasma 25(OH)D₃ was also found for total body fat and for men for hepatic fat.

Dr Davaasambuu Enkhmaa (National Center for Maternal and Child Health, Mongolia) presented data from a dose-ranging vitamin D supplementation trial (600, 2000, or 4000 IU of vitamin D₃/day) from early pregnancy to delivery in women with from Mongolia, where vitamin D status is generally low. A dose-dependent increase in maternal and cord plasma 25(OH)D₃ was found. Although all dosages reduced the percentage of women with a plasma concentration below 20 ng/ml compared to baseline (91%), a significantly higher proportion of women (56%) had a plasma 25(OH)D₃ below 20 ng/ml after supplementation with 600 IU/day compared to the higher dosages (16%). Cord blood concentrations of 25(OH)D₃ below 50 nmol/L were found in 83% of the 600 IU/day group, and was significantly lower with the higher doses. **Dr Rebecca Mason** (University of Sydney) provided evidence from *in vitro* and animal models that uptake of 25(OH)D₃ in muscle tissue and the cycling of 25(OH)D₃ between the muscle and blood pool may provide a mechanism to protect against 25(OH)D₃ catabolism and depletion of bodily stores. Phosphorus depletion disrupts this mechanism; phosphorus depleted animals not only have rachitic lesions but also and an increased rate of plasma

disappearance of 25(OH)D₃. Phosphorus depletion was shown to disrupt 25(OH)D₃ uptake and release from muscle and leads to altered concentrations of 25(OH)D₃ in muscle tissue.

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