

Highlights from the 22nd Workshop on Vitamin D in New York City, May 2019

James C. Fleet¹, Carlos Bernal-Mizrachi², Martin Hewison³, Rolf Jorde⁴, John White⁵,
Margherita Cantorna⁶, Inez Schoenmakers⁷, Reinhold Erben⁸

¹Department of Nutrition Science, College of Health and Human Sciences, Purdue University,
700 West State St., West Lafayette, IN 47906-2059, USA
e-mail: fleet@purdue.edu

²Division of Endocrinology, Metabolism & Lipid Research, School of Medicine, Washington
University, 660 S. Euclid Ave. Campus Box 8127, St. Louis, MO 63110
e-mail: cbernal@wustl.edu

³Institute of Metabolism and Systems Research, Centre for Endocrinology, Diabetes, and
Metabolism, Birmingham Health Partners, Level 2, IBR, Rom 225, The University of
Birmingham, Birmingham, B15 2TT, UK
e-mail: m.hewison@bham.ac.uk

⁴Endocrine Research Group, Department of Clinical Medicine, UiT The Arctic University of
Norway, 9037 Tromsø, Norway
e-mail: rolf.jorde@unn.no

⁵Department of Physiology, Department of Medicine, McGill University, 3655 Drummond St.
Room 1112, Montreal, QC H3G 1Y6, Canada
e-mail: john.white@mcgill.ca

⁶Department of Veterinary and Biomedical Sciences, College of Agricultural Sciences, 125
Henning Building, University Park, PA 16802
e-mail: mxc69@psu.edu

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

⁸Institute of Physiology, Pathophysiology, and Biophysics, Unit of Physiology, Pathophysiology,
and Experimental Endocrinology, Veterinärplatz 1, 1210 Wien, Vienna, Austria
e-mail: Reinhold.Erben@vetmeduni.ac.at

Overview

The 22nd Scientific Conference on the Biology of Vitamin D (aka the Vitamin D Workshop) was held in the Crowne Plaza Hotel in New York City from May 29-June 1, 2019. The workshop was organized by **Dr. James Fleet** (Chair, Purdue University, USA), **Dr. JoEllen Welsh** (Chief Financial Officer of the Vitamin D Workshop, Inc, SUNY Albany, USA), and **Roxanne Hall** (Meetings Plus). Topical sessions and speakers were chosen by the Vitamin D Workshop Executive Committee with input from the Program Advisory Committee (<https://vitamindworkshop.org/about>). The meeting attracted 262 delegates from around the globe. During the meeting, there were 42 podium presentations (16 invited speakers, 26 promoted abstracts) and 124 poster presentations. The meeting began on the evening of May 28 with an opening reception and 24 Plenary Poster presentations. The following two and a half days of meetings were organized into 13 sessions covering topics in basic science, clinical research, epidemiology, and policy. Flash poster sessions were held on May 30 and May 31 prior to an open poster session that was held concurrent with lunch. Delegates who presented primary research studies or invited presentations were invited to submit a manuscript 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 for the special issue were: **Dr. James Fleet**, **Dr. Paul Anderson** (University of South Australia), and **Dr. Peter Ebeling** (Monash University, Australia). Summaries of all oral sessions are presented below.

The first day of the meeting started with opening remarks by the Chair, Dr. James Fleet. This was followed by **Session I**, a keynote lecture on **The Year in Vitamin D: Basic Research** presented by **Dr. Carlos Bernal-Mizrachi** (Washington University, USA). This session was chaired by **Dr. JoEllen Welsh** (SUNY Albany, USA). Dr. Bernal-Mizrachi provided an excellent overview of some of the key publications within the vitamin D field from 2018 and 2019, focusing

on basic science research discoveries regarding vitamin D metabolism and its consequences. He first discussed two novel genetic forms of vitamin D deficiency. One publication described two patients with early-onset rickets due to heterozygous point mutations in CYP3A4, resulting in 10-fold higher enzyme activity and accelerated catabolism of vitamin D metabolites [1]. The second publication described a case of a middle-aged woman with debilitating ankylosing spondylitis with severe vitamin D deficiency that did not respond to supplementation because of to a homozygous deletion of the group-specific component (GC) of the vitamin D binding protein [2]. This patient, despite a lifelong deficiency of vitamin D-binding protein, did not have rickets or osteomalacia but rather mild bone disease. This suggests that cholecalciferol is locally hydroxylated to form the active, vitamin D hormone, which can then prevent adverse consequences of serum vitamin D deficiency.

Next, Dr. Bernal-Mizrachi described the discovery that environmental conditions such as prolonged food deprivation in mice can repress hepatic CYP2R1 expression and vitamin D 25-hydroxylation via marked activation of hepatic PGC1 α -ERR α and glucocorticoid receptor [3]. Additional data suggested that repression of hepatic CYP2R1 occurs in high-fat-diet-induced obesity, with insulin resistance, and in type 1 diabetes models, suggesting that the high prevalence of vitamin D deficiency occurring with these disease states could be secondary to prolonged hepatic energy deprivation. It is unclear whether vitamin D plays a role in either beta cell failure or tissue insulin action. Dr. Bernal-Mizrachi also discussed research that showed how the vitamin D receptor (VDR), by modulating islet stress, can have a protective role in beta cell survival [4]. This publication reported that VDR activation changes chromatin accessibility at consensus VDR binding elements and that this then suppresses expression of critical inflammatory genes and improves beta cell function and survival. Moreover, other research shows that VDR activation promotes insulin exocytosis from the beta cell under high-glucose conditions via increased expression of a voltage-gated calcium channel [5]. In diet-induced obesity and insulin resistance, intraperitoneal administration of active vitamin D reduces body

weight and improves muscle myosteatosis and insulin resistance by decreasing activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and by lowering tumor necrosis factor alpha (TNF α) level [6]. Interestingly, the knockout of the VDR gene also protected mice from diet-induced obesity, high serum triglycerides, fatty liver, and adipose inflammation [7]. These changes in lipid metabolism were partially reversed by intestine-specific Vdr overexpression, which increased intestinal luminal lipase. This suggests a new role for intestinal Vdr on systemic lipid homeostasis. Other publications addressed additional roles for gut VDR signaling in mice. Colonic-specific deletion of epithelial Vdr aggravates epithelial cell apoptosis, resulting in increased mucosal barrier permeability, luminal bacterial invasion, and dendritic cell activation of mucosal Th1 and Th17 responses [8]. Dr. Bernal-Mizrachi also described research on how the intestinal clock system affects VDR and regulates skeletal bone homeostasis [9]. Clock proteins bind to cell-specific enhancers and drive rhythmic expression of target genes. Mice lacking intestinal clock proteins have loss of rhythmic expression of intestinal VDR target genes with a resultant reduction in calcium absorption that contributes to bone loss. Research describing a novel mechanisms of bone fracture repair in mice with knockout of the CYP24 enzyme was also discussed [10]. In this work, the authors demonstrated that lactosylceramide generated by the interaction of 24R,25(OH) $_2$ D $_3$ with the transmembrane protein FAM57B2 is essential for endochondral ossification and strong callus formation after fracture. Conversely, 1,25(OH) $_2$ D treatment suppresses the osteogenic functions of pro-inflammatory M1 macrophages and this ultimately reduces mesenchymal stem cell abundance and impairs fracture repair [11]. Thus, this research suggests that 1,25(OH) $_2$ D treatment may be detrimental during fracture healing. Finally, Dr. Bernal-Mizrachi discussed research that showed Vitamin D exerts an anti-inflammatory effect in the pathogenesis of psoriasis [12]. In an experimental mouse model of psoriasis, activation of skin VDR with topical calcipotriol suppressed IL-36 α/γ and IL-23/IL-17 expression and inhibited neutrophil infiltration into skin lesions.

The topic of **Session II** was **Vitamin D receptor – Transcription/Mechanism of Action** and it was chaired by **Dr. Sylvia Christakos** (Rutgers New Jersey Medical School, USA). This session started with an invited lecture by **Dr. Moray Campbell** (Ohio State University, USA) who talked about the opportunities, challenges, and future prospects for the use of genomics approaches in vitamin D research. By alternating between a “20,000 ft” view and a “2000 ft” view, Dr. Campbell explained how genomic approaches can illuminate our understanding of vitamin D action through the vitamin D receptor (VDR) as well as how these regulatory events can relate to human health and disease. In the short talk that followed, **Dr. Takashi Ishii** (Ulster University, Ireland) reported how protein inhibitor of activated STAT4 (PIAS4)-mediated SUMOylation of the VDR could alter vitamin D transcriptional activity. They reported that SUMOylation represses VDR transcriptional activity at least in part by altering chromatin structure and VDR-coregulator interactions. Next, **Dr. Seong Min Lee** (University of Wisconsin, USA) reported the results of studies that used genetically modified mice to study the 1,25(OH)₂ D-mediated regulation of the Fgf23 gene, a critical step in the regulation of whole body phosphate metabolism. He demonstrated that 1,25(OH)₂ D uses a novel upstream enhancer region to regulate Fgf23 gene expression. In contrast, dietary phosphate restriction-mediated regulation of Fgf23 is more complex and does not rely on the vitamin D regulated upstream enhancer or several other putative regulators they tested. In the last talk in this session, **Dr. John White** (McGill University, Canada) reported a novel reanalysis of 80 published human transcript profiling studies that sought to provide a more clear understanding of the impact of vitamin D signaling on the genome. This analysis highlighted the cell-specificity of vitamin D signaling. In addition, it revealed that cells that are critical to the innate immune response were the richest source of vitamin D regulated genes. Thus, this data integration analysis provided a powerful resource for future mechanistic studies on cell-specific and immune modulatory effects of vitamin D signaling.

Session III on Vitamin D Metabolism was held in honor of **Dr. Helen Henry**, one of the pioneers of vitamin D metabolism who passed away in 2018. It was chaired by Dr Martin Hewison (University of Birmingham, UK) and was preceded by a tribute to Dr. Henry by **Dr. Dan Bikle** (University of California, San Francisco, USA). Dr. Bikle presented an overview of Dr. Henry's career achievements and her legacy to Vitamin D Workshop, thus providing a moving introduction to the area of vitamin D research she was most closely associated with. The session proper began with a talk by **Dr. Mark Myer** (University of Wisconsin, USA) describing the latest advances in our understanding of the molecular basis for regulation of serum $1,25(\text{OH})_2\text{D}_3$ levels [13]. Dr Meyer described mouse models that have been used to dissect the coordinated, reciprocal expression and activity of the enzymes vitamin D-1 α -hydroxylase (*Cyp27b1*) and 24-hydroxylase (*Cyp24a1*). In particular, these studies identified kidney-specific enhancer modules within introns of the *Met1* and *Met21b* genes that mediated basal, parathyroid hormone (PTH)-induced, and fibroblast growth factor 23 (FGF23)-suppressed *Cyp27b1* expression. Deletion of both of the intronic enhancer modules completely ablated renal synthesis of $1,25(\text{OH})_2\text{D}_3$ but had no effect on extra-renal synthesis. Enhancer module knockout also decreased levels of $24,25(\text{OH})_2\text{D}_3$ indirectly as a consequence of elevated PTH and suppressed FGF23. Meanwhile, direct regulation of *Cyp24a1* by PTH and FGF23 involved a set of downstream enhancers that were distinct from the *Met1/Met21b* intronic enhancers observed for *Cyp27b1*. These data, and related human studies, underline the exquisite molecular regulatory mechanism involved in controlling circulating levels of $1,25(\text{OH})_2\text{D}_3$.

The second talk in Session III by **Dr Jane Cleal** (University of Southampton, UK) described studies of the uptake and metabolism of vitamin D in the placenta. Experiments were carried out using villous fragments of fresh human term placentas incubated with C^{13} -labeled 25 hydroxyvitamin D_3 (25OHD_3) in a perfusion system. The resulting data indicated that 25OHD_3 was taken up by the placenta via a blockable endocytic mechanism, and then converted to $24,25(\text{OH})_2\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$. These vitamin D metabolites were then able to pass into the

maternal and fetal circulation systems. Through this mechanism, enhanced serum 25OHD₃ was able to regulate the expression of 493 genes and 246 proteins, notably including the suppression of inflammatory pathways. These data further underline the importance of maternal 25OHD₃ levels in maintaining optimal placental function and, in turn, healthy fetal development. In a separate but related study, **Dr JoEllen Welsh** (University of Albany, USA) used wild type (WT) and vitamin D binding protein (DBP) knockout mice to assess levels of vitamin D₃, and 25OHD₃ in the liver and breast tissues. Data indicated that in the vitamin D forms accumulate in both tissues but with higher accumulation in mammary glands of the DBP knockout mouse. Also, DBP was not required for release of vitamin D₃ or 25OHD₃ from mammary tissue. These data show that uptake of vitamin D metabolites is a crucial factor in target tissue responses to vitamin D. In the final presentation of Session III, **Dr Martin Kaufmann** (Queens University, Canada) presented data showing that the ratio of 25OHD₃ to 24,25(OH)₂D₃ is a better marker of renal vitamin D metabolism than simple measurement of 25OHD₃. Using a cohort of chronic kidney disease (CKD) dialysis patients and non-dialysis CKD patients, liquid chromatography-tandem mass spectrometry analysis of vitamin D metabolites showed that suppressed renal 1 α -hydroxylase and 24-hydroxylase activities in the dialysis patients dramatically decreased serum levels of 1,25(OH)₂D₃ but also increased the ratio of 25OHD₃ to 24,25(OH)₂D₃. These data suggest that, similar to what has been reported in subjects with *CYP24A1* gene mutations, measurement of the ratio of 25OHD₃ to 24,25(OH)₂D₃ may be a meaningful measure of vitamin D metabolism and function in patients with kidney disease.

Session IV was moderated by **Dr. James Fleet** (Purdue University, USA) and **Dr. Roger Bouillon** (KU Leuven, Belgium). The goal of this session was to provide an update on several large Vitamin D Clinical Trials that were recently completed or will be complete in the near future. These studies were the Vitamin D Assessment (ViDA) Study (presented by **Dr. Robert Scragg**, University of Auckland, NZ), the Vitamin D and Omega-3 (VITAL) study (presented by

Dr. JoAnn Manson, Harvard University, USA), and the Vitamin D and Type 2 Diabetes (D2D) Study (presented by **Dr. Anastassios Pittas**, Tufts University, USA). Each presenter reviewed the goals and design of their study. The primary goals of the ViDA trial were to assess the effect of monthly vitamin D₃ supplementation (100,000 IU) on non-skeletal diseases (i.e. cardiovascular disease, cancer, mortality) in adults from New Zealand. The VITAL study examined the impact of daily vitamin D₃ (2000 IU) and/or omega-3 fatty acid (1 g) on total invasive cancer and major cardiovascular events in US adults. These two trials are complete and summaries of these presentations are available in this special issue [14, 15]. The D2D examined whether daily vitamin D₃ (4000 IU) could reduce the risk of diabetes in adults at high risk for diabetes. Unfortunately, the results of the D2D trial were embargoed prior to their publication in June 2019 [16] so Dr Pittas was only able to discuss the study design and research goals. In addition to the reporting of the results of ViDA and VITAL, all three presenters participated in a lively discussion on the challenges faced during large vitamin D supplementation trials. These challenges included issues such as: the lack of pre-study vitamin D status levels, the cost-prohibitive nature of measuring serum 25OHD in all subjects, the inclusion of both vitamin D insufficient and vitamin D replete subjects in the trial, the high use of vitamin D supplement use in the study populations, the high cost of conducting trials with multiple doses of vitamin D, the expectation that intervention studies will be analyzed based on “intent to treat” despite the use of out-of-study vitamin D supplements by subjects or the level of subject compliance, and the existence of latent disease in the study population at the trial outset. There was also some discussion of the value that alternative analyses of the data have for interpreting the importance for vitamin D in human health as well as how the media report the complexities of study results.

The last session of the first day, **Session V: Eradication of rickets**, was chaired by **Dr Rolf Jorde** (UiT The Arctic University of Norway). This session was at the request of **Dr Roger**

Bouillon (KU Leuven, Belgium) who gave a brief overview of rickets and outlined how to convince the World Health Organization (WHO) to set up a plan to eradicate nutritional rickets before 2030. The reported prevalence of rickets varies considerably, from a few percent in some African countries to more than 50 % in Tibet and Mongolia [17]. The remedy for this is straightforward - vitamin D supplementation to infants and children - and there is no shortage of guidelines for such supplementation. At the Vitamin D workshop in Delft in 2015 a separate session was held with WHO representatives to discuss a potential eradication program for rickets, and at the International Congress of Endocrinology in Cape Town 2018 **Dr. Bouillon** received full support for an action plan to persuade WHO to implement such a program. This plan was presented in the present session and consists of the following steps: appointment of a task force on behalf of endocrine societies and Vitamin D workshop; convince leadership of WHO in Geneva; persuade member countries of WHO to ask WHO to implement an eradication plan; and get eradication of rickets on the agenda of the general assembly of WHO for approval. In the discussion following this presentation, there was unanimous support for this plan. The next step will be to establish the task force including a writing group for the WHO memorandum, and an advisory group to finalize this document.

Session VI examined the role of Vitamin D in Cancer and was chaired by **Dr. Eniko Kallay**

(University of Vienna, Austria) and **Dr. David Feldman** (Stanford University, USA). **Dr.**

Leonard Augenlicht (Albert Einstein College of Medicine, USA) opened the session with an invited talk on how the vitamin D and nutritional environment influence intestinal stem cells in ways that contribute to tumorigenesis [18]. His lecture built upon his earlier work that showed high fat “western diets” low in calcium and vitamin D increase the incidence of intestinal cancers in mice. In his lecture, Dr. Augenlicht reported that diet has an impact on the earliest stages of colon cancer through a complex interaction among stem-cell populations. Specifically, the “western diet” reprograms the transcriptome of the Lgr5+ intestinal stem cell and reduces its

contribution to normal intestinal tissue homeostasis. At the same time, the “western diet” reprograms Bmi1+ cells to make them function and persist as stem-cells in both normal intestinal homeostasis and tumorigenesis. These data suggest that diets low in vitamin D are an avoidable and modifiable stress that contribute to colon cancer initiation.

Dr. Augenlicht’s lecture was followed by five short research presentations. The first of these was by **Dr. Pamela Hershberger** (Roswell Park Cancer Center, USA), who examined the mechanism by which 1,25(OH)₂D exerts a protective effect on EGFR tyrosine kinase inhibitor (TKI) resistant lung cancer. By using multiple cell models of EGFR TKI resistance that undergo epithelial-mesenchymal-transition (EMT), she showed that 1,25(OH)₂D could enhance EGFR TKI-induced G0/G1 growth arrest and reverse the EMT phenotype of some models. In addition, in a small clinical study, she showed that patients with higher serum 25OHD levels (>30 ng/ml) had a longer benefit from EGFR TKI inhibition than patients with low serum 25OHD levels. This suggests vitamin D metabolites or analogs might be developed as adjunctive treatments for EGFR TKI therapy.

The second short talk in the session was by **Dr. James Fleet** (Purdue University, USA) who presented research on the impact that vitamin D signaling has on myeloid derived suppressor cells (MDSC), an immunosuppressive cell in the tumor microenvironment that promotes tumor growth. He showed that tumor MDSC express VDR and are vitamin D target cells. MDSC support tumor growth by suppressing proliferation of anti-tumor cytotoxic T cells. Using both 1,25(OH)₂D treatment and VDR deletion, Dr. Fleet showed that signaling through the VDR blocks the development of this immunosuppressive function. He presented data to show that this is in part due to vitamin D mediated effects on traditional mediators of MDSC immunosuppressive function like ARG1 and NOS2. Collectively, his data suggest that activation of vitamin D signaling could be used to suppress MDSC function and thereby enhance T-cell mediated immunotherapies.

The third short talk by **Dr. Jason Garcia** (University of Illinois-Chicago, USA) examined the role of megalin-mediated vitamin D uptake and its role in observed differences in intraprostatic androgens between African-American (AA) and Caucasian men. This group had previously found that megalin is expressed in prostate epithelium and that the serum and prostate levels of vitamin D metabolites are correlated in AA men. Consistent with a role for megalin in prostate steroid hormone uptake, 25OHD bound to the vitamin D binding protein and testosterone bound to the sex hormone binding globulin were both imported into primary prostate epithelial cells and human prostate tissue slices and both induced their respective hormone-driven gene expression. 25OHD also reduced expression of the megalin gene (LRP2) in these models. In human subjects, dihydrotestosterone (DHT) levels were inversely correlated to serum 25OHD level and DHT levels were higher in AA men, who normally have low serum 25OHD levels. This suggests that vitamin D deficiency could increase androgen import into the prostate through megalin.

In the fourth short talk, **Aparajita Verma** (Roswell Park Cancer Center, USA) reported on the impact of vitamin D signaling on immune checkpoint blockade (ICB) in head and neck (H&N) cancer. She noted that immune checkpoint inhibitors that block binding of PD-L1 to the programmed cell death protein (PD-1) work in only a subgroup of H&N cancer patients. However, since 1,25(OH)₂D increases intratumoral infiltration of CD4⁺ and CD8⁺ T cells in H&N cancer models and patients, they hypothesized that vitamin D signaling may regulate the PD-1/PD-L1 axis in vivo. Consistent with this hypothesis, 1,25(OH)₂D induced PD-L1 mRNA and protein expression in a novel mouse H&N cancer model (RP-mSCC1 cells). In mice with RP-mSCC1 tumors, 1,25(OH)₂D modestly reduced tumor growth but increased tumor CD4⁺ and CD8⁺ cells and PD-L1 levels. However, 1,25(OH)₂D also enhanced the anti-tumor effect of anti-PD-1 antibody immunotherapy. This suggests 1,25(OH)₂D might be an effective adjunct treatment to enhance ICB in humans.

The final talk of Session VI was by **Dr. Alicia Heath** (Imperial College London, UK). She reported the results of a case-cohort study nested within the Melbourne Collaborative Cohort Study that examined the association between serum 25OHD and cause-specific mortality. By using data on the 2307 participants who died during the 14 y follow-up, she found that 25OHD was inversely associated with the risk of cancer death, particularly colon cancer, but it wasn't associated with the risk of death due to breast, lung, pancreatic, or prostate cancer. Higher serum 25OHD levels were also associated with lower risk of death due to respiratory system diseases and digestive system diseases but there was no evidence of an association with cardiovascular disease mortality.

The **Session VII** topic **Vitamin D and the Microbiome or Immunity** was chaired by **Dr. Margherita Cantorna** (Pennsylvania State University, USA) and **Dr. John White** (McGill University, Canada). The session began with two presentations on the effects of vitamin D on the microbiota. **Dr. Tangpricha** (Emory University, USA) discussed the effects of a vitamin D intervention on the microbiome in patients with cystic fibrosis [19]. The double blind, placebo-controlled randomized clinical trial recruited patients with low vitamin D status (25(OH)D <30 ng/mL) and used either a 50,000 IU vitamin D/week or placebo for 12 weeks. A third group of cystic fibrosis patients with 25OHD levels over 30 ng/mL served as an additional control group. The vitamin D intervention improved 25OHD levels in the supplemented group compared to placebo. In addition, sequencing of the stool and sputum samples showed that the vitamin D insufficient group had different microbiota than the vitamin D sufficient group at the beginning of the study and that the vitamin D intervention changed the composition of the microbiota in both the gastrointestinal and respiratory tracts compared to placebo controls. Dr. Tangpricha concluded that vitamin D repletion favorably alters the microbiota in the lung and gut of cystic fibrosis patients.

The second presentation reported on the effects of vitamin D status on microbially induced regulatory T cells in the mouse. The paper was presented by **Juhi Arora** (Pennsylvania State University, USA), a graduate student in the **Cantorna** laboratory. The effect of vitamin D on the microbiota included changes in several microbial species important for the induction of regulatory T cells such as *Bacteroides*, and *Clostridium* XIVa and *Clostridium* XVII clusters. Transplantation of the microbes from vitamin D sufficient and vitamin D deficient mice into the intestine of germfree mice phenocopied the higher numbers of regulatory T cells and protection from colitis seen in vitamin D sufficient versus vitamin D deficient recipient mice [20]. Together the two presentations establish that vitamin D status affects the composition of the microbiome in both humans and mice. The session further demonstrated a beneficial effect of vitamin D through the regulation of the microbiota in the mouse.

The third talk from **Dr. Stefan Tukaj** (University of Gdansk, Poland) presented results showing that calcitriol treatment ameliorated the symptoms of a mouse model of epidermolysis bullosa acquisita (EBA), an anti-type VII collagen autoantibody-induced blistering skin disease. Calcitriol reduced immune effector cell activation and was associated with an increase in regulatory T and B cells as well as a reduction of pro-inflammatory T helper 17 cells in mice with immunization-induced EBA. These findings are significant because many patients with EBA present with vitamin D deficiency which suggest that vitamin D supplementation or treatment with analogues may be of therapeutic benefit.

The next presentation of the session by **Dr. Yanchun Li** (University of Chicago, USA) reported on the effects of vitamin D and the vitamin D receptor on the development of type 3 innate lymphoid cell (IL3C). He noted that both VDR and Cyp27b1 gene deletion markedly reduced colonic ILC3 levels and that there were impaired responses to *Citrobacter rodentium* infection in both knockout lines. The defects in IL3C in Cyp27b1 knockout mice could be restored by treatment with 1,25(OH)₂ D and this regulation was independent of T lymphocytes, B lymphocytes, and the gut microbiota. The new data presented by Dr. Li established a role for

vitamin D and 1,25(OH)₂D in the proliferation of the innate lymphoid cells, which was important for protection from gastrointestinal infection.

Dr. Masako Suzuki (Albert Einstein College of Medicine, USA) reported on research that tested whether prenatal vitamin D deficiency causes long-term effects on postnatal immunity in mice. Offspring of vitamin D deficient or sufficient females were fed vitamin D sufficient diets. The investigators found that vitamin D deficiency during pregnancy reduced the proportion of T cells (CD4+ and CD8+) among CD45+ cells, whereas there was no significant difference in B cells between groups. They also found that the total number of bone marrow cells per body weight was significantly lower in offspring of vitamin D-deficient females. In addition, MPP4 lymphoid progenitor and early-stage T cell progenitor cell populations were decreased by vitamin D deficiency during pregnancy. These results are important because vitamin D deficiency is common in reproductive-aged women and maternal vitamin D deficiency is strongly associated with immune-related conditions in offspring later in life.

The session closed with a presentation by **Grant Parnell** (Westmead Institute, Australia) who assessed changes in immune cell expression of potentially vitamin D responsive multiple sclerosis (MS) risk genes. Supplementation altered the expression genes such as *CYP24A1* *in vitro* in immune cells. However, in patients with clinically isolated syndrome (CIS), a precursor of MS, expression of these genes in whole blood was not altered upon supplementation with 25-hydroxyvitamin D₃ or given UV therapy. Notably, expression of marker genes, including *CYP27B1*, was lower in CIS than controls at baseline and expression of marker genes were correlated with each other, suggesting co-regulation. The researchers also found evidence of seasonal variation in marker gene expression, and correlation with serum 25D₃ and solar UVB. The lack of response of marker gene expression to supplementation may have been due to insufficient activation of the 25-hydroxyvitamin D₃ precursor due to limiting expression *CYP27B1* and/or insensitivity to supplementation due to homeostatic constraints.

Session VIII on Bone and mineral metabolism was held in honor of **Dr. Robert Wasserman**, a leader in the field of vitamin D-regulated calcium binding proteins, who passed away in 2018. The session was chaired by **Dr. Paul Anderson** (University of South Australia, Australia) and **Dr. Geert Carmeliet** (KU Leuven, Belgium). It was preceded by a tribute to Dr. Wasserman by **Dr. Sylvia Christakos** (Rutgers New Jersey Medical School, USA), who provided a thoughtful review of Dr. Wasserman's contribution to our understanding of the mechanism of vitamin D-mediated intestinal and renal calcium metabolism. Dr. Wasserman's research on the identification, induction by vitamin D, and function of the intestinal and renal calcium binding proteins (calbindin D_{9k} and D_{28k} in mammals, respectively) served as a foundation for the facilitated diffusion model of transepithelial calcium transport. After her tribute, Dr. Christakos continued to give an invited talk that described new research on vitamin D signaling in the intestine [21]. Her talk began with a review of her recent publication on the role of signaling through the VDR in the distal intestine. This work showed that transgenic expression of VDR that is limited to the epithelial cells of the cecum and proximal colon is sufficient to recover the abnormal calcium and bone phenotype of global VDR knockout mice. She then described research showing that there are large transcriptomic changes that occur in both the small intestine and colon following 1,25(OH)₂D treatment. In addition, she reported studies on human intestinal organoids to show that 1,25(OH)₂D regulates more than 300 genes in organoids that model either the intestinal crypt or the differentiated cells in the intestinal villus. This expansion of the number of vitamin D regulated genes in the mouse and human intestine suggest that vitamin D regulates more intestinal biology than simply intestinal calcium absorption. Finally, she explained that the high level of VDR expression that is typically seen in the intestine is dependent upon regulation mediated by the transcription factor Cdx-2.

In the second talk, **Dr. Eva Liu** (Brigham and Women's Hospital, USA) described her experiments on the role that 1,25(OH)₂D plays in the development of enthesopathy, an abnormal mineralization of tendon attachment sites, in X-linked hypophosphatemia (XLH).

Using the Hyp mouse model of XLH, she found that 1,25(OH)₂D treatment or a blocking antibody to FGF23 both prevent expansion of hypertrophic enthesopathy cell expansion. Next she showed that deletion of renal 1,25(OH)₂D production in CYP27b1 knockout mice causes enthesopathy and other phenotypes of XLH. Cell studies were presented that suggest 1,25(OH)₂D inhibits enthesopathy by suppressing BMP signaling in chondrogenic cells.

In the third presentation of the session, **Stephanie Doms** (KU Leuven, Belgium) reported research on a mouse model expressing a VDR that lacks the activation function 2 (AF2) domain (VDR^{ΔAF2}) and which is unresponsive to 1,25(OH)₂D. Initially, these mice looked similar to global VDR knockout mice with elevated serum PTH and 1,25(OH)₂D and low bone mass. However, when directly compared to the VDR global knockout mice, VDR^{ΔAF2} mice fed a high lactose, high calcium rescue diet have increased cortical porosity and a greater reduction in cortical thickness. These changes were not due to differences in the expression of intestinal or renal calcium transporter genes. This suggests that there must be direct negative effects of unliganded VDR in bone cells.

The final presentation in the session was by **Dr. Christopher Sempos** (Vitamin D Standardization Program, USA). In his talk, Dr. Sempos examined the role that serum 25OHD level plays in the prevention of rickets in Nigerian children. Using a case-control approach, his group showed that serum 25OHD levels were significantly different in children with rickets but that these children and the control children have similar, low dietary calcium intakes (~ 200 mg/d). Additional analysis showed that serum 25OHD levels strongly discriminates between rickets and controls whereby children with levels < 40 nmol/L have a significantly higher odds of having rickets.

Session IX, chaired by **Dr. Moray Campbell** (Ohio State University, USA) was on the **interaction of Genetics and vitamin D biology**. The first talk in this session was an invited

lecture by **Dr. Brent Richards** (McGill University, Canada) on the use of Mendelian Randomization (MR) as a tool to understand the role of vitamin D in disease. Dr. Richards explained that because genetic variants are randomly assigned at conception, MR can use the natural genetic variation in genes controlling serum 25OHD levels to strengthen causal inference by mimicking a life-long randomized controlled trial (RCT). The strengths of this approach is that it can be done in large populations of subjects, it decreases confounding, and it is not prone to reverse causation. However, MR assumes that the relationship between serum 25OHD and disease is linear, and this may not be true for all vitamin D-disease relationships. In addition, MR is limited by the strength of the relationship between specific gene polymorphisms and serum 25OHD levels and it does not currently apply to genetic variation controlling either serum 1,25(OH)₂D levels or action. Thus, Dr. Richards explained that while MR is a population level approach that has features that improve upon the weaknesses of association studies and traditional RCT with vitamin D supplementation, it is not a perfect method and readers should appreciate these weaknesses when reading the MR literature.

The second talk in the session was by **Dr. Folami Ideraabdullah** (University of North Carolina at Chapel Hill, USA). She discussed her recent studies on the epigenetic changes induced in offspring by maternal vitamin D deficiency that cause increases in male offspring bodyweight and adiposity. New studies were described where by mice were fed a vitamin D deficient diet during a reciprocal mating strategy between two genetically divergent recombinant inbred mouse lines from the Collaborative Cross (CC001 and CC011); this allows genetically identical offspring to develop in distinct maternal and uterine environments. She found that that both first and generation male offspring from the cross of female CC001 to male CC011 mice had higher bodyweight that was associated with reduced methylation of an imprinted gene in the liver. She also found that male offspring from this cross had 2-3 more epimutations in the liver and sperm than the other cross. This shows that despite identical dietary vitamin D intake, maternal

genetic background can strongly influence the molecular and physiologic responses to vitamin D deficiency through epigenetic effects on the offspring.

The third talk of the session was presented by **Dr. Amaud Molin** (Caen University Hospital, France) on idiopathic infantile hypercalcemia (IIH). He reported findings from a cohort of 8 IIH type 1 patients (7 children, 1 adult) that identified 13 variants in the SLC34A1 gene that encodes the renal phosphate transporter NaPi2A. There were a variety of mutation types: 7 mis-sense mutations, 2 inframe deletions, and 3 splice site mutations, that all presented as precocious nephrocalcinosis. Despite this, the spectrum of mutations caused both increases and reductions in serum phosphate. Other family members of the subjects were found to have some phenotypes of IIH and one sister of an affected subject carried the same genotype as her sibling but had no IIH phenotype. This suggests that other factors may affect the penetrance of the genetic effects on IIH.

The last presentation of the session was by **Rikeenkumar Dhaduk** (Loyola University, USA). He noted that serum 25OHD levels are lower among blacks than whites in the US and this led to the hypothesis that serum 25OHD levels are influenced by genetic factors in populations of African descent. To test this hypothesis, a cohort of ~1600 African heritage subjects from 5 countries were genotyped for 22 variants in 5 genes controlling vitamin D metabolism or action (GC, CYP2R1, CYP27B1, CYP24A1, VDR). Variants in two genes, GC and CYP2R1, were significantly associated with serum 25OHD in the cohort, even after correcting for multiple comparisons.

Session X was a keynote lecture on The Year in Vitamin D: Clinical/Population Research by **Dr. Inez Schoonmakers** (University of East Anglia, Norwich, UK). The session was chaired by **Dr. Malachai McKenna** (University College Dublin, Dublin, Ireland). Dr. Schoonmakers noted that as of early 2019, around 3000 trials evaluating the effect of vitamin D as mono- or co-intervention were registered, with a wide range of primary outcomes. Several of these are

mega-trials with over 5000 participants and also collect data for a large number of secondary outcomes. Around 500 are currently in the stage of data collection. The majority of studies investigated adults, with only 653 of studies designed specifically to recruit children. Inspection of the world-wide distribution of trials shows that the vast-majority of RCTs are or were conducted in the US and EU. Relatively few are taking place in North Asia, South America and Africa, continents from which there are also few population-representative data available on vitamin D status. Dr. Schoonmakers warned that the lack of data in these populations needs to be considered as the vitamin D research community interprets the emerging trial data in an international context. Dr. Schoonmakers then went on to summarize the results from many of the most influential publications.

Musculoskeletal. A meta-analysis and sequential analyses of vitamin D intervention trials (81 trials with vitamin D mono-therapy or with calcium if the comparator groups also received calcium; total N= 53,537 adults over 18 years of age) reported non-significant effects of vitamin D on total fractures, hip fractures and falls, as well as small differences in lumbar spine and hip BMD but not at the femoral neck and total body [22]. However, the vast majority of included trials were conducted in populations with a mean baseline 25OHD > 50 nmol/L (43%) and the majority of trials lasted less than 1 year (i.e. a duration generally considered the minimum necessary to observe a change in BMD). These authors wrote a narrative review of subgroup analyses by baseline 25OHD, but they did not conduct a formal meta-analysis or Individual Patient Data meta-analysis. In response to this publication, several authors cautioned that the findings of this meta-analysis are not contradictory to earlier meta-analyses that showed a reduction in hip fracture risk in older people receiving vitamin D and calcium supplementation and they emphasized the importance of the vitamin D and calcium as co-therapies when taking anti-resorptive medication.

Several RCTs evaluated the effect of various dosages of vitamin D on muscle function and strength in healthy adults and older adults with or without frailty [23-27]. In contrast to

observational studies that had showed negative muscle outcomes when vitamin D status was below 50 nmol/L or a meta-analyses of intervention studies that reported positive effects on muscle [28], the RCTs found no effects of supplementation on musculoskeletal endpoints.

Cancer. Dr. Schoonmakers noted that there is strong evidence for an association between vitamin D status and cancer incidence from observational studies. However, in the last year, two mega-trials reported vitamin D supplementation no effect on cancer incidence: VITAL, 2000 IU D₃/d with or without omega 3- fatty acids for 5.3 years [29]; ViDa: 100,000IU D₃/m for 3.3 years [30]. However, the US-based VITAL trial did find a significant reduction in cancer mortality in the last 3.3 years of the trial. No interaction with baseline 25OHD (both as categorized by 25OHD below or above 50 or 77.5 nmol/L) was found with any of the outcomes [29]. These data are consistent with other intervention trials on cancer incidence and mortality [31-36]. However, there was a significant interaction with BMI, with a protective effect of vitamin D supplementation on cancer incidence in those with a BMI <25 (HR 0.76). This suggests that adiposity is an important modifier of vitamin D in cancer. The results from several ongoing trials are anticipated in the next few years (D2dCa, D-Health and FIND [37, 38]), and these may provide additional insight on the interaction of body composition and vitamin D in cancer.

Type 2 and gestational diabetes: There is strong evidence for an association between vitamin D deficiency (serum 25OHD < 30 nmol/L) and increased risk of type 2 diabetes (T2D) from prospective cohort and observations studies. Two recent meta-analyses of intervention studies show small, but significant effects on glucose or insulin secretion and resistance in people with impaired glucose tolerance and vitamin D deficiency at baseline [39, 40]. In one of these, Li et al. [40] also showed a modifying effect of ethnicity (with effects particularly seen in Asian and Middle Eastern populations) and more pronounced effect of vitamin D status in patients with well-controlled T2D. Several recent, small RCTs conducted in overweight/obese or T2D patients also showed that vitamin D supplementation had benefits on glucose metabolism or insulin sensitivity with those these ethnic backgrounds [41-44]. In contrast, vitamin D supplementation

was found to have no effect on markers of glycaemic control in an Individual Patient Data based re-analyses of data and samples of 12 RCTs in diverse European populations. Also, no interaction with baseline 25OHD was detected in a study that used Vitamin D Standardization Program-harmonised methodology [45]. Finally, in the DALI study [46] there was a small, significant effect of vitamin D supplementation on fasting glucose in pregnant women at increased risk of gestational diabetes whose baseline 25OHD was > 50 nmol/L. However, vitamin D supplementation had no effect on insulin resistance or weight gain.

Reproductive function and infant status. Conflicting data was reported from several studies that examined the association between maternal or paternal status and the effect of vitamin D supplementation on chances of conception, early and late pregnant losses, and stillbirth [47-53]. Strong evidence for a negative association between vitamin D status (predominantly at lower 25OHD concentrations) and the risk of pregnancy complications is found in observational studies [54], but these were not confirmed in a meta-analysis of 43 intervention studies (N=8406 subjects)[55]. In contrast, for several measures of fetal and infant development, there was consistency between the findings from meta-analyses of observational and intervention studies that related maternal vitamin D deficiency or insufficiency to vitamin D supplementation, e.g. risk of pre-term birth, birth anthropometrics, mental development and wheezing [55-58]. However, the positive effects of maternal supplementation on fetal and infant development were not replicated in a dose ranging placebo-controlled RCT conducted in Bangladesh, a population with a high risk of maternal vitamin D deficiency and foetal and infant growth restriction [59]. Interestingly, this study showed that at 3-months of age, the vitamin D status of an infant was no longer influenced by gestational maternal supplementation. This shows that vitamin D stores are rapidly depleted during infancy and that while gestational vitamin D supplementation improves vitamin D status of term infants, additional vitamin D supplementation to the infant is required to maintain their status. A meta-analysis of the 25OHD response to vitamin D

supplementation in infants (61 studies; total N=1828) estimated a required intake of 400 IU/d to maintain 25OHD \geq 50 nmol/l in 97.5% of term infants [60].

Secular trends in population vitamin D status: Recent publications from national surveys in the US and UK indicate that the population prevalence of deficiency (serum 25OHD <30 or 25 nmol/L) has not decreased in recent years, although in the US the prevalence of inadequacy (serum 25OHD 30–49 nmol/L) did decline between 2003–2014 [61]. Disparities in vitamin D status and supplement use between ethnic groups and social-economic classes persist [62, 63]. Several professional societies have recently formulated position statements how to implement population recommendations and to prevent the burden of vitamin D deficiency in populations across the world [64-66].

After presenting her view of the recently literature on vitamin D and health, Dr. Schoonmakers concluded that the latest research indicates that an effect of vitamin D supplementation is mostly limited to those with low vitamin D status at baseline and/or at increased disease risk. She noted that several, current ongoing RCTs specifically recruit participants with vitamin D deficiency and that the field of vitamin D is anxiously awaiting the results of studies to determine if they confirm these findings. Dr. Schoonmaker also noted that recent findings also suggest that there are potential ethnic differences in the effect of vitamin D supplementation on outcomes. This may be related to differences in vitamin D status and disease risk between populations. Emerging research in different continents will address these population differences.

A session on **vitamin D and Public Health (Session XI)** was chaired by **Dr. Susan Whiting**, (University of Saskatchewan, Saskatoon, Canada). This session began with an invited lecture by **Dr. Stephanie Atkinson** (McMaster University, Hamilton, Canada). She summarized the rationale for, and process that led to, the vitamin D intake guidelines for persons affected by multiple sclerosis (MS) that was released by the MS Society of Canada in November 2018 <https://mssociety.ca/library/document/Vka6RXcnOizNm9slwuWvroxeilhLqTJ8/original.pdf> [67].

These guidelines were developed by an expert panel over a period of 18 months. She noted that although there was consistent and growing evidence for a causal link between MS risk and vitamin D status, there was a paucity of type of research needed to definitively define specific intake levels for health-related recommendations. As such, the guidelines for people at risk for MS, as well as for people living with MS, are similar to those for the public. In addition, Dr. Atkinson noted that the panel made a clear statement that vitamin D should not be used as a stand-alone treatment for MS.

The second talk of the session on food vehicles for vitamin D fortification was by **Dr. Kevin Cashman** (University College Cork, Ireland). His research group identified 7 low-middle income countries that have a high prevalence of vitamin D deficiency and who might benefit from a vitamin D food fortification program. They then modeled the potential impact that fortifying three different food vehicles (edible oil, wheat or maize flour, and milk) could have on mean vitamin D intake in all 7 of the countries. By using usual intakes of these foods in the 7 countries, they calculated the levels of fortification that could significantly increase vitamin D intake. Their data also demonstrated how different food vehicles need to be considered in the different countries to improve vitamin D intake.

Session XXII on New Functions of Vitamin D was chaired by **Dr Reinhold G. Erben** (University of Veterinary Medicine Vienna). The session was opened by **Dr Jacob Rullo** (Queen's University Ontario) who presented a study in 120 patients undergoing ophthalmic procedures, in which 25OHD concentrations were measured in blood and in the eye, together with ocular expression of CYP27B1, CYP24A1, and VEGF. 25OHD was clearly detected in the eye at varying and disease-dependent concentrations, together with the enzymes responsible for production and inactivation of the vitamin D hormone. He suggested that vitamin D production and signaling in the eye may be important for maintenance of the visual machinery and in ocular disease pathogenesis. Next, **Dr Erica Mandell** (University of Colorado) reported

the results from a rat study aimed at testing the role of maternal vitamin D deficiency on fetal lung development. Newborn pups from vitamin D deficient mothers showed reduced pulmonary vessel density and reduced radial alveolar counts. In addition, pulmonary endothelial cells cultured from these pups had reduced cell growth and tube formation. She speculated that maternal vitamin D deficiency may hamper normal lung development in the fetus, predisposing the offspring to respiratory disease later in life. Finally, **Dr Nejlja Latic** (University of Veterinary Medicine Vienna) reported on a study in male cardiomyocyte-specific VDR knock-out mice that were subjected to transverse aortic constriction as an afterload-induced left ventricular hypertrophy model. The sham-operated cardiomyocyte-specific VDR knockout mice had normal mineral homeostasis, and did not show cardiac hypertrophy or a cardiovascular phenotype. However, echocardiography revealed that compared with VDR floxed control mice, cardiomyocyte-specific VDR knockout mice had left ventricular functional impairment after transverse aortic constriction. The findings indicate that vitamin D signaling is dispensable in the heart under normal conditions, but that vitamin D signaling in cardiomyocytes has a protective function in the stressed heart.

Session XIII was the last scientific session of the meeting and it explored the controversy surrounding **vitamin D requirements during pregnancy**. The session was chaired and moderated by **Dr. Mairead Kiely** (University College Cork, Cork, Ireland) and included the participation of two discussants: **Dr. Daniel Roth** (The Hospital for Sick Children, Toronto, Canada) and **Dr. Carol Wagner** (Medical University of South Carolina, USA). Initially, Dr. Kiely explained the motivation for the session by noting that research on vitamin D and pregnancy is a neglected area. This is true despite the fact that low vitamin D status is common in pregnant women and that the evidence available for setting the vitamin D requirement during the 2011 US Dietary Reference Intakes (DRI) deliberations was weak [68]. She also noted that trials on vitamin D and pregnancy are often designed to test the benefit of additional vitamin D rather

than to answer the question “What level of vitamin D status or intake is sufficient to support a healthy pregnancy?” During a series of presentations by Drs. Roth and Wagner, and in an extended discussion that followed, the current state of the evidence based on considerations of vitamin D metabolism in pregnancy and the prevalence of gestational vitamin D deficiency worldwide was evaluated. The panel went on to discuss the challenge of translating laboratory research findings on the role of vitamin D in perinatal health to clinical studies; the challenges of designing and conducting clinical trials to establish prenatal vitamin D requirements; and results to date of major large randomized controlled trials of prenatal vitamin D supplementation. During the discussion, the speakers and audience also explored potential next steps towards generating robust clinical data in this field that would address both public health protection and patient care. Overall, there was a plurality of divergent viewpoints represented rather than a unified or consensus position by the speakers or the audience.

Acknowledgments

We would like to acknowledge the generous support of all the sponsors of the 22nd Workshop on Vitamin D in New York City, May 2019. The Workshop received a conference grant from the National Institute of Aging in the US National Institutes of Health (R13AG048689). This supported invited speaker presentations and contributed to the Young Investigator Award and the Trainee Travel Awards. Awards for junior scientists were also generously supported by Heartland Assays (Platinum sponsor). The Vitamin D Clinical Trials Update (Session IV) was supported by Abiogen Pharma (Platinum sponsor). We also want to thank our other sponsors including: Pfizer (Platinum sponsor), OPKO Health, and Elsevier (Gold Sponsors) and our exhibitors: BioTech Pharmacal, Inc., EndoTherm, Immundiagnostik, Diazyme, VitaminDWiki, and GrassrootsHealth. Without the support of these sponsors and exhibitors, the Vitamin D workshop would not be possible.

Literature Cited

- [1] J.D. Roizen, D. Li, L. O'Lear, M.K. Javaid, N.J. Shaw, P.R. Ebeling, H.H. Nguyen, C.P. Rodda, K.E. Thummel, T.D. Thacher, H. Hakonarson, M.A. Levine, CYP3A4 mutation causes vitamin D-dependent rickets type 3, *J Clin Invest*, 128 (2018) 1913-1918.
- [2] C.M. Henderson, S.L. Fink, H. Bassyouni, B. Argiropoulos, L. Brown, T.J. Laha, K.J. Jackson, R. Lewkonia, P. Ferreira, A.N. Hoofnagle, J.L. Marcadier, Vitamin D-Binding Protein Deficiency and Homozygous Deletion of the GC Gene, *N Engl J Med*, 380 (2019) 1150-1157.
- [3] S.M. Aatsinki, M.S. Elkhwanky, O. Kummu, M. Karpale, M. Buler, P. Viitala, V. Rinne, M. Mutikainen, P. Tavi, A. Franko, R.J. Wiesner, K.T. Chambers, B.N. Finck, J. Hakkola, Fasting-Induced Transcription Factors Repress Vitamin D Bioactivation, a Mechanism for Vitamin D Deficiency in Diabetes, *Diabetes*, 68 (2019) 918-931.
- [4] Z. Wei, E. Yoshihara, N. He, N. Hah, W. Fan, A.F.M. Pinto, T. Huddy, Y. Wang, B. Ross, G. Estepa, Y. Dai, N. Ding, M.H. Sherman, S. Fang, X. Zhao, C. Liddle, A.R. Atkins, R.T. Yu, M. Downes, R.M. Evans, Vitamin D Switches BAF Complexes to Protect beta Cells, *Cell*, 173 (2018) 1135-1149 e1115.
- [5] L. Kjalarsdottir, S.A. Tersey, M. Vishwanath, J.C. Chuang, B.A. Posner, R.G. Mirmira, J.J. Repa, 1,25-Dihydroxyvitamin D₃ enhances glucose-stimulated insulin secretion in mouse and human islets: a role for transcriptional regulation of voltage-gated calcium channels by the vitamin D receptor, *J Steroid Biochem Mol Biol*, 185 (2019) 17-26.

- [6] E. Benetti, R. Mastrocola, F. Chiazza, D. Nigro, G. D'Antona, V. Bordano, R. Fantozzi, M. Aragno, M. Collino, M.A. Minetto, Effects of vitamin D on insulin resistance and myosteatorsis in diet-induced obese mice, *PLoS One*, 13 (2018) e0189707.
- [7] D. Jahn, D. Dorbath, A.K. Schilling, L. Gildein, C. Meier, R.N. Vuille-Dit-Bille, J. Schmitt, D. Kraus, J.C. Fleet, H.M. Hermanns, A. Geier, Intestinal vitamin D receptor modulates lipid metabolism, adipose tissue inflammation and liver steatorsis in obese mice, *Biochim Biophys Acta Mol Basis Dis*, 1865 (2019) 1567-1578.
- [8] L. He, T. Liu, Y. Shi, F. Tian, H. Hu, D.K. Deb, Y. Chen, M. Bissonnette, Y.C. Li, Gut Epithelial Vitamin D Receptor Regulates Microbiota-Dependent Mucosal Inflammation by Suppressing Intestinal Epithelial Cell Apoptosis, *Endocrinology*, 159 (2018) 967-979.
- [9] M. Kawai, S. Kinoshita, M. Yamazaki, K. Yamamoto, C.J. Rosen, S. Shimba, K. Ozono, T. Michigami, Intestinal clock system regulates skeletal homeostasis, *JCI Insight*, 4 (2019).
- [10] C. Martineau, R.P. Naja, A. Hussein, B. Hamade, M. Kaufmann, O. Akhouayri, A. Arabian, G. Jones, R. St-Arnaud, Optimal bone fracture repair requires 24R,25-dihydroxyvitamin D3 and its effector molecule FAM57B2, *J Clin Invest*, 128 (2018) 3546-3557.
- [11] S. Wasnik, C.H. Rundle, D.J. Baylink, M.S. Yazdi, E.E. Carreon, Y. Xu, X. Qin, K.W. Lau, X. Tang, 1,25-Dihydroxyvitamin D suppresses M1 macrophages and promotes M2 differentiation at bone injury sites, *JCI Insight*, 3 (2018).

- [12] B. German, R. Wei, P. Hener, C. Martins, T. Ye, C. Gottwick, J. Yang, J. Seneschal, K. Boniface, M. Li, Disrupting the IL-36 and IL-23/IL-17 loop underlies the efficacy of calcipotriol and corticosteroid therapy for psoriasis, *JCI Insight*, 4 (2019).
- [13] M.B. Meyer, J.W. Pike, Mechanistic homeostasis of vitamin D metabolism in the kidney through reciprocal modulation of Cyp27b1 and Cyp24a1 expression, *J Steroid Biochem Mol Biol*, 196 (2020) 105500.
- [14] J.E. Manson, S.S. Bassuk, J.E. Buring, V.R. Group, Principal results of the VITamin D and OmegA-3 Trial (VITAL) and updated meta-analyses of relevant vitamin D trials, *J Steroid Biochem Mol Biol*, 198 (2019) 105522.
- [15] R. Scragg, The Vitamin D Assessment (ViDA) study - Design and main findings, *J Steroid Biochem Mol Biol*, 198 (2019) 105562.
- [16] A.G. Pittas, B. Dawson-Hughes, P. Sheehan, J.H. Ware, W.C. Knowler, V.R. Aroda, I. Brodsky, L. Ceglia, C. Chadha, R. Chatterjee, C. Desouza, R. Dolor, J. Foreyt, P. Fuss, A. Ghazi, D.S. Hsia, K.C. Johnson, S.R. Kashyap, S. Kim, E.S. LeBlanc, M.R. Lewis, E. Liao, L.M. Neff, J. Nelson, P. O'Neil, J. Park, A. Peters, L.S. Phillips, R. Pratley, P. Raskin, N. Rasouli, D. Robbins, C. Rosen, E.M. Vickery, M. Staten, D.d.R. Group, Vitamin D Supplementation and Prevention of Type 2 Diabetes, *N Engl J Med*, 381 (2019) 520-530.
- [17] A. Prentice, Nutritional rickets around the world, *J Steroid Biochem Mol Biol*, 136 (2013) 201-206.

- [18] W. Li, K. Peregrina, M. Houston, L.H. Augenlicht, Vitamin D and the nutritional environment in functions of intestinal stem cells: Implications for tumorigenesis and prevention, *J Steroid Biochem Mol Biol*, 198 (2019) 105556.
- [19] M. Kanhere, J. He, B. Chassaing, T.R. Ziegler, J.A. Alvarez, E.A. Ivie, L. Hao, J. Hanfelt, A.T. Gewirtz, V. Tangpricha, Bolus Weekly Vitamin D3 Supplementation Impacts Gut and Airway Microbiota in Adults With Cystic Fibrosis: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial, *J Clin Endocrinol Metab*, 103 (2018) 564-574.
- [20] M.T. Cantorna, Y.D. Lin, J. Arora, S. Bora, Y. Tian, R.G. Nichols, A.D. Patterson, Vitamin D Regulates the Microbiota to Control the Numbers of ROR γ mat/FoxP3+ Regulatory T Cells in the Colon, *Front Immunol*, 10 (2019) 1772.
- [21] S. Christakos, S. Li, J. De La Cruz, N.F. Shroyer, Z.K. Criss, M.P. Verzi, J.C. Fleet, Vitamin D and the intestine: Review and update, *J Steroid Biochem Mol Biol*, 196 (2020) 105501.
- [22] M.J. Bolland, A. Grey, A. Avenell, Effects of vitamin D supplementation on musculoskeletal health: a systematic review, meta-analysis, and trial sequential analysis, *Lancet Diabetes Endocrinol*, 6 (2018) 847-858.
- [23] R. Ranathunga, T.R. Hill, J.C. Mathers, R.M. Francis, A. Prentice, I. Schoenmakers, T.J. Aspray, D.i.O.P.S.g. Vitamin, No effect of monthly supplementation with 12000 IU, 24000 IU or 48000 IU vitamin D3 for one year on muscle function: The vitamin D in older people study, *J Steroid Biochem Mol Biol*, 190 (2019) 256-262.

- [24] A.M.M. Vaes, M. Tieland, N. Toussaint, R. Nilwik, L.B. Verdijk, L.J.C. van Loon, L. de Groot, Cholecalciferol or 25-Hydroxycholecalciferol Supplementation Does Not Affect Muscle Strength and Physical Performance in Prefrail and Frail Older Adults, *J Nutr*, 148 (2018) 712-720.
- [25] A.T. Carswell, S.J. Oliver, L.M. Wentz, D.S. Kashi, R. Roberts, J.C.Y. Tang, R.M. Izard, S. Jackson, D. Allan, L.E. Rhodes, W.D. Fraser, J.P. Greeves, N.P. Walsh, Influence of Vitamin D Supplementation by Sunlight or Oral D3 on Exercise Performance, *Med Sci Sports Exerc*, 50 (2018) 2555-2564.
- [26] J.F. Aloia, M. Mikhail, M. Fazzari, S. Islam, L. Ragolia, J. Guralnik, Physical Performance and Vitamin D in Elderly Black Women-The PODA Randomized Clinical Trial, *J Clin Endocrinol Metab*, 104 (2019) 1441-1448.
- [27] M. Apaydin, A.G. Can, M. Kizilgul, S. Beysel, S. Kan, M. Caliskan, T. Demirci, O. Ozcelik, M. Ozbek, E. Cakal, The effects of single high-dose or daily low-dosage oral colecalciferol treatment on vitamin D levels and muscle strength in postmenopausal women, *BMC Endocr Disord*, 18 (2018) 48.
- [28] A. De Spiegeleer, D. Beckwee, I. Bautmans, M. Petrovic, G. Sarcopenia Guidelines Development group of the Belgian Society of Geriatrics, Pharmacological Interventions to Improve Muscle Mass, Muscle Strength and Physical Performance in Older People: An Umbrella Review of Systematic Reviews and Meta-analyses, *Drugs Aging*, 35 (2018) 719-734.
- [29] J.E. Manson, N.R. Cook, I.M. Lee, W. Christen, S.S. Bassuk, S. Mora, H. Gibson, D. Gordon, T. Copeland, D. D'Agostino, G. Friedenber, C. Ridge, V. Bubes, E.L.

Giovannucci, W.C. Willett, J.E. Buring, V.R. Group, Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease, *N Engl J Med*, 380 (2019) 33-44.

- [30] R. Scragg, K.T. Khaw, L. Toop, J. Sluyter, C.M.M. Lawes, D. Waayer, E. Giovannucci, C.A. Camargo, Jr., Monthly High-Dose Vitamin D Supplementation and Cancer Risk: A Post Hoc Analysis of the Vitamin D Assessment Randomized Clinical Trial, *JAMA Oncol*, 4 (2018) e182178.
- [31] J. Lappe, P. Watson, D. Travers-Gustafson, R. Recker, C. Garland, E. Gorham, K. Baggerly, S.L. McDonnell, Effect of Vitamin D and Calcium Supplementation on Cancer Incidence in Older Women: A Randomized Clinical Trial, *JAMA*, 317 (2017) 1234-1243.
- [32] J. Wactawski-Wende, J.M. Kotchen, G.L. Anderson, A.R. Assaf, R.L. Brunner, M.J. O'Sullivan, K.L. Margolis, J.K. Ockene, L. Phillips, L. Pottern, R.L. Prentice, J. Robbins, T.E. Rohan, G.E. Sarto, S. Sharma, M.L. Stefanick, L. Van Horn, R.B. Wallace, E. Whitlock, T. Bassford, S.A. Beresford, H.R. Black, D.E. Bonds, R.G. Brzyski, B. Caan, R.T. Chlebowski, B. Cochrane, C. Garland, M. Gass, J. Hays, G. Heiss, S.L. Hendrix, B.V. Howard, J. Hsia, F.A. Hubbell, R.D. Jackson, K.C. Johnson, H. Judd, C.L. Kooperberg, L.H. Kuller, A.Z. LaCroix, D.S. Lane, R.D. Langer, N.L. Lasser, C.E. Lewis, M.C. Limacher, J.E. Manson, Calcium plus vitamin D supplementation and the risk of colorectal cancer, *N.Engl.J.Med.*, 354 (2006) 684-696.
- [33] R.T. Chlebowski, K.C. Johnson, C. Kooperberg, M. Pettinger, J. Wactawski-Wende, T. Rohan, J. Rossouw, D. Lane, M.J. O'Sullivan, S. Yasmeeen, R.A. Hiatt, J.M. Shikany, M. Vitolins, J. Khandekar, F.A. Hubbell, Calcium plus vitamin D

supplementation and the risk of breast cancer, *J Natl Cancer Inst.*, 100 (2008) 1581-1591.

- [34] N. Keum, D.H. Lee, D.C. Greenwood, J.E. Manson, E. Giovannucci, Vitamin D supplementation and total cancer incidence and mortality: a meta-analysis of randomized controlled trials, *Ann Oncol*, 30 (2019) 733-743.
- [35] N. Keum, E. Giovannucci, Vitamin D supplements and cancer incidence and mortality: a meta-analysis, *Br J Cancer*, 111 (2014) 976-980.
- [36] G. Bjelakovic, L.L. Gluud, D. Nikolova, K. Whitfield, J. Wetterslev, R.G. Simonetti, M. Bjelakovic, C. Gluud, Vitamin D supplementation for prevention of mortality in adults, *Cochrane Database Syst Rev*, (2014) CD007470.
- [37] R. Chatterjee, J.K. Erban, P. Fuss, R. Dolor, E. LeBlanc, M. Staten, P. Sheehan, A. Pittas, D.d.R. Group, Vitamin D supplementation for prevention of cancer: The D2d cancer outcomes (D2dCA) study, *Contemp Clin Trials*, 81 (2019) 62-70.
- [38] R.E. Neale, B.K. Armstrong, C. Baxter, B. Duarte Romero, P. Ebeling, D.R. English, M.G. Kimlin, D.S. McLeod, O.C. RL, J.C. van der Pols, A.J. Venn, P.M. Webb, D.C. Whiteman, L. Wockner, The D-Health Trial: A randomized trial of vitamin D for prevention of mortality and cancer, *Contemp Clin Trials*, 48 (2016) 83-90.
- [39] Z. Hu, J. Chen, X. Sun, L. Wang, A. Wang, Efficacy of vitamin D supplementation on glyceic control in type 2 diabetes patients: A meta-analysis of interventional studies, *Medicine (Baltim)*, 98 (2019) e14970.
- [40] X. Li, Y. Liu, Y. Zheng, P. Wang, Y. Zhang, The Effect of Vitamin D Supplementation on Glycemic Control in Type 2 Diabetes Patients: A Systematic Review and Meta-Analysis, *Nutrients*, 10 (2018).

- [41] V. Upreti, V. Maitri, P. Dhull, A. Handa, M.S. Prakash, A. Behl, Effect of oral vitamin D supplementation on glycemic control in patients with type 2 diabetes mellitus with coexisting hypovitaminosis D: A parallel group placebo controlled randomized controlled pilot study, *Diabetes Metab Syndr*, 12 (2018) 509-512.
- [42] D.M. Khan, A. Jamil, F.A. Randhawa, N.F. Butt, U. Malik, Efficacy of oral vitamin D on glycated haemoglobin (HbA1c) in type 2 diabetics having vitamin D deficiency - A randomized controlled trial, *J Pak Med Assoc*, 68 (2018) 694-697.
- [43] D. Scott, A. Mousa, N. Naderpoor, M.P.J. de Courten, R. Scragg, B. de Courten, Vitamin D supplementation improves waist-to-hip ratio and fasting blood glucose in vitamin D deficient, overweight or obese Asians: A pilot secondary analysis of a randomised controlled trial, *J Steroid Biochem Mol Biol*, 186 (2019) 136-141.
- [44] M. Niroomand, A. Fotouhi, N. Irannejad, F. Hosseinpanah, Does high-dose vitamin D supplementation impact insulin resistance and risk of development of diabetes in patients with pre-diabetes? A double-blind randomized clinical trial, *Diabetes Res Clin Pract*, 148 (2019) 1-9.
- [45] K.M. Swart, P. Lips, I.A. Brouwer, R. Jorde, M.W. Heymans, G. Grimnes, M.R. Grubler, M. Gaksch, A. Tomaschitz, S. Pilz, G. Eiriksdottir, V. Gudnason, L. Wamberg, L. Rejnmark, C.T. Sempos, R.A. Durazo-Arvizu, K.G. Dowling, G. Hull, Z. Skrabakova, M. Kiely, K.D. Cashman, N.M. van Schoor, Effects of vitamin D supplementation on markers for cardiovascular disease and type 2 diabetes: an individual participant data meta-analysis of randomized controlled trials, *Am J Clin Nutr*, 107 (2018) 1043-1053.

- [46] R. Corcoy, L.C. Mendoza, D. Simmons, G. Desoye, J.M. Adelantado, A. Chico, R. Devlieger, A. van Assche, S. Galjaard, D. Timmerman, A. Lapolla, M.G. Dalfrà, A. Bertolotto, J. Harreiter, E. Wender-Ozegowska, A. Zawiejska, A. Kautzky-Willer, F.P. Dunne, P. Damm, E.R. Mathiesen, D.M. Jensen, L.L.T. Andersen, M. Tanvig, D.J. Hill, J.G. Jelsma, F.J. Snoek, H. Kofeler, M. Trotsmuller, P. Lips, M.N.M. van Poppel, The DALI vitamin D randomized controlled trial for gestational diabetes mellitus prevention: No major benefit shown besides vitamin D sufficiency, *Clin Nutr*, 39 (2020) 976-984.
- [47] S. Abedi, M. Taebi, M.H. Nasr Esfahani, Effect of Vitamin D Supplementation on Intracytoplasmic Sperm Injection Outcomes: A Randomized Double-Blind Placebo-Controlled Trial, *Int J Fertil Steril*, 13 (2019) 18-23.
- [48] L. Jiang, L. Ji, J. Song, K. Qian, The effect of serum vitamin D levels in couples on embryo development and clinical outcomes, *Reprod Biomed Online*, 38 (2019) 699-710.
- [49] K. Jueraitetibaik, Z. Ding, D.D. Wang, L.P. Peng, J. Jing, L. Chen, X. Ge, X.H. Qiu, B. Yao, The effect of vitamin D on sperm motility and the underlying mechanism, *Asian J Androl*, 21 (2019) 400-407.
- [50] M. Blomberg Jensen, J.G. Lawaetz, J.H. Petersen, A. Juul, N. Jorgensen, Effects of Vitamin D Supplementation on Semen Quality, Reproductive Hormones, and Live Birth Rate: A Randomized Clinical Trial, *J Clin Endocrinol Metab*, 103 (2018) 870-881.
- [51] E. Azizi, M. Najji, M. Shabani-Nashtaei, A. Aligholi, A. Najafi, F. Amidi, Association of serum content of 25-hydroxy vitamin D with semen quality in normozoospermic

and oligoasthenoteratozoospermic men, *Int J Reprod Biomed (Yazd)*, 16 (2018) 689-696.

[52] S.L. Mumford, R.A. Garbose, K. Kim, K. Kissell, D.L. Kuhr, U.R. Omosigho, N.J. Perkins, N. Galai, R.M. Silver, L.A. Sjaarda, T.C. Plowden, E.F. Schisterman, Association of preconception serum 25-hydroxyvitamin D concentrations with livebirth and pregnancy loss: a prospective cohort study, *Lancet Diabetes Endocrinol*, 6 (2018) 725-732.

[53] A.K. Amegah, M.K. Klevor, C.L. Wagner, Maternal vitamin D insufficiency and risk of adverse pregnancy and birth outcomes: A systematic review and meta-analysis of longitudinal studies, *PLoS One*, 12 (2017) e0173605.

[54] G. Santorelli, D. Whitelaw, D. Farrar, J. West, D.A. Lawlor, Associations of maternal vitamin D, PTH and calcium with hypertensive disorders of pregnancy and associated adverse perinatal outcomes: Findings from the Born in Bradford cohort study, *Sci Rep*, 9 (2019) 1205.

[55] D.E. Roth, M. Leung, E. Mesfin, H. Qamar, J. Watterworth, E. Papp, Vitamin D supplementation during pregnancy: state of the evidence from a systematic review of randomised trials, *BMJ*, 359 (2017) j5237.

[56] M. Tous, M. Villalobos, L. Iglesias, S. Fernandez-Barres, V. Arija, Vitamin D status during pregnancy and offspring outcomes: a systematic review and meta-analysis of observational studies, *Eur J Clin Nutr*, 74 (2020) 36-53.

[57] A. Maugeri, M. Barchitta, G. Favara, M.C. La Rosa, C. La Mastra, R. Magnano San Lio, A. Agodi, Maternal Dietary Patterns Are Associated with Pre-Pregnancy Body

Mass Index and Gestational Weight Gain: Results from the "Mamma & Bambino" Cohort, *Nutrients*, 11 (2019).

- [58] L. Rejnmark, L.S. Bislev, K.D. Cashman, G. Eiriksdottir, M. Gaksch, M. Grubler, G. Grimnes, V. Gudnason, P. Lips, S. Pilz, N.M. van Schoor, M. Kiely, R. Jorde, Non-skeletal health effects of vitamin D supplementation: A systematic review on findings from meta-analyses summarizing trial data, *PLoS One*, 12 (2017) e0180512.
- [59] D.E. Roth, S.K. Morris, S. Zlotkin, A.D. Gernand, T. Ahmed, S.S. Shanta, E. Papp, J. Korsiak, J. Shi, M.M. Islam, I. Jahan, F.K. Keya, A.R. Willan, R. Weksberg, M. Mohsin, Q.S. Rahman, P.S. Shah, K.E. Murphy, J. Stimec, L.G. Pell, H. Qamar, A. Al Mahmud, Vitamin D Supplementation in Pregnancy and Lactation and Infant Growth, *N Engl J Med*, 379 (2018) 535-546.
- [60] A. Zittermann, S. Pilz, H.K. Berthold, Serum 25-hydroxyvitamin D response to vitamin D supplementation in infants: a systematic review and meta-analysis of clinical intervention trials, *Eur J Nutr*, 59 (2020) 359-369.
- [61] K.A. Herrick, R.J. Storandt, J. Afful, C.M. Pfeiffer, R.L. Schleicher, J.J. Gahche, N. Potischman, Vitamin D status in the United States, 2011-2014, *Am J Clin Nutr*, (2019).
- [62] A.E. Cowan, S. Jun, J.J. Gahche, J.A. Tooze, J.T. Dwyer, H.A. Eicher-Miller, A. Bhadra, P.M. Guenther, N. Potischman, K.W. Dodd, R.L. Bailey, Dietary Supplement Use Differs by Socioeconomic and Health-Related Characteristics among U.S. Adults, NHANES 2011(-)2014, *Nutrients*, 10 (2018).
- [63] A.L. Darling, D.J. Blackbourn, K.R. Ahmadi, S.A. Lanham-New, Vitamin D supplement use and associated demographic, dietary and lifestyle factors in 8024

South Asians aged 40-69 years: analysis of the UK Biobank cohort, *Public Health Nutr*, 21 (2018) 2678-2688.

- [64] D.E. Roth, S.A. Abrams, J. Aloia, G. Bergeron, M.W. Bourassa, K.H. Brown, M.S. Calvo, K.D. Cashman, G. Combs, L.M. De-Regil, M.E. Jefferds, K.S. Jones, H. Kapner, A.R. Martineau, L.M. Neufeld, R.L. Schleicher, T.D. Thacher, S.J. Whiting, Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries, *Ann N Y Acad Sci*, 1430 (2018) 44-79.
- [65] P. Lips, K.D. Cashman, C. Lamberg-Allardt, H.A. Bischoff-Ferrari, B. Obermayer-Pietsch, M.L. Bianchi, J. Stepan, G. El-Hajj Fuleihan, R. Bouillon, Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: a position statement of the European Calcified Tissue Society, *Eur J Endocrinol*, 180 (2019) P23-P54.
- [66] S. Pilz, W. Marz, K.D. Cashman, M.E. Kiely, S.J. Whiting, M.F. Holick, W.B. Grant, P. Pludowski, M. Hilgsmann, C. Trummer, V. Schwetz, E. Lerchbaum, M. Pandis, A. Tomaschitz, M.R. Grubler, M. Gaksch, N. Verheyen, B.W. Hollis, L. Rejnmark, S.N. Karras, A. Hahn, H.A. Bischoff-Ferrari, J. Reichrath, R. Jorde, I. Elmadfa, R. Vieth, R. Scragg, M.S. Calvo, N.M. van Schoor, R. Bouillon, P. Lips, S.T. Itkonen, A.R. Martineau, C. Lamberg-Allardt, A. Zittermann, Rationale and Plan for Vitamin D Food Fortification: A Review and Guidance Paper, *Front Endocrinol (Lausanne)*, 9 (2018) 373.
- [67] S.A. Atkinson, J.C. Fleet, Canadian recommendations for vitamin D intake for persons affected by multiple sclerosis, *J Steroid Biochem Mol Biol*, 199 (2020) 105606.

[68] A.C. Ross, Taylor, C. L., Yaktine, A. L., Del Valle, H. B. , Dietary Reference Intakes for Calcium and Vitamin D, Institute of Medicine 2011.