Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

J Steroid Biochem Mol Biol. Author manuscript; available in PMC 2024 April 01.

Published in final edited form as:

J Steroid Biochem Mol Biol. 2023 April ; 228: 106247. doi:10.1016/j.jsbmb.2023.106247.

Highlights from the 24th workshop on vitamin D in Austin, September 2022

Mark B. Meyer[1], **Carlos Bernal-Mizrachi**[2], **Daniel D. Bikle**[3], **Madhu Biyani**[4], **Moray J. Campbell**[5], **Snehal N. Chaudhari**[6], **Sylvia Christakos**[7], **Sue A. Ingles**[8], **Megan M. Knuth**[9], **Seong Min Lee**[1], **Thomas S. Lisse**[10], **Eva S. Liu**[11], **Isabelle Piec**[12], **Lori A. Plum**[13], **Sudhaker D. Rao**[14], **Carmen J. Reynolds**[15], **Tom D. Thacher**[16], **John H. White**[17], **Margherita T. Cantorna**[18,*]

[1]Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, WI, USA.

[2]Department of Medicine VA Medical Center and Division of Endocrinology, Metabolism and Lipid Research, Washington University School of Medicine, St. Louis, MO, USA.

[3]Departments of Medicine and Endocrinology, University of California San Francisco and Veterans Affairs Medical Center, San Francisco, CA, USA.

[4]Nano Life Science Institute (WPI-NanoLSI) and Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Japan.

[5]Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH, USA.

[6]Department of Biological Chemistry and Molecular Pharmacology, Blavatnik Institute, Harvard Medical School, Boston, MA, USA.

[7]Department of Microbiology, Biochemistry, and Molecular Genetics, Rutgers, The State University of New Jersey, New Jersey Medical School, Newark, NJ, USA.

[8]Department of Preventive Medicine, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA, USA.

[9]Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

[10]Department of Biology and Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Coral Gables, FL, USA.

[11]Harvard Medical School and Division of Endocrinology, Diabetes, Hypertension, Brigham and Women's Hospital, Boston, MA, USA.

[12]Medical School, University of East Anglia, Norwich, UK.

[*]To whom correspondence should be addressed: Margherita T. Cantorna, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA, USA. mxc69@psu.edu. Phone: (814)863-2819.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[13]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.

[14]Director, Bone & Mineral Research Laboratory, Henry Ford Health, Detroit, MI, USA.

[15]Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA.

[16]Department of Family Medicine, Mayo Clinic, Rochester, MN, USA.

[17]Departments of Physiology and Medicine, McGill University, Montreal, QC, Canada.

[18]Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA, USA.

Overview

The 24th Workshop on Vitamin D was held at the Thompson Conference Center on the campus of University of Texas at Austin in Austin, Texas from September 7 – 9, 2022. The Workshop was organized by **Dr. Margherita Cantorna** (Scientific Chair, Pennsylvania State University), **Dr. Mark Meyer** (Assistant Scientific Chair, University of Wisconsin-Madison), **Dr. James Fleet** (President of the Vitamin D workshop, University of Texas at Austin), **Dr. JoEllen Welsh** (Chief Financial Officer of the Vitamin D workshop, Inc, SUNY Albany, NY) and **Nichole Ballard** (YesEvents, Inc.). The subject matter of the invited presentations were chosen by the Workshop Executive Committee as well as the Program Advisory Committee (http://www.vitamindworkshop.org). This was the first in-person workshop held in three years due to the COVID-19 global pandemic and associated restrictions (2020 was cancelled, 2021 was held virtually). The meeting drew 125 attendees from 17 countries with attendees from the United States, Western Europe, South America, Japan, Nepal, Mongolia, South Africa, Mexico, and others. The organizing committee invited 17 speakers leading the field with exciting publications in the vitamin D field over the past two calendar years. There were over 100 abstracts submitted for this meeting and from those abstracts the awardees, promoted talks, and plenary posters were selected. The committee was pleased to promote 27 of the top scoring abstracts to the podium, whose research are detailed in the sections below. The plenary poster session opened the meeting at the Opening Reception on Tuesday evening September 6th with 20 selected Plenary Posters covering a wide range of topics related to the actions of vitamin D in pregnancy, the intestine, the immune system, those with kidney disease, and cancer. Forty additional posters were presented on September 7th and 8th during the lunchtime break. The meeting was sponsored by the National Institutes of Health (NIH Institute on Aging), Heartland Assays, OPKO Health, Thermo-Fisher Scientific, the journal Nutrients, Faes Farma, and BioTech Pharmacal, Inc. These sponsors provided support for young investigator awardees, travel awards, and meeting costs.

There were 14 awards given out based on the scientific merit of the submitted abstracts or presented poster. These awards were in three categories: Tony Norman Young Investigator Awards (3), Ron Horst Awards (10), and the Nutrients Poster Award (1). The Anthony (Tony) Norman Young Investigator awards were given to the top scoring abstracts submitted by a Junior Faculty Member, Post-Doctoral Fellow, and Graduate Student. The 2022 Anthony Norman Young Investigator Junior Faculty awardee was **Dr. Madhu Biyani**

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(Kanazawa University, Japan) and her work on "A novel DNA aptamer for CYP24 inhibition exerts a therapeutic effect by enhancing anti-proliferative function of vitamin D_3 in lung cancer cells. The 2022 Anthony Norman Young Investigator Post-Doctoral Fellow awardee was **Dr. Megan Knuth** (University of North Carolina – Chapel Hill, USA) and her work "Developmental vitamin D deficiency alters adult liver energy metabolism pathways". The 2022 Anthony Norman Young Investigator Graduate Student awardee was **Stephanie Doms** (KU Leuven, Belgium) and her work "The vitamin D_3 analog WY1048 affects cortical bone directly through VDR induced signaling in osteoblast precursors". The Ron Horst travel awards, sponsored by Heartland Assays, were given to **Juhi Arora** (Penn State University, USA), **Shelby Bollen** (University of Nottingham, UK), **Cydney Dennis** (Virginia Commonwealth University, USA), **Nicole Froelich** (Penn State University, USA), **Sonya Ketchens** (Medical University of South Carolina, USA), **Satoko Kise** (Toyama Prefectural University, Japan), **Vanessa McGaughey** (University of Miami, USA), **Martyna Stachowicz-Suhs** (Hirszfeld Institute, Poland), **Serra Ucer Ozgurel** (University of Texas at Austin, USA), and **Natalie Watkins** (University of Texas at Austin, USA). Finally, **Kirsten Krieger** (University of Illinois at Chicago, USA) was the unanimous choice for the journal Nutrients' Best Poster and Presentation Award.

The meeting kicked off ***Session I*** with the keynote speaker **Dr. George Georgiou** from University of Texas at Austin and his talk entitled: "Molecular level decomposition of the identity, dynamics, and function of the constituent antibodies comprising the serum antibody repertoire in response to infection of vaccination". Dr. Georgiou's research is focused on understanding the molecular attributes of the B cell response in human health and disease and the discovery and preclinical development of enzyme and antibody therapeutics for cancer and inborn errors of metabolism [1, 2] Dr. Georgiou has received many prestigious accolades over his career including being named one of the "Top 20 Translational Researchers" by Nature Biotechnology and in the list of "100 Eminent Chemical Engineers of the Modern Era". Dr. Georgiou gave an excellent seminar detailing the immune response to infection as well as vaccination, which is an area of great interest due to the global COVID-19 pandemic. We learned that each individual has a specific B cell and T cell enrichment following either infection or vaccination and that vaccination drives specific B and T cell responses over time that may not always match with the current infection strain [3]. Dr. Georgiou's group has been cataloging these repertoires over the course of SARS-CoV2 and influenza infections to better guide therapeutics and treatments [3, 4]. Dr. Georgiou presented an out-of-field perspective for the Vitamin D Workshop on greater immunity challenges to infection, which was an excellent primer to the remainder of the first day of the Meeting.

Session II focused on ***Immunoregulation: from Autoimmunity to Infection*** and was chaired **by Dr. Margherita Cantorna** (Veterinary and Biomedical Sciences, Pennsylvania State University) and **Dr. Isabelle Piec** (Bioanalytical Facility, University of East Anglia). The session opened with an excellent overview of vitamin D regulated mechanisms of immunoregulation from **Dr. John White** (McGill University, Montreal). Dr. White focused on mechanisms of vitamin D regulation in innate and acquired immune cells (discussed below) and an exciting clinical study that provides evidence that clinically vitamin D

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

is effective in humans with autoimmune disease. Dr. White reported the results of the 25,000+ cohort of the VITAL randomized controlled trial [5]. The results revealed a 22% reduction in autoimmune disease over a 5-year period in the vitamin D treatment arm of the study, with the strongest effects observed over the last 3 years of the trial [5]. The effects of vitamin D to downregulate immune function would be consistent with the clinical effects reported in the VITAL randomized control trial [5]. Dr. White discussed the role of vitamin D as a regulator of T cell responses and introduced Dr. Behdad Afzali's work that showed T helper cells induced local production of $1,25(OH)_2D_3$ and stimulated a gene expression program that attenuated inflammation [6]. Dr. White summarized the expanding roles of $1,25(OH)_2D_3$-induced antimicrobial peptide gene expression in innate immune responses, with an emphasis on the emerging roles of antimicrobial peptides anti-viral as well as anti-bacterial peptides. It has been proposed that induction of cathelicidin by vitamin D is one mechanism whereby $1,25(OH)_2D_3$ might protect the lungs from influenza or SARS-CoV-2. **Dr. Juhi Arora** (Pennsylvania State University) showed the effects of vitamin D on animal models of influenza and SARS-CoV-2 infections [7]. Mice that were unable to produce $1,25(OH)_2D_3$ (*Cyp27b1* knockout) had decreased survival and increased lung damage compared to wild type mice following influenza infection [7]. Infection with SARS-CoV-2 caused upregulation in the vitamin D 1-a hydroxylase (*Cyp27b1*) and the vitamin D 24 hydroxylase (*Cyp24a1*) genes in the lungs [7]. High dose vitamin D treatments decreased lung damage and reduced the expression of IFNβ in the lung of the SARS-CoV-2 infected mice [7]. Vitamin D treatments and the ability to produce $1,25(OH)_2D_3$ were shown to be protect the lung by reducing inflammation following infection with influenza or SARS-CoV-2 [7]. **Dr. Lori Plum** (University of Wisconsin, Madison) discussed the role of vitamin D in regulating the B cell response. Antibodies are important for host-resistance to infections. Dr. Plum presented work that tested the impact of nutritional vitamin D-deficiency and vitamin D receptor expression in mice [8]. Dr. Plum found no negative ramifications on the production of antibodies in vitamin D deficient mice [8]. Similar results were found in mice in which the vitamin D receptor was eliminated [8]. Dr. Plum concluded that B-cell production of antibodies does not require vitamin D or the vitamin D receptor (VDR). **Dr. Isabelle Piec** (University of East Anglia) presented results of a human observational open-label cohort study evaluating the antibody response to Pfizer SARS-CoV-2 immunization in healthcare workers [9]. The production of antibodies against the virus increased sharply after the first dose of the Pfizer vaccine [9]. Although the Ab levels decreased, the timing of the second vaccine dose was effective for inducing additional antibodies before protection was lost [9]. Furthermore, Dr. Piec showed that higher 25(OH)D levels correlated with a better response to the first dose of the Pfizer vaccine in SARS-CoV-2 naïve participants [9]. However, higher 25(OH)D status did not increase protection from infection with omicron or other potential novel variants [9]. **Dr. Behdad Afzali** (National Institute of Diabetes and Digestive and Kidney Diseases, NIDDK, Bethesda) presented the last talk of the session and reported on the details of that work. Local complement signals at sites of inflammation regulated T cell-mediated inflammation [6]. Ligation of complement receptors induced *CYP27B1* locally, which resulted in the inhibition of the inflammatory program and the induction of *IL-10* in Th1 cells [6]. Mechanistically, $1,25(OH)_2D_3$ reprogrammed the Th1 transcriptome via epigenetic changes and the formation of super-enhancers that included the recruitment of VDR, c-JUN, STAT3,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and BACH2. In addition, genetic deficiency of either BACH2 or STAT3 inhibited the ability of vitamin D to induce *IL-10* in the Th1 cells [6]. Vitamin D and $1,25(OH)_2D_3$ reprogram Th1 cells to reduce the inflammatory signature and induce *IL-10* [6]. The session provided an update on the most recent information about the role of vitamin D as a regulator of T and B cells and the host response to infection.

Session III: Life Cycle – Pregnancy was moderated by **Dr. John White**, McGill University and **Dr. Megan Knuth**, University of North Carolina. Session III opened with **Dr. Margherita Cantorna's** (Pennsylvania State University) talk entitled "The role of the VDR and the effect of vitamin D deficiency on the development of immune cells". The talk focused on the role of VDR expression and the effect of vitamin D deficiency on immune cell development [10]. Using VDR reporter mice expressing tdTomato protein under the control of the VDR promoter, the authors investigated the effect of vitamin D deficiency on immune cell expression of tdTomato in early immune cell progenitors in the fetal livers of embryonic day 15.5 mice. Developmental vitamin D deficiency (D-) significantly reduced the frequency of the tdTomato reporter in fetal livers from 39% to less than 5%. The authors data suggested that vitamin D deficiency during development could affect the ability of the immune system to be regulated by vitamin D. The second talk in session III was delivered by **Dr. Carol Wagner's** (Medical University of South Carolina) gave a talk on "The effect of maternal vitamin D status during lactation on the human milk proteome" . Here the authors sought to determine if maternal vitamin D status during lactation was associated with measurable differences in the breastmilk proteome. In this study, 40 women were randomized to receive a daily multivitamin containing 400 IU vitamin D_3 and either placebo gummies (0 IU vitamin D_3) or 6400 IU D_3 given as gummies starting at one-month post-partum until 4 months. 1,250 milk proteins were identified using LC-MS/MS and the authors found that the levels of these proteins responded to maternal vitamin D status with distinct patterns in the milk of women who have low vs. higher total circulating 25(OH)D concentrations. **Dr. Sonya Ketchen**, also from the Medical University of South Carolina, presented a study titled "Supplementation of vitamin D in Black American pregnant women to decrease adverse pregnancy outcomes". Women who presented for prenatal care at ≤14 weeks' gestation were randomized into one of two treatment regimens of vitamin D_3 [400 IU (control) or 4400 IU of vitamin D_3/day; n=66]. The primary outcome was comorbidities of pregnancy as a function of maternal 25(OH)D area under the curve. The authors concluded that vitamin D supplementation may have utility in reducing adverse pregnancy outcomes in at-risk black American women. The session closed with a talk by **Dr. Jane Cleal** (University of Southampton, UK) titled "Placental uptake and metabolism of 25(OH)D determines its activity within the fetoplacental unit" [11]. The talk emphasized that the fetal supply of 25(OH)D was dependent on placental function rather than simply maternal 25(OH)D levels. The data suggested that the transcriptional responses to $1,25(OH)_2D$ on the placental transcriptome and proteome mapped to gene pathways central to placental function and thereby fetal development [11]. There is a complex interplay between vitamin D and the placenta that controls to support fetal development and maternal adaptations during pregnancy [11]. Overall the session outlined several key factors controlled by vitamin D during pregnancy and potential impacts for the health of the fetus and mother during development.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The topic of ***Session IV*** was ***Life Cycle: Aging and Metabolism***, and it was chaired by **Dr. Carlos Bernal-Mizrachi** (St. Louis VA Medical Center and Washington University, St. Louis, MO). This session emphasized the effects of the VDR signaling on skeletal muscle and energy homeostasis in humans and rodents. The first talk was presented by **Dr. Philip Atherton** (University of Nottingham, UK), who described the importance of the VDR in skeletal muscle mass, function, and regeneration using mouse genetic models of gain and loss of VDR function [12]. He showed that overexpression of VDR in the anterior tibialis muscle induces increased protein synthesis, causing skeletal muscle hypertrophy. Moreover, Dr. Atherton showed that loss of VDR function was associated with muscle atrophy with less protein content and lower myofiber area by activating autophagy in myocytes with no changes in protein synthesis [12]. These data suggest that targeting VDR signaling in skeletal muscle could be a therapeutic alternative for muscle homeostasis. The second talk was presented by **Dr. Megan Knuth** (University of North Carolina at Chapel Hill, NC) reported on the developmental effects of vitamin D deficiency that result in higher adult adiposity. Using a previously published mouse model, the transient effects of vitamin D deficiency during development resulted in increased body weight and liver transcriptional and metabolic changes in energy metabolism pathways [13]. The data suggests that there are parental mediated effects of developmental vitamin D deficiency that modify offspring susceptibility to adiposity. In the third talk, **Dr. Jeffrey Roizen** (University of Pennsylvania, PA) showed that increasing vitamin D in mice within the normal range from 20-30 ng/dL to above 30 ng/dL increased lean mass without any change in weight by redistribution of calories from fat to muscle. Thus, high-dose dietary vitamin D affects increased calorie allocation to the muscle by manipulating energy needs and storage. Finally, **Dr. Shelby Bollen** (University of Nottingham, UK) presented a talk titled “VDR and vitamin D binding protein polymorphisms are associated with skeletal muscle function and physiology in elite master athletes”. The influence of 6 VDR single nucleotide polymorphisms (SNPs) and 2 vitamin D binding protein SNPs upon neuromuscular performance and musculoskeletal characteristics in elite master athletes and non-athletic controls was determined. The data demonstrated that there one VDR SNP (A1012G) was associated with greater handgrip strength and increased levels of serum sex hormone-binding globulin. Individuals carrying the vitamin D binding protein SNP, rs704, had reduced bone mineral density. Together the data confirmed the influences of VDR and vitamin D binding protein SNPs on muscle physiology and function.

Session V was titled ***Vitamin D Does That?*** and was chaired by **Dr. Lori Plum** (Department of Biochemistry, University of Wisconsin, Madison, WI) and **Dr. Sudaker Rao** (Director, Henry Ford Health, Bone & Mineral Research Laboratory, Detroit, MI). **Dr. Luke Peppone** from the University of Rochester Medical Center (Rochester, NY), reported on data from 2 randomized control studies on the effects of vitamin D on cancer induced bone loss. The study demonstrated that high dose vitamin D treatment effectively reduced bone loss in the hip and the largest benefit was in individuals with lower 25(OH)D levels at baseline. The authors concluded that vitamin D therapy may be important in preventing bone loss in cancer patients on sex-steroid inhibitors. **Dr Sakoto Kise** from Toyama Prefectural University (Imizu, Japan) presented data from a rat model of type II rickets. The data further demonstrated the preclinical potential of gene therapy to treat alopecia in the rats

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

with type II rickets. The final presentation of the session was from **Dr. Daniel D. Bikle** in the Department of Medicine and Endocrinology at the University of California, San Francisco, CA. Dr. Bikle presented work that investigated the role of VDR in the regulation of epidermal regeneration [14]. Deleting the VDR in keratinocytes prevented wound healing. VDR expression in keratinocytes regulates normal epidermal stem cell activation through interactions with p53/p63 signaling [14]. Together the session reported on the effects of vitamin D to prevent cancer induced bone loss in the clinic and the effects of VDR in reversing alopecia and as a regulator of wound healing in the skin.

Session VI focused on ***VDR and the Signals We Keep – Genomics and Signaling*** which was chaired by **Dr. Moray Campbell** (Ohio State University). The application of genomic approaches has massively increased in the vitamin D receptor field, as in all of biology. This was evident at the 2022 meeting with multiple talks across the workshop built around insights generated by high dimensional data approaches to capturing and defining the biology of this nuclear receptor. Within this focused session the three talks likewise yielded novel insights into some well-appreciated signaling aspects of the VDR. First was **Dr. Ayse Kilic** and team from Brigham and Women's Hospital. These investigators investigated the effects of vitamin D on the regulation of two genomic loci associated with chronic inflammation and the risk for asthma. By combining clinical samples from the VDAART clinical trial of maternal vitamin D supplementation and a murine model of lung inflammation they were able to develop an impactful statistical framework to link VDR genomic interactions that regulated Th2 immunity and asthma risk. The second presentation was from **Dr. Morgan Ritter** and colleagues at North Carolina State University who examined how VDR impacts behavioral development in Zebrafish. The zebrafish model provides unique opportunities in terms of genetic manipulation and behavioral analyses [15]. Remarkably, in larvae with a dominant negative *Vdr*, heat shock challenge led to changes in behavior in light/dark responses that persisted to adulthood, demonstrating potential neural impact of VDR on the earliest stages of development. Finally, **Dr. Nicole Ball** and team from University of East Anglia, UK investigated one of the most highly-studied VDR-regulated genes, the *CYP24A1*. Using convergent resources, the team combined insights from a pivotal clinical cohort of patients with idiopathic infantile hypercalcemia with mechanistic studies in vitro. The work showed that patients without coding mutations in the *CYP24A1* gene instead had mutations in the 3'UTR that impacted mRNA folding structure of the transcript, and impacted the cell's ability to metabolize $1\alpha,25(OH)_2D_3$. Understanding the structure-function relationships that affect *CYP24A1* RNA translation and protein expression is needed for a better understanding of the molecular basis of disease pathogenesis and potential treatments.

The title of ***Session VII*** was ***What the Gut! Microbes and the Intestine*** and (Moderators: **Dr. Sylvia Christakos** and **Dr. Seong Min Lee**) started with a presentation by **Dr. James Fleet** (University of Texas at Austin) on the integration of genomic and physiologic data to understand how vitamin D signaling regulates intestinal biology. Dr. Fleet provided an excellent overview of recent collaborative studies (between the Fleet, Christakos and Verzi labs) using RNA-seq, VDR ChIP-seq and ATAC-seq to examine $1,25(OH)_2D_3$ target genes and mechanisms involved in $1,25(OH)_2D_3$ molecular actions across the proximal-distal

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and crypt villus axes of the intestine [16]. Gene ontology analysis indicated vitamin D-mediated regulation of unique biological functions that are independent of those that control calcium homeostasis [16]. This includes regulation of RNA metabolic processes, metabolism of xenobiotics and lipid metabolic processes. The findings showed that the majority of $1,25(OH)_2D_3$ regulated transcripts in crypts, villi, small intestine and colon have compartment-specific regulation patterns of expression that may account for intestine compartment-specific functions of $1,25(OH)_2D_3$ [16]. This was followed by a presentation by **Dr. Natalie Watkins** (University of Texas at Austin) that showed how intestinal epithelial cell deletion of *Cyp24a1* reduces renal *Cyp27b1* and enhances intestinal *Trpv6* mRNA levels. These findings suggest a role for CYP24A1 both in local regulation of $1,25(OH)_2D_3$ action and also in the modulation of renal synthesis of $1,25(OH)_2D_3$. The last presentation in this session by **Dr. Snehal Chaudhari** (Harvard University/University of Wisconsin-Madison) showed that in the sleeve gastrectomy mouse model of bariatric surgery, lithocholic acid is increased in the mouse portal vein post sleeve gastrectomy and by activating the vitamin D receptor induces hepatic sulfotransferase expression to drive cholic acid-7-sulfate production, a molecule with anti-diabetic properties [17]. These findings suggest a mechanism by which vitamin D supplementation may improve post-sleeve gastrectomy patient outcomes.

Session VIII was entitled ***Expanding our understanding of vitamin D metabolism*** and was chaired by **Dr. Carmen Reynolds** (Mayo Clinic, Rochester) and ***Dr. Eva Liu*** (Brigham and Women's Hospital, Harvard Medical School, Boston). **Dr. Mark Meyer** (University of Wisconsin, Madison) opened session 8 with a talk entitled "The rapid genomic mechanisms controlling renal vitamin D metabolism." Dr. Meyer reviewed the detailed genomic mechanisms by which the *Cyp27b1* gene was found to be regulated in the kidney via distinct enhancers for parathyroid hormone (PTH) activation and fibroblast growth factor (FGF)23 and $1,25(OH)_2D_3$ suppression [18, 19]. Through elimination of these enhancers using CRISPR/Cas9, their group was able to create an animal model with extremely low circulating $1,25(OH)_2D_3$ and skeletal phenotypes similar to the *Cyp27b1* global knockout mouse [18, 19]. This rescued mouse still had the ability to regulate *Cyp27b1* expression in non-renal tissues, more specifically the immune system in response to inflammation. They are currently using these animals to probe non-renal production of $1,25(OH)_2D_3$ through mass spectrometry imaging techniques and through supplementation studies. Dr. Meyer went on to show that the activating cascade of coactivators powering the PTH upregulation of *Cyp27b1*, may also be involved in the suppressive mechanism of $1,25(OH)_2D_3$ on *Cyp27b1* [18, 19]. Next, **Dr. Martin Kaufmann** (Queen's University, Kingston) presented a talk for **Dr. Glenville Jones** (Queen's University, Kingston) entitled "R396W mutation of CYP24A1: a humanized preclinical model of infantile Hypercalcemia Type I"[20]. The team developed a model for Infantile hypercalcemia Type 1 (HCINF-1) by expressing a variant of CYP24A1 containing the common R396W loss-of-function mutation in mice [20]. The phenotypical resemblance of this knock-in mouse to HCINF-1 patients was particularly striking, and included hypercalcemia, nephrolithiasis and impaired bone healing [20]. The R396W knock-in mouse model may more-accurately resemble HCINF-1 patients than the *Cyp24a1* knockout mouse first published in 2000; because the *Cyp24a1* gene is expressed, recapitulating the effect of hypomorphic mutations, and one strain exhibited incomplete

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

penetrance [20]. Further assessments will follow for determining appropriate treatment for HCINF-1. **Dr. Etienne Sochett** (Hospital for Sick Children, Toronto) presented a talk titled “Rifampin use in children with idiopathic infantile hypercalcemia.” Dr. Sochett and his group treated 5 children with idiopathic infantile hypercalcemia, characterized by high levels of $1,25(OH)_2D$ leading to hypercalcemia and hypercalciuria independent of PTH action, with 5 mg/kg/day rifampin and 10 mg/kg/day rifampin each for two months and separated by a two-month washout. Both doses of rifampin decreased serum levels of $1,25(OH)_2D$, $1,25(OH)_2D/25(OH)D$ ratio and the $24,25(OH)_2D/25(OH)D$ ratio, but did not normalize hypercalcemia or hypercalciuria. It was observed that rifampin was effective for patients with CYP24A1 mutations, supporting future studies with prolonged use of rifampin in this cohort of children and suggesting that the genetic cause of idiopathic infantile hypercalcemia may affect the response to therapy. This session was concluded by **Dr. Martin Kaufmann** (Queen’s University, Kingston) presenting “Clinical utility of measuring the serum vitamin D metabolome including $24,25(OH)_2D_3$ by liquid chromatography-tandem mass spectrometry.” Dr. Kaufmann and colleagues developed a LC-MS/MS assay which can measure multiple serum vitamin D metabolites in pathways of bioactivation and catabolism; termed ‘vitamin D metabolomics’. The diagnostic value of elevated ratios of 25-OH-D_3-to-24,25-$(OH)_2D_3$ in identifying patients with hypercalcemia due to CYP24A1 mutation (HCINF-1) was shown in over 50 cases. When hypercalcemia is caused by other factors, the ratio remains normal. The group showed that altered levels of other lower-abundance metabolites such as 1,25-$(OH)_2D_3$ and 25-OH-D_3-26,23-lactone were important for identifying certain hypercalcemia patients, when CYP24A1 mutation was ruled out. These rare cases of hypercalcemia appear to be caused by hypersensitivity to normal vitamin D levels. The assay has also been used to study vitamin D metabolism *in vitro* and in mouse models; work that has been important for understanding vitamin D metabolism in humans. Vitamin D metabolomics is useful for helping to determine the cause of vitamin D-related hypercalcemia, and in the selection of appropriate treatment.

In ***Session IX***, titled “***The Bones Have It,***” investigators presented research findings related to the effects of vitamin D in bone. These studies expanded our understanding of the metabolic effects of vitamin D and its metabolites on bone. **Dr. Eva Liu (Brigham and Women’s Hospital/Harvard)** presented the “Role of 1,25 dihydroxyvitamin D in regulating enthesopathy development in a mouse model of X-linked hypophosphatemia”. Adults with X-linked hypophosphatemia develop a painful mineralization of the bone-tendon attachment site (enthesis), called enthesopathy. In this presentation, Liu, *et. al.* examined entheses from mice lacking CYP27B1, FGF23, and compared them to the mouse model of X-linked hypophosphatemia [21]. The data show that although $1,25(OH)_2D$ therapy can prevent enthesopathy if started early in post-natal development, initiation of $1,25(OH)_2D$ therapy after enthesopathy has already developed does not attenuate the abnormal enthesis phenotype. These studies demonstrate that impaired $1,25(OH)_2D$ action contributes to the enthesopathy seen in X-linked hypophosphatemia and emphasize the importance of early $1,25(OH)_2D$ therapy in those affected with X-linked hypophosphatemia. **Stefanie Doms** (KU Leuven) presented a talk titled “The vitamin D3 analog, WY1048, affects cortical bone directly through VDR induced signaling in osteoblast precursors”. Ms. Doms was the graduate student recipient of the Tony Norman award. **Dr. Seong Min Lee (University of**

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Wisconsin-Madison) presented a talk titled “A complex genomic mechanism governs the regulation of FGF23 expression in response to $1,25(OH)_2D_3$, phosphate and inflammation”. The work presented described the molecular mechanisms through which FGF23 expression is increased in response to $1,25(OH)_2D_3$ in bone *in vivo* in advances from his previous work [22]. The data show that a 170 bp long regulatory region near the FGF23 gene is responsible for $1,25(OH)_2D_3$-mediated FGF23 induction *in vivo*. The work further demonstrated that several *in vitro* identified vitamin D response elements were inactive *in vivo*. **Dr. Serra Ucer Ozgurel** (University of Texas at Austin) presented a talk titled “Male LRP5 A214V mutant mice with genetically programmed high bone mass have disruption of the vitamin D endocrine system”. The talk was focused on describing how environmental and genetic factors interact to impact bone. Mice with genetically programmed high bone formation were challenged with inadequate dietary calcium, but the mice still maintained high bone mass. Other data showed that the mice ($LRP5^{A214V}$) were able to maintain high bone mass by limiting pro-resorptive serum $1,25(OH)_2D_3$ signals, increasing expression of molecular events controlling renal Ca retention and increasing intestinal Ca absorption. The $LRP5^{A214V}$ mutation induces changes in vitamin D production and signaling that permit mice to retain more Ca that results in a high bone mass phenotype. **Dr. Lieve Verlinden** (KU Leuven) talk was titled “Osteoblast-specific deletion of neuropilin 2 results in trabecular and cortical bone loss in male mice”. Dr. Verlinden’s talk reported on the sex specific effects of the $1,25(OH)_2D_3$ target-neuropilin 2 in bone cells for the control of bone homeostasis. **Dr. Sudhaker Rao** (Henry Ford Health) presented the final talk of the session titled “Effect of vitamin D metabolites on bone histomorphometry in healthy black and white women: an attempt to unravel the so-called vitamin D paradox in blacks”[23]. The talk discussed the vitamin D paradox in black individuals that have low serum 25(OH)D levels but high bone mineral densities. The study reported that although serum 25(OH)D were lower in black women versus white women, black women had higher serum $1,25(OH)_2D$ levels and greater trabecular thickness than white women [23]. However, there were no significant differences in other bone structural histomorphometric variables between the two groups of women [23]. Dr. Rao concluded that the higher serum $1,25(OH)_2D$ levels in blacks may help preserve bone mass and explain the presence of low 25(OH)D and higher trabecular bone mineral in black versus white women [23].

The final day of the Vitamin D Workshop began with ***Session X***, which was chaired by **Dr Sue Ingles** (USC, Keck School of Medicine) and **Dr Thomas Lisse** (University of Miami Florida) and addressed recent developments in ***Vitamin D Cancer - Health Disparities and Treatments*. Dr. Clayton Yates** (Tuskegee University) opened the session with a talk titled “Prostate cancer and vitamin D: GWAS, Skin Color, and Health Disparities”. Dr. Yates described his teams work on the prevalence, incidence, and treatments of prostate cancer in populations with dark skin colors from different global locations. **Dr. Madhu Biyani** (Kanazawa University, Japan) presented a talk titled “A novel DNA aptamer for CYP24 inhibition exerts a therapeutic effect by enhancing anti-proliferative function of vitamin D in lung cells” [24]. The talk described a method for identifying and optimizing DNA-derived aptamers capable of sensitizing the anti-cancer effects of vitamin D on cancer cells. Low vitamin D levels, as well as an increased CYP24A1 activity in cancer patients metabolically inactivates vitamin D and is linked to a poor prognosis. Molecules

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

that inhibit CYP24A1 activity can be used as antiproliferative agents in cancer therapy. Dr. Biyani demonstrated that an endocytosed aptamer that targeted CYP24A1 activity sensitized lung adenocarcinoma cells to the anti-proliferative effects of $1,25(OH)_2D$ [24]. Overall, the findings indicate that treatment strategies involving CYP24A1-targeting DNA aptamers could be a promising cancer therapy in the clinic. **Dr. Cydney Dennis** (Virginia Commonwealth University) presented her research on the anti-tumorigenic effects of the vitamin D metabolite, 24,25-dihydroxycholecalciferol (24R,25), on laryngeal cancer cells. Dr. Dennis reported that after treating estrogen-responsive laryngeal cancer cell lines with 24R,25, apoptosis markers and caspase 9 activity decreased, whereas estrogen-resistant cells showed the opposite results. To better understand the potential membrane-mediated signaling processes underlying these findings, 24R,25 was found to increase phospholipase D activity in estrogen-responsive cells but not in estrogen-resistant cells. The findings suggest that 24R,25 may be used to treat various types of laryngeal cancer depending on their molecular profile. **Dr. Rocio Garcia-Becerra** (Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico) gave the final talk of Session X, titled "Reestablishment of the anti-tumoral effects of anti-estrogens by $1,25(OH)_2D$ in triple-negative breast cancer". The talk described the effects of $1,25(OH)_2D$ and the $1,25(OH)_2D$ analog EB1089 on expression of the estrogen receptor alpha in triple-negative breast cancer models [25]. The results showed that EB1089 and $1,25(OH)_2D$ induced estrogen receptor alpha expression in both *in vitro* and *in vivo* models, which sensitized the cancer cells to treatment with the anti-estrogen fulvestrant that inhibited cancer cell proliferation, tumor volume, and cellular metabolism. Thus, treating triple-negative breast cancer patients with anti-estrogens and vitamin D agonists in combination may represent a novel and effective therapeutic approach.

Session XI: Not Just Another Micronutrient – Nutrition and Vitamin D (Moderators: **Dr. Dan Bikle** (University of San Francisco) and **Dr. Snehal Chaudhari** (Harvard Medical School) was the last session of the 2022 Vitamin D Workshop and delved into investigating the role of nutritional vitamin D in supporting a healthy metabolism. **Dr. Susan A. Lanham-New** from the University of Surrey, UK presented a talk titled "Differential effects of vitamin D_2 and vitamin D_3 on vitamin D metabolism in health and disease". Dr. Lanham-New and her research group conducted the largest (to date) randomized controlled trial in healthy European and South Asian adults and found that vitamin D_3 was twice as effective as vitamin D_2 for raising total 25(OH)D levels [26]. Furthermore, analyses of the effect of vitamin D_3 supplementation on the human transcriptome showed that the majority of changes in gene expression reflected a downregulation in transcriptional levels of genes involved in innate and adaptive immune pathways in circulating blood [26]. Next, **Dr. Tom Thacher** from the Mayo Clinic discussed their findings on associations between vitamin D metabolites and rickets in children. Using a multivariable logistic model accounting for serum vitamin D metabolite levels, the study discovered that children with rickets associated with significantly higher circulating levels of $1,25(OH)_2D$ and lower 25(OH)D levels. Mechanistically, elevated $1,25(OH)_2D$ resulted from calcium deficiency in children, leading to increased FGF23 levels further inducing progression of rickets. Continuing the investigation on the importance of vitamin D on bone development, **Dr. Sue Shapses** from Rutgers University showed that vitamin D deficiency impaired biomechanical

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

strength of bone and led to weight gain in older mice. While vitamin D deficiency did not result in overt bone mineral density or content changes, the study showed that lack of vitamin D in mice associated with stiffer bones and higher energy intake. A key question in the study of vitamin D deficiency is whether obesity is a consequence or cause of impaired vitamin D expenditure. To address this question, **Dr. Sophie Davies** from the University of Bath measured vitamin D expenditure in healthy and obese individuals using a stable isotope tracer technique. Using analytical methods to quantify plasma levels of deuterated 25(OH)D_3, the group found that even in individuals with large differences in adiposity and physical activity, vitamin D expenditure is consistent. Whether exercise can beneficially modulate vitamin D expenditure was proposed as ongoing/future work. Lastly, to close out the session and the Vitamin D Workshop, **Dr. Roger Bouillon** from Leuven, Belgium presented evidence to support the use of calcifediol (25(OH)D) in fortification programs in place of vitamin D [27, 28]. With superior intestinal absorption and more potent bioactivity, calcifediol administration resulted in a faster increase in serum levels, correcting for deficiency rapidly. Further, Dr. Bouillon discussed the consistency in calcifediol absorption irrespective of prevalence of metabolic diseases, including obesity, intestinal fat malabsorption or renal failure. This session tied mechanistic insights with future perspectives on nutritional fortification of vitamin D metabolites in correcting metabolic diseases.

Acknowledgements

The meeting was sponsored by the National Institutes of Health (NIH Institute on Aging) grant R13 AG48689-01, Heartland Assays, OPKO Health, Thermo-Fisher Scientific, the journal Nutrients, Faes Farma, and BioTech Pharmacal, Inc.

References

1. Delidakis G, et al. , Improving Antibody Therapeutics by Manipulating the Fc Domain: Immunological and Structural Considerations. Annu Rev Biomed Eng, 2022. 24: p. 249–274. [PubMed: 35363537]
2. Cantor JR, et al. , Engineering reduced-immunogenicity enzymes for amino acid depletion therapy in cancer. Methods Enzymol, 2012. 502: p. 291–319. [PubMed: 22208990]
3. Goike J, et al. , Synthetic repertoires derived from convalescent COVID-19 patients enable discovery of SARS-CoV-2 neutralizing antibodies and a novel quaternary binding modality. bioRxiv, 2021.
4. Kuraoka M, et al. , Infant Antibody Repertoires during the First Two Years of Influenza Vaccination. mBio, 2022. 13(6): p. e0254622. [PubMed: 36314798]
5. Hahn J, et al. , Vitamin D and marine omega 3 fatty acid supplementation and incident autoimmune disease: VITAL randomized controlled trial. BMJ, 2022. 376: p. e066452. [PubMed: 35082139]
6. Chauss D, et al. , Autocrine vitamin D signaling switches off pro-inflammatory programs of TH1 cells. Nat Immunol, 2022. 23(1): p. 62–74. [PubMed: 34764490]
7. Arora J, et al. , Vitamin D and the Ability to Produce 1,25(OH)2D Are Critical for Protection from Viral Infection of the Lungs. Nutrients, 2022. 14(15).
8. Plum LA, et al. , Antibody production in mice requires neither vitamin D, nor the vitamin D receptor. Front Immunol, 2022. 13: p. 960405. [PubMed: 36341456]
9. Piec I, et al. , Age and vitamin D affect the magnitude of the antibody response to the first dose of the SARS-CoV-2 BNT162b2 vaccine. Curr Res Transl Med, 2022. 70(3): p. 103344. [PubMed: 35390564]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

10. Arora J, et al. , Novel insight into the role of the vitamin D receptor in the development and function of the immune system. J Steroid Biochem Mol Biol, 2022. 219: p. 106084. [PubMed: 35202799]
11. Ashley B, et al. , Placental uptake and metabolism of 25(OH)vitamin D determine its activity within the fetoplacental unit. Elife, 2022. 11.
12. Bollen SE, et al. , The Vitamin D/Vitamin D receptor (VDR) axis in muscle atrophy and sarcopenia. Cell Signal, 2022. 96: p. 110355. [PubMed: 35595176]
13. Xue J, et al. , Maternal Liver Metabolic Response to Chronic Vitamin D Deficiency Is Determined by Mouse Strain Genetic Background. Curr Dev Nutr, 2020. 4(8): p. nzaa106. [PubMed: 32851199]
14. Bikle DD, Role of vitamin D and calcium signaling in epidermal wound healing. J Endocrinol Invest, 2022.
15. Knuth MM, et al. , Vitamin D deficiency promotes accumulation of bioactive lipids and increased endocannabinoid tone in zebrafish. J Lipid Res, 2021. 62: p. 100142. [PubMed: 34673019]
16. Aita R, et al. , Genomic analysis of 1,25-dihydroxyvitamin D(3) action in mouse intestine reveals compartment and segment-specific gene regulatory effects. J Biol Chem, 2022. 298(8): p. 102213. [PubMed: 35779631]
17. Chaudhari SN, et al. , A microbial metabolite remodels the gut-liver axis following bariatric surgery. Cell Host Microbe, 2021. 29(3): p. 408–424 e7. [PubMed: 33434516]
18. Meyer MB, et al. , Rapid genomic changes by mineralotropic hormones and kinase SIK inhibition drive coordinated renal Cyp27b1 and Cyp24a1 expression via CREB modules. J Biol Chem, 2022. 298(11): p. 102559. [PubMed: 36183832]
19. Meyer MB, et al. , Targeted genomic deletions identify diverse enhancer functions and generate a kidney-specific, endocrine-deficient Cyp27b1 pseudo-null mouse. J Biol Chem, 2019. 294(24): p. 9518–9535. [PubMed: 31053643]
20. St-Arnaud R, et al. , Vitamin D and Diseases of Mineral Homeostasis: A Cyp24a1 R396W Humanized Preclinical Model of Infantile Hypercalcemia Type 1. Nutrients, 2022. 14(15).
21. Yuan Y, et al. , Impaired 1,25 dihydroxyvitamin D3 action and hypophosphatemia underlie the altered lacuno-canalicular remodeling observed in the Hyp mouse model of XLH. PLoS One, 2021. 16(5): p. e0252348. [PubMed: 34043707]
22. Lee SM, et al. , A Control Region Near the Fibroblast Growth Factor 23 Gene Mediates Response to Phosphate, 1,25(OH)2D3, and LPS In Vivo. Endocrinology, 2019. 160(12): p. 2877–2891. [PubMed: 31599948]
23. Qiu S, Divine G, and Rao SD, Effect of vitamin D metabolites on bone histomorphometry in healthy black and white women: An attempt to unravel the so-called vitamin D paradox in blacks. Bone Rep, 2023. 18: p. 101650. [PubMed: 36588780]
24. Biyani M, et al. , Novel DNA Aptamer for CYP24A1 Inhibition with Enhanced Antiproliferative Activity in Cancer Cells. ACS Appl Mater Interfaces, 2022. 14(16): p. 18064–18078. [PubMed: 35436103]
25. Santos-Martinez N, et al. , Calcitriol induces estrogen receptor alpha expression through direct transcriptional regulation and epigenetic modifications in estrogen receptor-negative breast cancer cells. Am J Cancer Res, 2021. 11(12): p. 5951–5964. [PubMed: 35018235]
26. Durrant LR, et al. , Vitamins D(2) and D(3) Have Overlapping But Different Effects on the Human Immune System Revealed Through Analysis of the Blood Transcriptome. Front Immunol, 2022. 13: p.790444. [PubMed: 35281034]
27. Bouillon R, Antonio L, and Olarte OR, Calcifediol (25OH Vitamin D(3)) Deficiency: A Risk Factor from Early to Old Age. Nutrients, 2022. 14(6).
28. Quesada-Gomez JM, et al. , Vitamin D Endocrine System and COVID-19: Treatment with Calcifediol. Nutrients, 2022. 14(13).