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Chapter 1

The History and Modern Controversies of Vitamin D Fortification and Supplementation

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Abstract

Clinical manifestations of insufficient vitamin D have been described since ancient times. The term rickets, the classic childhood disease of impaired skeletal development caused by hypovitaminosis D, was first described in the 1600s. As early as the 19th century, research identified sunlight and cod liver oil as cures for rickets but it was not until 1922 that an anti-rachitic factor in cod liver oil was isolated and named vitamin D. Harry Steenbock of the University of Wisconsin developed, published and patented a process of food fortification by UV irradiation. Thus began the standardized cereal/milk fortification used in the United States and the availability of pharmaceutical vitamin D supplementation. Vitamin D can be ingested or it can be cutaneously synthesized with skin exposure to UV light. However, concerns regarding skin cancer have promoted skin covering and/or sunscreen use, both of which interfere with vitamin D synthesis. Additionally, geographic location, air pollution, season, and ethnicity and age limit the ability of certain populations to

derive sufficient vitamin D from cutaneous synthesis. Over the past several years, studies have begun to investigate both the skeletal and non-skeletal effects of hypovitaminosis D. Dosing recommendations for vitamin D supplementation vary considerably. Several key organizations have made recommendations regarding vitamin D needs including the Institute of Medicine, the United States Endocrine Society, the United States Preventative Services Task Force, the American Academy of Pediatrics, the American College of Obstetricians and Gynecologists, the National Osteoporosis Foundation, the North American Menopause Society and the World Health Organization. Most commonly cited by others, the Institute of Medicine updated their calcium and vitamin D recommendations in 2011 based purely on skeletal impacts. Adequate intake was defined at 400 IU of vitamin D daily for children under the age of one year; recommend daily allowances were established at 600 IU of vitamin D daily for ages 1-70 years and at 800 IU daily for individuals over the age of 70 years. The IOM's recommendations were met with surprise from many leading researchers in the field, as studies had suggested much higher supplementation regimens, especially for potential non-skeletal benefits. Additionally, the American Academy of Pediatrics recommends vitamin D supplementation for all children, especially exclusively breastfed infants. Most agree that additional research is needed to better identify the optimal vitamin D supplementation for individuals of all ages for both skeletal and non-skeletal benefits.

Introduction

Recently, vitamin D-related research has spread virally throughout the medical literature. Supplementation regimens for a variety of clinical conditions are hotly debated within both the medical communities and the lay media. However, questions and controversies regarding vitamin D and the clinical manifestations of its deficiency have existed for centuries.

First Observations of Rickets

Long before anyone knew of vitamin D’s existence, observations regarding the implications of deficiency were recorded. Now known as rickets, vitamin D deficiency can lead to abnormal bone formation and growth plate development. Although the condition did not gain any meaningful attention until the 17th century, modern examination of historical writings and art have

revealed evidence that the manifestations of rickets were observed much earlier. In the second century Soraneus, a Roman physician, and Galen both wrote of the bony deformities noted in many infants. [1, 2] Recent examination of pre-Columbian Peruvian skeletal remains identified pathological changes indicative of rickets. During the early 20th century, John Foote examined paintings throughout history. He concluded that artists were inequitably depicting royal and not common children since the royal families were often the ones commissioning the work. However, among the limited paintings of peasant children he did identify evidence of rickets in several 15th century Dutch and German paintings [1].

Although this suggests that rickets had been present for centuries, the disease did not find its name until the mid-17th century. London began recording cause of death of each deceased citizen in 1629. Five years later the term "rickets" debuted in print in the Annual Bill of Mortality as the cause of death for 14 children. [3] Later scholars attempted to deduce the creation of this term. No Greek derivatives could be found. A not uncommon surname of the day, it was hypothesized that "rickets" was named for an apothecary who may have treated the condition.

Later in the 17th Century, four publications touched upon the topic of rickets. Daniel Whistler penned the first description of rickets in his medical doctoral thesis, "Inaugural medical disputation on the disease of English children which is popularly termed the rickets" in 1645 while attending medical school at Leyden [4]. Historians have questioned the originality of this work and some have even accused Whistler of plagiarizing the more experienced Francis Glisson, though Glisson did not publish his findings for five more years [2, 3, 5].

In 1649 Arnold Boote published a book ("Medicae de Affectibus Omissis", later translated "Medical Observations on Neglected Ailments") which briefly touched on rickets in a chapter on several different conditions. [3] In 1650, Cambridge physician Francis Glisson published the most extensive work regarding rickets, "De Rachitide", describing his clinical and autopsy observations of the disease. Glisson extensively described his pathological findings and went on to hypothesize poor climate as a causative factor. His treatment suggestions included drawing out bad humors by cautery, incisions or blistering; oral therapy of crow or frog liver; and correcting bony deformities with wool wraps, splits, or artificial suspension [2, 3]. Finally, John Mayow's "Concerning Rickets" was published in 1668. He extensively quoted Glisson as well as describing his own autopsy findings.

Despite these back to back rickets-related publications, little else entered the professional literature for almost two centuries. The causes of rickets became a hotly debated and highly speculative topic throughout the late 18th through early 20th centuries. Although a dietary deficiency and lack of sunlight eventually emerged as the agreed upon causes of childhood rickets, a plethora of other potential etiologies were considered. One popular theory supported by Parrot and criticized by Cheadle argued that rickets was a symptom of congenital syphilis [6]. Other infectious sources including strep or diarrhea illnesses were blamed [7 8].

After observing enlarged fontanelles and costochondral junctions in newborns, Kassowitz and Siegert suggested a congenital etiology. Schmoryl argued against this theory after examining over a hundred unaffected newborns and diagnosing the earliest case at the age of 1.5 months although Dunham, Yllpo and Hamilton found earlier cases in premature infants [7]. Relationships with several different organs were suggested including the thyroid (supported by Lanz), adrenal (supported by Stoeltzner), thymus (supported by Basch, Klose, Vogt, and Matti; argued against by Nordmann, Pappenheimer, Renton, Robertson, McClure and Park), parathyroid (supported by Erdheim and Pappenheimer), parathyroid, and thymus (supported by Park) [7]. Several others argued a variety of environmental conditions may contribute including improper hygienic conditions, lack of fresh air or lack of exercise.

Other dietary theories included overeating (supported by Glisson and Jundell), underfeeding, fermentation of yeast to lactic acid causing interruption of skeletal ossification (supported by Heitzman, Klose, Vogt and Pritchard), fermentation of carbohydrate to produce toxins (supported by Ashby), deficiency of earthly salts (supported by Chossat and Milne-Edwards), deficiency of lime salts (supported by Wegner and Katz), lack of dietary fat and/or protein (supported by Holt, Cheadle and Bland-Sutton), or excessive carbohydrates [6-8].

Both not nursing and prolonged nursing were blamed [8]. It has been recently argued that some of the earliest described cases of rickets may have resulted from deficient calcium intake related to the practice of “wet nursing” among wealthy English families [9].

In a 1888 paper, Cheadle discussed many of these theories and concluded that the causative factor was most likely dietary (animal fat, phosphorus, protein) and accentuated by “evil external hygienic conditions” [6]. In a 1908 paper, Findlay also discussed the varying theories and stated “Almost every

possible quality and combination of the different constituents have been accredited with the power of inducing the disease" [8].

Finding the Cause of Rickets – Early Observations with Light and Cod Liver Oil

Peasants and fisherman frequently used cod liver oil, especially in England, Germany and Holland. Occasionally they would introduce medical professionals to this lay therapy. Several physicians wrote of the successes they observed and the theory of cod liver oil's healing effect on rickets slowly trickled throughout Europe. After cod liver oil became part of the British Pharmacopoeia in 1771 [10], Manchester Infirmary physicians (Drs. Darbey in 1789, Percival in 1790 and Bardsley in 1807) suggested its use for the treatment of rheumatism [2, 10]. Similarly, in 1824 German Schenck wrote of cod liver oil as therapy for rheumatism and gout [2, 10]. Modern speculation has suggested that some of the ailments labeled as rheumatism or gout may have actually been rickets or osteomalacia. Cod liver oil continued to gain attention when, in 1822, the Society of Science and Arts of Utrecht chose an essay regarding cod liver oil for the treatment of scrofula and rickets as their award winner. In 1824, Schutte reported a case of cured rickets from cod liver oil treatment; he went on to describe four case reports of cure in 1826 [10].

In 1827 Bretonneau was unsuccessfully treating a 15-month-old Dutch baby in Tours with standard French therapies. Frustrated by the lack of improvement, the baby's father suggested cod liver oil, a therapy that had been used in Holland on their older son. Bretonneau administered cod liver oil and noted improvement with "incredible speed." Bretonneau continued to test the therapy on an additional four French children with success [11]. Up until that time, nutrition's only goal had been to achieve caloric sufficiency. The concept that the lack of a specific dietary factor could promote health or that its absence could cause disease was transformative. It was with great interest that these "vital substances" (later vitamins) deficiencies were identified as the causes of scurvy, beriberi and pellagra [12].

Sunlight and diet experiments began in the mid-19th century. Some investigators focused on a single potential causative/curative agent while others noted both diet and environment in their studies. Jules Guerin separated a litter of puppies into two groups in 1838. Half lived in the basement and ate raw meat while the rest nursed from their mothers in the sunlight. After a few

weeks, he noted the basement pups began to show signs of rickets including developing misshapen bones and "looking sad." The pups reared with their mothers in the sun had no such symptoms [11].

Based on Guerin's puppy studies, Parisian physician Armand Trousseau, a student of Bretonneau (the French physician who first used the Dutch cod liver oil therapy upon the suggestion of his patient's father), hypothesized that faulty diet and lack of sun exposure caused rickets. He also stated that cod liver oil, which he defined as a beneficial food and not a drug, cured the condition. He found some evidence that other oils – ray liver, herring liver, whale liver, seal liver but not vegetable oil – provided protection as well [11].

In 1878, Hugh Owen Thomas treated rachitic children by taking them out on the sunny balconies of the Sea Side Hospital in Rhyl, Wales. [12] In the 1880's both Kassowitz and Schmorl separately observed the influence of season on the incidence of rickets. Similarly, Hansemann noted that babies born in autumn months who died in the springtime frequently exhibited signs of rickets whereas spring-borne infants dying in the fall had no such findings [7].

John Bland-Sutton, a lecturer of comparative anatomy and surgery at Middlesex Hospital in London, began observing animals at the London Zoo in 1889. He first noted that within months of entering captivity, the monkeys would develop skeletal changes. Captive bears fed a diet of biscuits and lean meat died with severe skeletal changes suggestive of rickets. He noted similar skeletal changes and early deaths in lion cubs. He added cod liver oil and crushed bones to the lions' standard diets of lean meat. With no other dietary or environmental changes, these captive lions began showing skeletal improvement and lived. Bland-Sutton hypothesized that fat represented the healing dietary component. His observations were noted by his contemporaries and inspired many further investigations. Interestingly, it was later noted that in addition to the dietary deficiencies of these zoo animals, the monkeys were enclosed in UV-blocking glass [2, 6, 13].

After graduating with a medical degree from the Edinburgh University School of Medicine, Theobald Adrian Palm entered into the Edinburgh Medical Mission and traveled to Japan. There he astutely observed an absence of rickets compared to his British homeland. Just prior to the turn of the 19th into 20th centuries, he collected observations from fellow missionaries throughout Asia and Africa and compared the perceived incidences of rickets to that of Europe. He observed that children living in the filth and often unsafe conditions of the tropics succumbed to a variety of infectious conditions but were spared from rickets. Additionally, he noted that even within Europe,

rickets disproportionally plagued children in the industrial (coal burning), crowded cities. He deduced that sunshine provided the protection from rickets and suggested regular sun baths for prevention and therapy [2, 12].

Over the next several years, several light-exposure studies observed a protective affect for rachitic children and lab animals. Although he received little attention for it at the time, Buchholz may have been the first to successfully treat rickets with artificial light when he cured 16 rachitic children with exposure to lamp light in 1904. [7] After noting the seasonal tendency for the development of rickets, Jan Raczynski watched as a puppy reared in a basement developed rickets symptoms whereas the one developing in the light had no symptoms [12]. In 1919 Berlin pediatrician Kurt Huldschinsky treated rachitic orphans with artificial light via a quartz mercury vapor lamp and noted cures. He then irradiated only a single arm of an affected child but demonstrated whole-body cure via follow up x-rays. He hypothesized that the light exposure produced a chemical within the body that provided healing [2, 12].

New York pediatrician Alfred Fabian Hess compared different wavelengths and found UVB (289-302 nm) but not UVA (>320 nm) wavelengths curative [12]. With Unger, they also demonstrated that UV exposure to one arm produced x-ray evidence of bilateral wrist improvements (via epiphyses calcification) of previously rachitic children [14]. In 1919 Winkler used medium soft tube x-rays to treat the craniotabes (skull abnormalities from rickets). X-ray findings post-treatment showed lime salt deposition on the radius, indicative of rickets healing [7].

Shortly after World War I, Dr. Harriette Chick analyzed both cod liver oil and light effects at the Kinderklinik in Vienna. Rachitic infants and children were divided into groups receiving varying diets and sunlight exposures. Staff completed careful records of intake, exposure, and clinical outcomes. The six children administered cod liver oil as part of their diet showed radiographic evidence of healing in two to four weeks. The seven children treated with mercury vapor quartz lamp exposure (increasing taper to 30 minutes of exposure three to four times per week) healed in two to four weeks (two to five total hours of UV exposure) with radiographic findings indistinguishable from the children treated with cod liver oil. She observed that the healing occurred regardless of whether a single limb, half of the body or the entire body received the UV exposure.

The twelve children taken out on the sunny balcony (with various levels of direct sunlight, shade, and clothing) also improved but the ones who also received cod liver oil healed the most rapidly. Children with no sunlight

exposure, mercury vapor quartz lamp treatment, or cod liver oil supplementation healed slowly after May; no one developed rickets during the summer [15 13, 16].

Isolation of the Anti-Rachitic Factor

Native Kansan, Elmer McCollum began his nutritional research at the Wisconsin College of Agriculture. He bought twelve albino rats from a pet dealer with six of his own dollars to develop his first rat colony [17]. For years, he studied the clinical implications of nutritional deficiencies by feeding rats various restricted diets. In 1917 he joined the faculty at Johns Hopkins and began collaborating with fellow chemists Nina Simmonds and Helen Parsons, pediatrician John Howland and orthopedic histopathologists Edward Park and Paul Shipley. Combining their collective expertise, the group developed a diet that consistently induced clinical and histopathological findings of rickets in rats. They further demonstrated that these rachitic changes would heal with the addition of cod liver oil and concluded that a deficiency in fat soluble A, calcium or a similar metabolite was to blame. They also developed the "line test", deposition of lime salts in the cartilage of the metaphysis junction, as a marker of rachitic healing [2, 18, 19, 20 21]

English physician Edward Mellanby induced rickets in puppies by utilizing this diet. He then introduced a single food to observe the clinical impact on the rickets symptoms. Yeast and orange juice provided no improvement whereas cod liver oil, butter and whole milk did. He concluded that "the cause of rickets is a diminished intake of an antirachitic factor which is either fat-soluble A or has a somewhat similar distribution to fat-soluble A." [2, 22] His work was noted by the British Medical Research Committee and they publically committed to the theory that a deficiency of "an antirachitic factor" caused rickets [7].

Both Mellanby and McCollum's groups sought to determine if the anti-rachitic factor was indeed vitamin A or a distinct other element. Mellanby oxidized butter fat and cod liver oil to destroy the vitamin A and found that the oxidized cod liver oil maintained its anti-rachitic effect on puppies but that the oxidized butter did not. He queried whether the calcium of milk played a role in this observed difference [7]. Similarly, McCollum's group found that oxidized cod liver oil maintained anti-rachitic properties while losing anti-xerophthalmic properties [23]. They concluded that the anti-rachitic factor was indeed separate from vitamin A. As this was the fourth nutritional factor

identified it was named "vitamin D." [2] (Vitamins A, B and C had already been discovered as impacting sight, pellagra and beriberi and scurvy, respectively.)

Development of Fortification

While Mellanby and McCollum's works identified the nutritional source of rickets protection, the question as to why sunlight seemed to also play a role in rickets had not yet been determined. In 1922 Alfred Hess, Lester Unger and Alwin Pappenheimer (Columbia University) irradiated rats with UV light shone through window glass and found that this method failed to protect the animals from rickets [24]. The next year Hume and Smith performed experiments irradiating rats (outside of their cages) or irradiating the glass jars in which the rats lived (without the rats present during the irradiation). Both groups of rats showed improvement in rickets compared to control animals (neither the animal nor its glass jar received irradiation). Webster and Hill tried to replicate this experiment but did not show rachitic improvement in the non-irradiated rats living in the irradiated cages. It was later realized that the latter experiment replaced the cage bedding after irradiation. Although not understood at the time, some irradiated organic products like rat bedding can synthesize vitamin D when exposed to UV light. Rats ingesting even small amounts of the irradiated bedding likely led to the difference in outcomes between the two studies [16].

Back at the University of Wisconsin, Harry Steenbock built on these findings. Son of a Wisconsin dairy farmer and student of Edward McCollum, Steenbock earned both his bachelors and doctoral degrees from the University of Wisconsin and remained on as faculty in the Department of Agricultural Chemistry. His experiments fed two rats living in the same cage the previously discovered rickets-inducing diet. Once both rats had developed clinical manifestations of rickets, one of the rats would be temporarily removed from the cage for UV irradiation and then replaced. Both the irradiated rat and its non-irradiated cage mate improved in terms of their rachitic symptoms. However, if the rats were placed in separate cages in near vicinity to each other, the non-irradiated rat did not improve. Hypothesizing that the non-irradiated rat may have been consuming the irradiated rat's excrement, they repeated the experiments but included a screen floor through which excrement would pass. Both the irradiated and non-irradiated cage-mate still improved. Once they then began cleaning or replacing the screen after the single rat was

irradiated, the cage-mate no longer improved. They hypothesized that the non-irradiated rat consumed either residual excrement on the screen floor (via exposure on paws and subsequent cleaning rituals) or irradiated bedding. Wondering if the rachitic-protective factor was being imparted onto previously vitamin D-deficient substances via irradiation, Steenbock irradiated hog millet and added it to the rat feed. These rats, too, improved, showing that vitamin D could be imparted into previously deficient foods [16, 25, 26, 27].

Although Alfred Hess and Mildred Weinstock were finding similar results at Columbia University by irradiating cottonseed and linseed oil by mercury vapor lamp [24], Steenbock was the first to patent the process. Early on, he realized the potential commercial implications of the discovery that the anti-rachitic properties could be generated in certain food products (containing appropriate sterols) with UV irradiation. His university president denied funding for a patent, so Steenbock filed for the patent with his own $300. As one of the first university professors to obtain a patent, Steenbock was publically criticized, but he astutely argued that other professors received royalties from their books. Realizing that managing a patent was beyond the abilities of the university and learning from the successes of other universities' patent management boards (namely Columbia, the University of Minnesota and the University of Toronto), Steenbock and the University of Wisconsin administration created the Wisconsin Alumni Research Foundation (WARF). In 1927 Steenbock sold the patent to the foundation for $10. [17]

The foundation licensed the process of fortification to Quaker Oats for breakfast cereals. Believing the fortification process should only include nutritional foods, they refused licenses to manufacturers of chewing gum, tobacco, lipstick, beer, and soft drinks. Addition of irradiated yeast provided an easy way to fortify cereals but laws at the time prevented the addition of anything (including chocolate or vitamins) to milk. Located in dairy farmland, the University recognized the potential impacts of milk fortification and created a process of directly irradiating flowing milk [28]. The milk was fortified to 400 IU per liter because that was the amount found in one teaspoon of cod liver oil [12, 17]. In addition to the license to Quaker Oats, the WARF licensed the vitamin D development process to pharmaceutical companies which developed Viosterol, the first medicinal vitamin D preparation [28]. Thus, vitamin D supplements and fortified foods were born.

A workaholic bachelor, Steenbock accepted no royalties initially. In order to set a precedent for future scientists who may need the salary boost, he eventually agreed to receive 15%; however he only accepted a fraction of this as the earnings became larger and larger. The profits from this patent funded

the University's research endeavors not only through the great depression but for decades to come [17].

Isolation of Vitamin D Metabolites

For almost four decades, vitamin D was recognized only as a single entity. However, in 1964 a group also from the University of Wisconsin identified five distinct metabolites. They administered radio-labeled vitamin D either orally or by intraperitoneal injection to rats and identified five separate peaks on chromatography of extracts from the intestine and kidney. Only one of these peaks was identical to the parent vitamin D compound administered to these animals [29]. Morii and colleagues administered one of these metabolites to calcium-deficient rats and noted stimulation of intestinal calcium transport, an increase in serum calcium, and improvement in rickets symptoms. Additional studies further elucidated that the more-polar metabolite existed in the chromatin fraction of chick intestine and that the radio-labeled vitamin D metabolite would not be taken up animals previously fed vitamin D rich diets or supplements [30]. Using ultraviolet spectra, gas-liquid partition chromatography and mass spectrometric and nuclear magnetic resonance spectrometric analyses, Blunt and his colleagues at the University of Wisconsin identified the structure of the metabolite, 25-hydroxycholecalciferol (25(OH)D), in hog serum and found its ability to cure rickets 1.4 times stronger than the parent vitamin D compound [31]. Noting the incidence of bony diseases in patients with advanced liver disease, University of Wisconsin researchers (Ponchon, Kennan and DeLuca) hypothesized the liver as the site of 25-hydroxylation. By studying rats after surgical ligation of the hepatic artery and bile duct (effectively eliminating all hepatic function), they found no further production of the 25(OH)D metabolite and deduced that the liver was the site of this conversion [32].

Although initially it was thought that 25(OH)D was the final product in vitamin D metabolism, Lawson and his Cambridge colleagues identified a yet more-polar metabolite. They also found that regardless of the dose of cholecalciferol administered to a chick, the concentration of this more-polar substance did not exceed one nanogram per gram of chick intestine tissue. Additionally, this metabolite seemed to have a rapid half-life with full clearance from the blood or adipose within 16 hours of the original cholecalciferol administration [33, 34]. In an attempt to localize the source of this metabolite's production, liver, adrenal, parathyroid, thyroid,

ultimobranchial body, thymus, bone, and blood samples were incubated with 25(OH)D but no subsequent extra-polar metabolite was produced. Additionally, observational studies conducted in this same Cambridge lab continued to note this metabolite's production even after surgical removal of the gut, pancreas, spleen, thyroid, parathyroid, ultimobranchial bodies, adrenals or thymus [35]. This was confirmed by Anthony Norman and his colleagues at the University of California [36]. Finally, two studies identified the kidney as the production location. First, the more-polar metabolite in question was produced by in-vitro kidney tissues exposed to 25(OH)D. Additionally, post-nephrectomy rats were found to produce 25(OH)D but not the more-polar metabolite [35]. In the early 1970s it was found that this kidney-produced, more-polar compound was superior to the precursor vitamin D metabolites in increasing calcium absorption in the gut.

In 1971, all three labs (University of Wisconsin, Cambridge University and the University of California) concurrently and independently identified the structure of this most-polar metabolite as 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$) by utilizing mass spectrometry, ultraviolet absorption spectrophotometry and chemical reactions [37-40]. Injecting radio-labeled $1,25(OH)_2D$ into vitamin D deficient rats identified $1,25(OH)_2D$ receptors in the nuclei of the gut (duodenal, jejunal, ileal and colonic luminal and crypt epithelium), kidney (nuclei of distal tubule epithelium and glomeruli podocytes), epidermis, stomach, pituitary and parathyroid [41]. Since its location of action was distant from its location of production, the $1,25(OH)_2D$ metabolite was reclassified as a hormone and not a true vitamin. Subsequent work allowed scientists to chemically synthesize $1,25(OH)_2D$ and in the 1970s Dr. Uskovick developed the ability to produce synthetic $1,25(OH)_2D$ for commercial use in renal osteodystrophy patients [42].

The Lighter Side of Vitamin D

All of this work successfully isolated vitamin D as the nutritional factor able to heal rickets as well as identified the sun's role in imparting vitamin D to food. Historical observation studies of children and complex research projects on animals had shown rickets healing with exposure to UV but the mechanism had not been established. Back during the 1920 experiments that showed that UV-irradiated foods had rickets-healing effects, Hess and Weinstock had also fed irradiated human and calf skin to rachitic rats and found that the rat's rachitic clinical features healed. It was hypothesized that

the skin, like the rat feed, could synthesize vitamin D with UV exposure [43]. The role of the skin was not fully characterized, though, for almost sixty years. University of Wisconsin Medical School graduate, Michael Holick, and his colleagues at Massachusetts General Hospital identified 7-dehydrocholesterol and previtamin D compounds within rat skin after UV exposure [44] Subsequent studies using human cadaver skin, human subjects and rats identified that the 7-dehydrocholesterol (sometimes called provitamin D) was converted to previtamin D in the epidermis with exposure to UV light. Over the course of about three days, warm (i.e. physiologic) temperatures promoted previtamin D conversion to vitamin D3 in a light-independent mechanism [45 46].

Into the 21st Century

With Steenbock's development of vitamin D fortification, the days of rickets were thought to be numbered. However, even with our modern understanding of vitamin D's role in skeletal health and the ability to impart vitamin D into our diet, rickets and hypovitaminosis D continue to be prevalent worldwide with estimates of six to nine cases of rickets per million children in the US and over one billion cases of vitamin D deficiency worldwide [47-51]. An estimated 9% of children, 24% of adolescents , 32-36% of young adults, 47.8% of lactating women, 25-57% of adults and 40-100% of elderly adults in the US have insufficient vitamin D status (<20 ng/mL) [50,51]. In light of this, the U.S. Centers for Disease Control and Prevention (CDC) expressed concern regarding the continued prevalence of childhood rickets [48, 52].

One reason for continued hypovitaminosis D involves our limited exposure to sunlight. UV radiation is considered a carcinogen which plays a role in the 1.5 million skin cancers, including 8,000 metastatic melanoma deaths, occurring annually in the US. [53] Recognizing this role in skin cancer, The American Academy of Dermatology recommends that vitamin D be obtained through dietary and supplement sources and not via UV exposure [54]. The American Academy of Pediatrics (AAP) recommends no direct sunlight exposure for infants under six months of age and consistent sunscreen use thereafter [55]. The United States Preventative Services Task Force (USPSTF) recommends limiting UV exposure in adolescents and adults 10-24 years (B recommendation) with no recommendation due to insufficient evidence (I statement) for adults older than 24 [56]. Public health campaigns

promote sunscreen to prevent cancers, sunburns and premature aging. However, sunscreen also prevents cutaneous vitamin D synthesis with an SPF of 8 or 15 decreasing the skin's vitamin D production by 97.5 and 99%, respectively [42, 48, 57]. In addition to lifestyle factors keeping people indoors, the public recognizes these potential negative impacts of sunlight. The Indoor Air Quality Act of 1989 found that Americans spend about 93% of their time indoors [55]. In addition to purposeful avoidance of sunlight, air pollution from the combustion of biomass of fossil fuels can also impact the available UV [58, 59]. Geographic location with latitudes above 35°N or below 35°S receive sunlight at too oblique of an angle for the UVB to penetrate the ozone during winter months [42]. Melanin decreases the skin's ability to absorb the UVB in darker skinned individuals [60, 61]. Finally, as skin ages, it loses 7-dehydrocholesterol, decreasing an elderly individual's ability to cutaneously synthesize vitamin D. [42, 57, 62, 63].

Lack of sunlight exposure represents only part of the story of limited vitamin D. Standard diets often do not contain adequate vitamin D. The National Health and Nutrition Examination Survey (NHANES) 2005-2006 found that average daily dietary intake of vitamin D was just 204-288 IU in men and 144-276 IU in women [53]. Similarly, the United Kingdom's National Diet and Nutrition Survey found a daily intake of 170 IU for men and 150 IU for women [64].

Part of the reason for this low intake is the limited number of foods that contain vitamin D. Certain ocean dwelling fatty fish naturally contain vitamin D due to their ingestion of plankton (566 IU per three ounces of swordfish, 447 IU per three ounces of cooked salmon, 154 IU per three ounces of canned tuna) [53]. Source of salmon can impact the vitamin D content dramatically with three ounces of ocean-dwelling salmon containing as much as 500-1000 IU of vitamin D but three ounces of canned salmon or farm-raised salmon containing just 250-500 or 85-220 IU, respectively [49]. Additionally one egg yolk or three ounces of cooked beef liver each contain about 40 IU of vitamin D. Fortified products may vary depending on the manufacturer but include about 100 IU per 8 ounces of milk, orange juice or yogurt; 50 IU per 3.5 ounces of butter; 430 IU per 3.5 ounces of margarine, 100 IU per 3 ounces of certain cheeses and 40 IU per serving of fortified cereal [49, 53]. Additionally, even when attempting to consume vitamin D fortified products, the amount of vitamin D present may vary from expectations. Although vitamin D fortified milk is usually advertised as containing 400 IU per quart (100 IU per cup), studies investigating the actual amount have shown varying results with only 29% of samples in a single study containing 320-480 IU per quart (50% of

samples tested at less than 320 IU per quart, 14% of samples with less than 20 IU per quart, and 21% of skim milk samples had no vitamin D) [48, 63, 65].

Finally, chronic co-morbidities (i.e. malabsorptive disorders including celiac disease, pancreatitis, cystic fibrosis, Crohn's or history of gastric bypass; chronic liver or kidney diseases; parathyroid disease), medication use and obesity (vitamin D is sequestered in adipose tissue) have contributed to the modern vitamin D deficiency epidemic [66].

Determining How Much Vitamin D We Need – What Does Vitamin D Do?

As many of the early scientists hypothesized, humans receive vitamin D through either sun exposure or ingestion. When UVB radiation with wavelengths of 290-315 nm (optimal 297 nm) strikes human skin, 7-dehydrocholesterol (provitamin D) is converted to previtamin D3 within the epidermis. The body's physiologic warm temperature promotes conversion from previtamin D3 to D3 (cholecalciferol). Alternatively, the compound can be ingested from a food naturally containing vitamin D, a fortified food or a supplement. Irradiation of yeast ergosterol produces vitamin D2 (ergocalciferol) which is found naturally in plants including certain mushrooms. As in human skin, irradiation of 7-dehydrocholesterol produces D3 in animals [53]. Within the digestive tract, vitamin D is solubilized by bile salts, incorporated into chylomicrons and diffuses through the water layer of the brush-border membrane of the small intestine. Regardless of whether the parent compound is ingested or cutaneously synthesized, the liver hydroxylates the vitamin D2 or D3 parent compound at the C-25 position via vitamin-D-25-hydroylase enzyme into 25(OH)D (calcidiol, 25-hydroxyvitamin D). This metabolite is frequently tested within the serum to determine nutritional status (i.e. deficiency) but requires further hydroxylation by $1,25(OH)_2$D-1α-hydroxylase enzyme within the kidney to $1,25(OH)_2$D (calcitriol, 1,25-dihydroxyvitamin D), the active hormonal form. The $1,25(OH)_2$D binds to vitamin D receptors (VDR) throughout the body (intestine, skin, kidney, bone, pancreas, parotid, parathyroid, brain, stomach, pituitary, thymus, testes, ovary, uterus, placenta, breast, lymphocytes, monocytes, macrophages, embryonic cells, etc.). Once activated by the $1,25(OH)_2$D, the VDR forms a heterodimer with the retinoid X receptor (RXR) in the cell nucleus. With coactivators and coreceptors, the VDR-RXR

recognizes vitamin D responsive elements in the DNA and, thus, controls gene transcription for a variety of functions. One of vitamin D's biggest roles is within the skeletal system to maintain calcium and phosphorus homeostasis. The activated VDR-RXR induces several genes including RANK-L which binds to nuclear factor κβ which promotes maturation of osteoclast precursors into osteoclasts. The osteoclasts dissolve skeletal matrix, releasing calcium into the bloodstream. The elevated serum calcium levels feed back onto calcium receptors on the parathyroid, decreasing parathyroid hormone (PTH) production. The decreased PTH subsequently decreases renal conversion of 25(OH)D into $1,25(OH)_2D$ in a complex feedback loop. Additionally, $1,25(OH)_2D$ liganded to VDR receptors in the parathyroid directly act on gene transcription to decrease PTH release. Under the influence of osteoclasts, bone secretes fibroblast growth factor 23 (FGF23) which further suppresses $1,25(OH)_2D$ synthesis in the kidney. The $1,25(OH)_2D$ also promotes calcium absorption through the gastrointestinal tract, although this genetic mechanism is less well described. Vitamin D receptors have been found in a variety of body tissues and control more than 200 genes. Active VDRs interact with the genome to promote cellular proliferation, angiogenesis, differentiation and apoptosis. They also inhibit renin production, promote insulin production, induce the production of macrophages and improve cardiac contractility. The $1,25(OH)_2D$ also promotes expression of the enzyme CYP24R, which promotesits own catabolism and clearance [49, 50, 65-68].

In order to accurately define how much vitamin D the body needs, all of the health impacts from vitamin D must be understood. The bony effects of vitamin D are well established. Newer studies have suggested implications far beyond skeletal health. Vitamin D sufficiency may decrease the incidence of falls [49, 52, 69-75], improve muscle strength [49, 52, 76, 77] and/or improve musculoskeletal pain [49, 70, 78].

Additionally vitamin D sufficiency and/or supplementation may decrease all-cause mortality [51, 61, 70, 79-84], blood pressure [51, 52, 82, 84-93], cholesterol [51, 86, 94-97], frequency of respiratory infections [98-100], incidence of type 1 diabetes mellitus [52, 101, 102], incidence of schizophrenia [103] and risk of pre-eclampsia [104] as well as improve outcomes for cardiovascular disease [51, 52, 105-109], type 1 and 2 diabetes mellitus [82, 86, 110, 111], chronic kidney disease [112-115], multiple sclerosis [52, 116-119], depression [120, 121] and a variety of cancers [52, 122-135]. With varying degrees of research support, vitamin D supplementation is sometimes also used to treat or augment therapy for asthma, autoimmune diseases, HIV, myelodysplastic syndrome, cystic fibrosis,

senile warts, sexual dysfunction, pigmented skin lesions, vitiligo and post-menopausal weight gain [136]. Topical and oral preparations are used to treat psoriasis [65].

Serum Testing and Goals

In order to make meaningful recommendations for vitamin D requirements, it's important to set a measureable physiological biomarker goal. Although serum concentrations of all of the vitamin D metabolites can be analyzed, 25(OH)D is most commonly measured. Within days of ingestion or UV exposure, the serum concentration of 25(OH)D rises with a half-life of about two to three weeks [66,70]. With a half-life of just 3.5-21 hours and multiple feedback loops controlling its conversion, $1,25(OH)_2D$ poorly represents overall sufficiency status [66]. While measuring $1,25(OH)_2D$ may be helpful for individuals with renal disease or extra-renal production of this hormone, 25(OH)D is considered the appropriate test to determine nutritional status. Laboratory methods for measurement of 25(OH)D vary with common tests including chemiluminescent assays, competitive protein binding assays, high performance liquid chromatography, radioimmunoassays and liquid chromatography-tandem mass spectrometry. Not all assays detect both D2 and D3 and inter-laboratory precision has demonstrated variation [70]. Even with accurate measurements, there is no universal agreement regarding the optimal serum concentration of serum 25(OH)D. Limited data regarding 25(OH)D levels which do not further cause additional PTH suppression or calcium absorption from the GI tract have attempted to identify optimal 25(OH)D concentrations. PTH has been shown to plateau at 25(OH)D concentrations ranging from 10-50 ng/mL with the strongest evidence at 30-40 ng/mL. However, these studies have been criticized due to the substantial variation in individual responses and absence of a true inflection point [67, 137, 49, 70, 138, 139]. Gastrointestinal calcium absorption was noted to improve at 25(OH)D concentrations of 32 ng/mL compared to 20 ng/mL [57, 67, 137].

A recent autopsy study found histologic evidence of rickets or osteomalacia in more than 25 individuals with serum 25(OH)D concentrations of 30-32 ng/mL, suggesting the need for even higher concentrations for bone health. However, subsequent analysis questioned the validity of these serum measurements [140].

With these varying results, it's no surprise that there are no universally accepted definitions for vitamin D deficiency, insufficiency and sufficiency.

One of the most commonly used descriptions define <12 ng/mL (<30 nmol/L) as deficient, <20 ng/mL (<50 nmol/L) as insufficient and ≥20 ng/ml (≥50 nmol/L) as sufficient. The Institute of Medicine, National Osteoporosis Foundation, American Society of Bone and Mineral Research and the United Kingdom's National Osteoporosis Society use these definitions [141-143] Among others, the US Endocrine Society and the World Health Organization define deficient as <20 ng/mL, insufficient as 21-29 ng/mL and sufficient as ≥30 ng/mL. [67] Still others argue that sufficiency goals should be set at concentrations >40 ng/mL or even higher [61]. No pediatrics references values have been established [55].

In 2009, 419 scientists from 35 counties participated in the 14th Workshop on Vitamin D. During the conference, participants debated this sufficiency definition. Some argued for 20 ng/ml, given the skeletal data that shows poorer outcomes at lower concentrations. Others argued for higher values (30-40 ng/mL), citing newer evidence that non-skeletal benefits, namely cardiovascular disease, multiple sclerosis, cancer, muscle strength and immunity, occur at higher concentrations. No consensus was reached as to the optimal serum concentration for overall health; however the group did recognize that frank deficiency is common worldwide and that determining the optimum supplementation regimen is important [144].

Of note, another point of potential confusion is the variety of units used in measuring vitamin D. Serum concentrations are usually measured in either nanograms per milliliter or nanomoles per liter. Conversion of 25(OH)D concentrations from ng/mL to nmol/L can be accomplished by multiplying by a factor of 2.496. [70] Additionally, food contents of vitamin D are frequently reported in either international units or micrograms; one microgram is equivalent to 40 IU.

Supplement Options

Pharmaceutical supplemental vitamin D exists as D2 (ergocalciferol, derived from plant sources) and D3 (cholecalciferol, derived from animal sources). Prescription preparations in the United States usually contain D2 whereas over-the-counter vitamin supplements may contain either. There is some debate regarding whether these two preparations have equivalent efficacy with some evidence that D3 supplementation may result in a more pronounced 25(OH)D response [49, 145]. Preparations exist in oral drops, oral pills and capsules, and intramuscular preparations. Most multivitamins and

combined calcium/vitamin D supplements contain 200-600 IU of vitamin D per dose. D2 is available in 400 and 50,000 IU capsules and 8,000 IU/mL solution. D3 is available in 400, 800, 1,000, 2,000, 4,000, 5,000, 10,000 and 50,000 IU oral preparations and a 400 IU/drop liquid concentration [57]. Although variation exists among individuals, oral daily supplementation with vitamin D3 will cause a serum rise about 0.4-0.5 ng/mL per 40 IU of ingested vitamin D3. However, the increase in 25(OH)D concentration is inversely related to the baseline serum concentration of 25(OH)D with rises ranging from 0.28 to 1.38 ng/mL per 40 IU oral vitamin D in sufficient (>28 ng/mL) or markedly deficient (<4 ng/mL) individuals, respectively [66,146].

A Variety of Supplementation Recommendations

Providers and patients looking for recommendations to guide their vitamin D supplementation will find a plethora of recommendations, but not universal agreement. Unique from all other nutrients, vitamin D can be obtained from not just dietary sources but sunlight exposure. Additionally, rather than directly exerting its nutritional effect like true vitamins, it is simply a precursor to the production of an active hormone. This makes estimations of nutritional needs quite complex and difficult.

Recommendations and guidelines have a historical basis. Throughout the 19th century, a teaspoon of cod liver oil (with 400 IU of vitamin D) was routinely administered to children to prevent or treat rickets. In the 1930s the US government established an agency tasked with promoting sunlight exposure to prevent childhood rickets [57].

Recommended dietary allowances (RDA) debuted in 1941. An RDA now represents the daily intake needed to meet the nutritional needs of 97.5% of the healthy population. With time, additional reference values were created. Estimated average requirements (EAR) are the estimated intakes needed to meet the requirements of half of the population. Therefore, for any given nutrient, the RDA is set two standard deviations above the EAR. Occasionally, adequate intakes (AI) are used instead of RDAs if insufficient evidence exists to establish an EAR. No observed adverse event levels (NOAEL) are the highest doses not associated with any harm in the literature. Finally, the tolerable upper intake level (UL) defines the maximum daily intake not expected to pose any harm to the general population and was set at half of the

observed NOAEL for each age. The Institute of Medicine (IOM) released their first guidelines for vitamin D in 1997. Daily AIs were set at 200 IU for children, pregnant or lactating women, and adults through the age of 50; 400 IU for adults ages 51 to 70 and 600 IU for elderly adults over the age of 70 years. Daily ULs were set at 1,000 IU for infants up to one year of age and 2,000 IU for children over the age of one year, pregnant and lactating women and all adults [147]. With the overwhelming amount of research and attention vitamin D received over the next decade, the guidelines needed to be readdressed. In November of 2010, at the urging of the US and Canadian governments, the IOM made public their updated recommendations regarding calcium and vitamin D requirements.

After reviewing available literature and hearing expert testimonials, the committee of experts tasked with this assignment decided that the data regarding non-skeletal benefits was inconclusive. Therefore, recommendations were made purely on the skeletal evidence. They based these recommendations on the presumption that serum 25(OH)D concentrations of 16 ng/mL should be sufficient for about half the population and 20 ng/mL should be sufficient for 97.5% of the population. The AI for children under one year of age was raised to 400 IU of vitamin D per day. EARs and RDAs were established with the 2010 guidelines. For everyone over the age of one year, the EAR was defined at 400 IU per day. The RDA was set at 600 IU per day for everyone ages 1-70 and 800 IU per day for those over the age of 70 years. Citing concern for potential side effects at serum concentrations of 25(OH)D exceeding 50 ng/mL, daily ULs were set at 1,000 IU for infants up to six months of age, 1,500 IU for infants 6-12 months, 2,500 IU for children ages 1-3 years, 3,000 IU for children 4-8 years, 4,000 IU for children 9-13 years and adults 19 years and older. No UL was reported for children 14-18 years. The report did acknowledge a NOAEL of 10,000 IU per day with no hypercalcemia or acute intoxication identified in adults supplemented at that dose [60, 148].

With the plethora of publications regarding studies investigating non-skeletal benefits using much higher doses, many within the medical community found these recommendations surprisingly low. However, the United Kingdom's National Osteoporosis Society and the American Society of Bone and Mineral Research agreed with these guidelines [141, 143] Specifically, the UK's National Osteoporosis Society recommended 400 IU supplementation of vitamin D daily for pregnant and breastfeeding women, people over the age of 65 and those not exposed to sufficient sunlight. [141] Similarly, the National Osteoporosis Foundation (NOF) recommends 400-800

IU vitamin D daily in healthy adults under the age of 50 years of age and 800-1,000 IU per day in adults over the age of 50 [142]. Further, the North American Menopause Society recognized the current controversies regarding optimal dosing but ultimately agreed with NOF's recommendations for 800-1,000 IU of vitamin D per day for adults over 50 years [146].

The US Endocrine Society also created a task force to make recommendations regarding vitamin D supplementation. Unlike the IOM, this group focused their recommendations on those with deficiency risk factors and considered not only the skeletal effects of vitamin D but also fall prevention. Therefore, they set a goal of achieving 25(OH)D serum concentrations ≥30 ng/mL and defined sufficiency as such. Their guidelines recommend 400-1,000 IU of vitamin D per day for children under one year, 600-1,000 IU daily for children over the age of one year, and 1,500-2,000 IU per day for adults over the age of 19, including pregnant women. Tolerable upper intake levels of daily vitamin D ingestion were set at 2,000 IU for infants under one year, 4,000 IU for children and adolescents ages 1-18 years, and 10,000 IU for adults. These guidelines also recommend that obese children and adults and individuals on certain medications (i.e. anticonvulsants, glucocorticoids, antifungals, HAART) be given two to three times the recommended supplementation dose. The task force did recommend prescribing vitamin D for fall prevention but did not recommend supplementation to improve cardiovascular disease, all-cause mortality or quality of life. The committee also recommends screening patients at higher risk of deficiency including those with rickets, osteomalacia, osteoporosis, chronic kidney disease, malabsorptive syndromes (cystic fibrosis, inflammatory bowel disease, Crohn's disease, history of bariatric surgery, radiation enteritis), hyperparathyroidism, use of certain medications (antiseizure medications, glucocorticoids, HAART, antifungals, and cholestyramine), African American or Hispanic ethnicity, pregnancy, lactation, a history of falls or nontraumatic fractures in older adults, BMI >30 kg/m2, granuloma-forming disorders (sarcoidosis, tuberculosis, histoplasmosis, coccidiomycosis, berylliosis) and lymphoma, but not the general population [67, 149].

The United States Preventative Services Task Force updated their calcium and vitamin D recommendations in 2013. For fracture prevention in post-menopausal, noninstitutionalized women, they found insufficient evidence for daily supplementation with more than 400 IU vitamin D and 1200 mg calcium (I statement) and recommended against daily supplementation with less than 400 IU vitamin D and 1200 mg calcium (Grade B recommendation). Additionally, the task force found insufficient evidence to assess vitamin D

and calcium supplementation to prevent fractures in premenopausal women and men (I statement).

These recommendations only assess fracture-related evidence and no other potential health outcomes. They do not apply to higher risk groups such as persons with a history of fractures or osteoporosis. [150] Additionally, although not part of the USPSTF's assessment, they acknowledged a meta-analysis published after their review which concluded that fractures may be reduced in individuals taking higher doses of vitamin D – 30% lower incidence of hip fracture with doses 792-2,000 IU vitamin D per day and no reduction in hip fractures at doses less than 792 IU daily [143, 150, 151] Of note, the task force had previously recommended vitamin D supplementation – along with exercise or physical therapy – to prevent falls in community dwelling adults over the age of 65 (Grade B recommendation). The Task Force did not specify a dosing regimen, recognizing that the data included a variety of supplementation regimens (median dose of 800 IU vitamin D daily for a median of 12 months) but did cite IOM's recommendations of 600 IU vitamin D per day for adults 51-70 and 800 IU per day for adults over the age of 70 years and the American Geriatric Society's recommendation of 800 IU daily for persons at increased risk of falls [69].

Like the IOM recommendations in 2010, these new 2013 USPSTF recommendations were met with quite a bit of controversy, not only among proponents of higher supplementation doses that were surprised by the IOM recommendations but by those who had accepted the IOM guidelines as well. Editorials abounded but reminded critics that these assessments were limited – the IOM only considered skeletal impacts and the newest USPSTF recommendations only assessed fracture risk [143].

Additionally, none of the aforementioned guidelines consider any outcome other than skeletal health, falls and fractures.

Neither the IOM nor the US Endocrine Society recommends any additional supplementation for pregnant or lactating women beyond the doses recommended for all adults. The American College of Obstetricians and Gynecologists' (ACOG) Committee Opinion concluded that insufficient evidence exists to recommend routine screening for vitamin D deficiency during pregnancy or to routinely supplement vitamin D beyond that which is contained within a prenatal vitamin. ACOG recognized that vitamin D deficiency is likely common during pregnancy and that maternal status impacts the infant's vitamin D status at birth. They acknowledged that new data has suggested a role for vitamin D supplementation to prevent preterm birth and preeclampsia but found the evidence insufficient to date. They did

state that if vitamin D deficiency is identified, 1,000-2,000 IU of vitamin D per day is safe during pregnancy [152]. The United Kingdom's National Institute for Health and Clinical Excellence recommends counseling pregnant and breastfeeding women to take 400 IU vitamin D daily (the amount contained in most US prenatal vitamins) [153]. The World Health Organization Food and Agriculture Organization of the United Nations (WHO/FAO) set the recommended nutrient intake (RNI) at 200 IU of vitamin D per day for pregnant women. The WHO's 2012 guidelines regarding vitamin D supplementation in pregnant women recommended against vitamin D supplementation during pregnancy to prevent pre-eclampsia (strong recommendation) or as a routine practice to improve maternal or infant health (conditional recommendation) with the latter recommendation being due to lack of evidence to fully assess potential benefits and harms [154].

In line with the IOM's AI and EAR but below the IOM's RDA and the US Endocrine Society's recommendations, the American Academy of Pediatrics (AAP) also recommends vitamin D supplementation for children. The AAP based their original 2003 recommendations on the IOM's 1997 vitamin D recommendation of 200 IU per day. Although pediatric reference ranges did not exist, adult sufficiency was later defined as serum concentrations of 20-30 ng/mL, and the daily 200 IU supplementation failed to achieve this in infants. Breastfed infants receiving 400 IU of vitamin D daily supplementation did achieve serum concentrations of 25(OH)D greater than 20 ng/mL. In terms of convenience, the AAP noted that the only commercially available liquid infant preparation of supplemental vitamin D came in 400 IU drops. Also recognizing that rickets continued to exist within Western society, especially among exclusively breastfed and/or darker pigmented infants, in 2008 the AAP published updated recommendations to supplement infants within days of birth, and children and adolescents with 400 IU of vitamin D daily. In addition to rickets prevention, the AAP based this new recommended dose on studies that had confirmed safety of 400 IU per day as well as early evidence for a role of vitamin D in innate immunity and disease prevention (including diabetes and cancer).

The World Health Organization has stated that vitamin D supplementation in children may prevent rickets (Category 2 intervention), but actual guidelines have yet to be developed [155].

Daily consumption of 32 ounces of vitamin D fortified milk would provide a child with the 400 IU of vitamin D recommended; however, the AAP recognizes that most children and adolescents do not consume the necessary dietary amounts of vitamin D fortified products. From the 1970s to

the 1990s milk consumption decreased by 36% in adolescent girls. Thus, the AAP recommends universal daily vitamin D supplementation with 400 IU for children and adolescents [55]. Although dietary sources are often not considered a reliable source of sufficient vitamin D, formula fed infants usually do receive enough vitamin D from their diet. Since the 1980 Infant Formula Act established the vitamin D fortification mandate, infant formula has included substantial vitamin D. [156]. Presently, the Food and Drug Administration has set a requirement of 40-100 IU vitamin D per 100 kcal of formula. Standard infant formulas are 20 kcal per ounce which means that they would contain 258-266 IU of vitamin D per liter of formula [157]. Most commercially available infant formulas in the US advertise 400-500 IU vitamin D per liter; therefore, consumption of one liter of infant formula provides an infant with the recommended 400 IU of vitamin D. By one month of age, most formula-fed infants are consuming at least one liter of formula daily and, therefore, do not require additional vitamin D supplementation. Infants consuming less than one liter of formula per day should, per the AAP recommendation, receive an additional 400 IU of supplemental vitamin D per day. [55] Breastfeeding is encouraged as a healthy practice for most infants. Healthy People 2010 set the goal that 75% of infants breastfeed for the first six months of their lives [158]. However, human milk is universally vitamin D deficient with an average of 15.9 +/- 8.6 IU/L [67] which is why the AAP recommends universal vitamin D supplementation for breastfed infants [55].

Some find all of these recommendations too conservative and suggest much higher dosing regimens. While the concern of hypervitaminosis D is legitimate; hypercalcemia has only been reported with serum concentrations of 25(OH)D exceeding 150 ng/mL. Models have suggested that to achieve that concentration would require daily oral supplementation of 40,000 IU of vitamin D [51].

Although cautiously, given concerns for skin cancer and the recommendations against it, some do suggest limited but regular sunlight exposure to meet physiologic needs. Full body exposure to UVB in an adult with fair complexion produces about 20,000 IU of vitamin D in 30 minutes [61].

With the rapidly changing recommendations and varying guidelines, it's no surprise that compliance with these recommendations is poor. Studies of supplementation rates in exclusively breastfed infants (per the AAP guidelines) have been shown to be as low as 5-16% [159, 160]. Evaluation of elderly individuals with hip fractures revealed that only 24% complied with the vitamin D and calcium supplementation recommendations [52].

A Variety of Replacement Regimens

Although controversy exists regarding whether or not to supplement a healthy individual, most are in agreement that an individual with vitamin D deficiency needs replacement. The best route, schedule, and dose remain to be determined, though. Oral and intramuscular routes are both used. Schedules include administration twice daily, daily, twice weekly, weekly, monthly, quarterly or even annually. A chart review at an Atlanta Veterans Administration (VA) identified 36 different vitamin D replacement regimens [161].

Recent assessment has suggested that either intermittent high dose replacement for two months or regular lower dose supplementation schedules are both acceptable [145]. Oral doses of 400-50,000 IU per day for six weeks to several years, 8,400-100,000 IU per week, and 300,000 IU quarterly have all been used.

Additional regimens include intramuscular administration of 300,000 IU quarterly or 600,000 IU annually [136,145 144]. The most commonly prescribed regimens at the aforementioned VA included oral vitamin D 50,000 IU weekly for four weeks followed by monthly administration for five months; oral 50,000 IU monthly for six months; and oral 50,000 IU three times weekly for six weeks. Although all of the VA's regimens improved vitamin D status, only the 50,000 IU monthly dose for six months achieved a serum concentration exceeding 30 ng/mL in greater than half of recipients (82% compared to 38 and 42%, respectively) [161].

The US Endocrine Society recommends treatment of vitamin D deficiency with either 2,000 IU per day or 50,000 IU per week for infants and children from birth to the age of 18 years; 6,000 IU per day or 50,000 IU per week for adults; and 6,000-10,000 IU per day for at risk individuals (including those with obesity or malabsorptive syndromes). Once high dose treatment had achieved a serum concentration of greater than 30 ng/mL (or the person had completed six weeks of treatment for children or eight weeks of treatment in adults), long term maintenance therapy was suggested with daily supplementation of 400-1,000 IU for infants less than one year of age, 600-1,000 IU for children and adolescents ages 1-18, 1,500-2,000 IU for adults, and 3,000-6,000 IU for at-risk individuals [67].

The United Kingdom's National Osteoporosis Society recommends oral replacement therapy with either fixed loading doses or just maintenance therapy for vitamin D deficient individuals. Suggested loading doses included 50,000 IU weekly for six weeks, 20,000 IU twice weekly for seven weeks or

4,000 IU daily for 10 weeks. Maintenance therapy recommendations included 800-2,000 IU daily [141].

Micromedex® currently recommends treatment of dietary vitamin D deficiency with 1,000 IU daily or 100,000 IU quarterly [162].

Conclusion

From the first observations of rachitic findings in ancient times to the initial studies of rickets in the 1600s to the discovery of vitamin D in the early 20th century to modern investigations of its role in the genome, vitamin D has already created a rich and complex history. Over the years controversies have abounded – the etiology of rickets, the ethics of a professor or university holding and profiting from a patent, what products to fortify, the definition as vitamin or hormone, the role outside of the skeleton, whether to recommend UV exposure, whether to test, how to define sufficiency, whether to supplement, how to treat deficiencies and what to study next. Although there may be lack of consensus regarding supplementation doses or replacement regimens, most agree that we need to learn more.

Organized by the National Institute of Child Health and Human Development and the National Institutes of Health Office of Dietary Supplements, the 2003 "Vitamin D and Health in the 21st Century: Bone and Beyond" conference included more than 200 attendees representing multiple national institute health offices, the US Department of Health and Human Services, the CDC, the Department of Agriculture and the National Dairy Council. They concluded that a number of topics needed to be better addressed including defining adequacy and sufficiency, establishing a qualitative measurement for UV exposure, exploring more thoroughly the benefits versus harms of UV exposure, determining bony and non-bony outcomes based on vitamin D status, reevaluating daily reference intake, providing the public with better information regarding vitamin D (and avoiding the mixed messages that can easily occur, especially with breastfeeding and UV exposure), studying genetic polymorphisms, improving prevalence estimates, determining what type of surveillance high risk groups may need, better explaining the relationship between vitamin D and obesity, and establishing more complete data for vitamin D in food [163].

Similarly, the attendees of IOM's 2011 "A Vitamin D Expert Panel Meeting" (including representatives from the CDC, AAP, academics, government agencies and laboratories) suggested that future recommendations

should consider health effects other than rickets and identified the need for more long term studies [48]. Within their most recent guidelines, the IOM also concluded that additional research and better understandings are urgently needed [60, 148, 164].

While some of these ambitions set forth by these conferences have already been and continue to be explored, the fact that each of these conferences developed lists of additional research needs is quite telling of our still elementary understanding of vitamin D. As we look back on the insightful observations and ground-breaking experiments of scientists such as Francis Glisson, Theobald Palm, Harriette Chick, Elmer McCollum, Edward Mellanby, and Harry Steenbock (just to name a very select few), one can only wonder what the next century will bring for the sunshine vitamin.

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