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Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride¹

Two university researchers presented information about Dietary Reference Intake (DRI) research recommendations contained in the Calcium and Related Nutrients Report (IOM, 1997). Bess Dawson-Hughes of Tufts University, who served on the panel for the report, highlighted a number of research recommendations that were considered to be of major importance for each of the five nutrients covered in the report and then made some comments about progress in those areas. Bruce W. Hollis of Medical University of South Carolina focused on the dietary requirement for vitamin D, which is his area of research.

DRI REPORT ON CALCIUM AND RELATED NUTRIENTS

Presenter: Bess Dawson-Hughes

Calcium

Major research recommendations for calcium included defining (1) the impact of calcium intake on mineral metabolism and peak bone mass; (2) genetic and calcium intake interactions; (3) the impact of calcium intake by adults on skeletal end points and on nonskeletal end points

¹This chapter is an edited transcript of remarks and slides by Drs. Bess Dawson-Hughes and Bruce Hollis at the workshop. Comments made by Dr. Dawson-Hughes represent a composite of input from many of the panel members including Stephanie Atkinson, Steve Abrams, Bob Heany, Robert Rude, Connie Weaver, and Gary Whitford, and from James Penland (regarding magnesium).

such as blood pressure, cancer risk, and diabetes; and (4) the impact of calcium intake on the risk of developing a first or recurrent kidney stone.

The most notable progress is a much better understanding of the link between calcium intake and absorption, particularly in adolescents. In addition, data now relate some of the vitamin D polymorphisms to mineral metabolism in children. Information from Goulding and others (1998) indicates that milk intake in childhood influences fracture rates both in childhood and later in life in women.

The longest of several recent calcium intervention trials is a 7-year intervention study conducted by Matkovic et al. (2005). That study found that the bone mineral density of girls who were taller at the end of the 7-year interval benefited from supplementation. The results raise the question as to whether calcium intake should be set on the basis of body size, at least in children and adolescents.

Racial differences in bone turnover and calcium metabolism have been described. Over the last nine years, numerous randomized controlled trials of adults have been published. The studies that had a placebo group with a low calcium intake and a reasonable level of compliance generally have shown beneficial effects of calcium supplementation on bone.

The Dietary Approaches to Stop Hypertension (DASH) Study has looked at dairy food intakes to reduce blood pressure. Several studies identify a small (3% to 5%) inverse effect of calcium on weight. That is controversial, but there seems to be a low-level association.

Data on the effect of high calcium intake on the occurrence of first kidney stone now are available from approximately 36,000 women in the Women's Health Initiative. Reportedly, a comprehensive analysis of the stone risk is underway. The report by Borghi et al. (2002) provides data on associations of calcium intake and recurring stones.

One recent 5-year calcium intervention trial (Prince et al., 2006) was conducted in western Australia. The trial included approximately 1,500 women with a mean age of 75 years. Usual calcium intakes averaged approximately 900 milligrams per day. This was a placebo-controlled trial in which the supplement provided 1,000 mg of calcium in the form of calcium carbonate. Table 3-1 shows that only about 57 percent of the subjects in the cohort had an adherence rate of 80 percent or higher. This actually is a fairly good degree of adherence for a very long intervention study. The subset of adherents had a statistically significant reduction in all fracture risk. Those in the intent-to-treat group had a similar trend, but it was not significant.

TABLE 3-1 Effect of 1,200 mg of Calcium Supplement Versus Placebo on Fracture Risk

	Percent of Subjects	Hazard Ratio [95% CI]
All subjects	100%	0.87 [0.67–1.12]
Adherent subjects ^b	57%	0.66 [0.45–0.97]

^aCI = confidence interval.

^bSubjects maintaining an adherence rate of at least 80 percent.

SOURCE: Prince et al., 2006.

Vitamin D

Among the primary research recommendations for vitamin D are to (1) define optimal serum 25-hydroxyvitamin D [25-(OH)D] concentration according to age group, race, and latitude; and (2) define relationships between intake of vitamin D and serum 25-(OH)D.

The process of defining optimal vitamin D intake has been unfolding along the following lines over the last nine years. Three steps in the process are identifying the biological action affected by vitamin D, determining the amount of 25-(OH)D needed to optimize function for that biological action, and then defining the relationship between intake and serum 25-(OH)D.

Previously, parathyroid hormone (PTH) suppression was the primary gauge of optimum vitamin D status. Then, considerable development occurred in the biological action arena, particularly regarding musculoskeletal end points. Bone density, muscle performance, falls, and fractures now are key vitamin D musculoskeletal end points. A burgeoning area involves associations of vitamin D intake and 25-(OH)D with a wide variety of other very important end points, including the risk of developing diabetes, cancer, infection, periodontal disease, and osteoarthritis. These health problems involve a large proportion of the population.

A cross-sectional study out of Iceland, in over 900 individuals, examined the relative contributions of calcium and vitamin D to PTH suppression. Figure 3-1 shows that subjects were sorted by three categories of calcium intake, shown in the three types of bars and by categories of 25-(OH)D concentrations, shown on the X axis. In persons with very low 25-(OH)D concentrations, below 25 nmol/L, PTH was suppressed by increasing calcium intake. However, as the vitamin D concentration increases above 25 nmol/L, calcium plays a less obvious role in suppress-

ing PTH. In Iceland, two-thirds of the people were at 25 nmol/L or more of 25-(OH)D, and in populations residing closer to the equator, one would expect to find a greater proportion with vitamin D concentrations greater than 25 nmol/L.

Table 3-2 lists vitamin D placebo-controlled intervention trials relevant to estimating the optimal 25-(OH)D concentration. The table includes the author, the dosage of vitamin D administered compared with placebo (some with calcium, some not), the 25-(OH)D concentration achieved on supplementation, and the changes in parathyroid hormone. It shows that four of the eight studies demonstrated a positive effect on nonvertebral fracture risk reduction. Based in part on these study results, many are estimating that a 25-(OH)D concentration of 75 nmol/L or higher will reduce the risk of fracture more than would lower concentrations of 25-(OH)D.

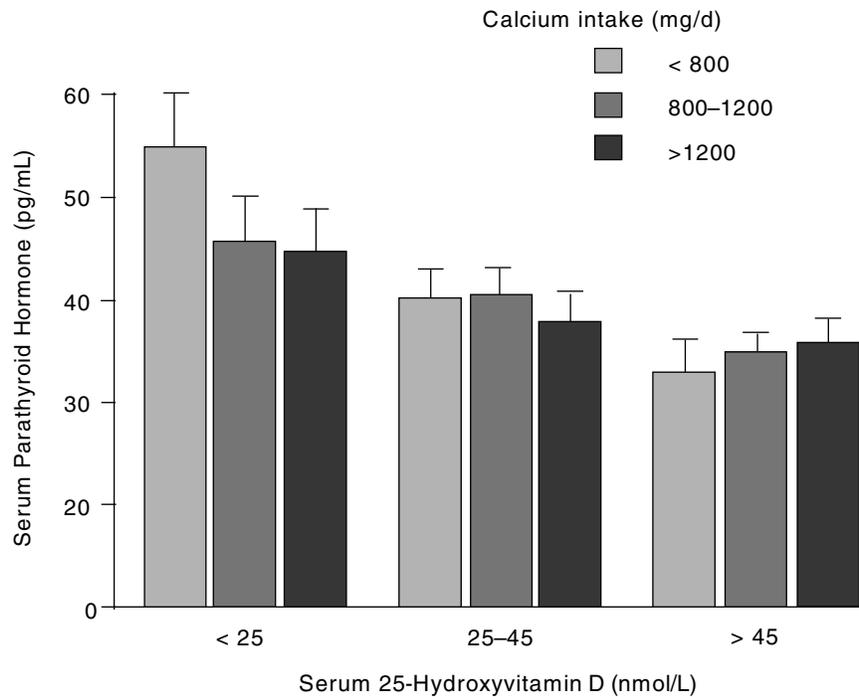


FIGURE 3-1 Serum parathyroid hormone concentration by serum 25-hydroxyvitamin D level and by level of calcium intake.

SOURCE: Steingrimsdottir et al. 2005. *JAMA*. 294: 2336-41. Copyright © 2005, American Medical Association. All rights reserved.

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TABLE 3-2 Serum 25-Hydroxyvitamin D, Parathyroid Hormone, and Nonvertebral Fracture Responses to Supplementation with Vitamin D₃

Author, Year	Dose (IU/d)	25-(OH)D (nmol/L)	Δ PTH (%)	Effect on Nonvertebral Fracture
Chapuy et al., 2002	800	100	-47	+
Chapuy et al., 1992	800	100	-33	+
Dawson-Hughes et al., 1997	700	112	-28	+
Trivedi et al., 2003	820	74	?	+
Grant et al., 2005	800	63*	0	NS
Jackson et al., 2006	400	59*	?	NS
Lips et al., 1996	400	54	-6	NS
Meyer et al., 2002	400	64	?	NS

NOTE: Δ PTH = Change in parathyroid hormone concentration; NS = no statistically significant effect.

*Estimates in subsets of subjects.

It appears more useful to consider the 25-(OH)D concentration than the supplement dose. Take the case, for example, of the Randomized Evaluation of Calcium or Vitamin D (RECORD) Trial (Grant et al., 2005). The investigators gave the same dose of vitamin D as was given in many of the positive trials. Their compliance, however, was low: only half the subjects were taking any supplements at all at the 2-year mark into the 5-year trial. Consequently, the dose consumed could not have been as high as the amount being delivered.

Progress has been made regarding the safety of vitamin D. In a study involving daily dosing with either 1,000 or 4,000 IU of vitamin D per day over a 5-month period, no toxicity was invoked. In panel B (the 4,000 IU dose of vitamin D₃) in Figure 3-2, the mean serum 25-(OH)D concentration achieved was 100 nmol/L. Nearly all subjects were in the desired range of 75 to 80 nmol/L or higher. Although one subject's 25-(OH)D value rose to 138 nmol/L, that is a typical level for a person who works outdoors in the sun in the summertime. An average lifeguard will have a 25-(OH)D concentration of approximately 160 nmol/L. There is no sense of concern about the serum vitamin D concentrations shown in Figure 3-2.

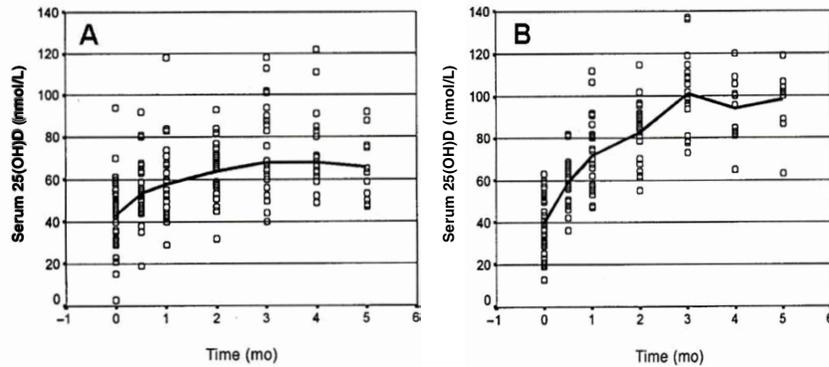


FIGURE 3-2 Serum 25-hydroxyvitamin D responses to two levels of vitamin D₃ supplementation over 6 months.

NOTE: A = 1000 IU/d of vitamin D₃; B = 4000 IU/d of vitamin D₃.

SOURCE: Veith et al. (2001). Reproduced with permission from *The American Journal of Clinical Nutrition*.

Data are just beginning to appear concerning vitamin D needs of children. However, much information still is needed, particularly for children in the age range of 6 months to 8 years. A very recent intervention study with 200 or 400 IU of vitamin D looked at the change in bone mineral content in young adolescent girls over a 1-year interval. Results showed step increases in the change in bone mineral content with more vitamin D supplementation.

For adults, more data are needed to refine estimates that 75 to 80 nmol/L of serum 25-(OH)D are needed for the musculoskeletal end points. There also is a great need to obtain threshold estimates for the chronic disease end points mentioned earlier. An enormous amount of work is being conducted in this area now.

Additional needs include standardization of 25-(OH)D assays and better quantitation of the vitamin D content of foods. For example, the vitamin D values for salmon (one of the main food sources of this vitamin) are based on wild salmon. Does farm salmon, which currently is widely consumed, have the same vitamin D content?

Phosphorus

For phosphorus, there were two key research recommendations: (1) for ages 1 to 18 years, to define the intake needed to optimize bone accretion; and (2) for children and adults, to define the relationship between phosphorus intake and the concentration of phosphorus in the blood.

Relatively little progress appears to have occurred in this area. Current identified research needs concern the potential problem of low phosphorus intake, especially by older individuals in the United States; the extent to which the increasing use of calcium supplements may decrease the bioavailability of phosphorus; and a potential increased need for phosphorus related to the introduction of anabolic agents for the treatment of osteoporosis.

Magnesium

The key research recommendations for magnesium were to (1) identify the magnesium intake needed for maximum accretion of bone in children and for preservation of bone in adults; (2) identify associations between magnesium intake and the development of other conditions such as hypertension, cardiovascular disease, and diabetes; and (3) identify and validate an accurate indicator of magnesium status.

Some interesting animal and epidemiological studies have been published recently, as mentioned briefly here. Rude and coworkers (2004, 2005, 2006) conducted several elegant magnesium depletion studies in rats. Table 3-3 shows that at half of the magnesium requirement, trabecular bone volume was decreased, and osteoclast number was higher. With further and further magnesium depletion, these effects became more exaggerated in the rats and, in addition, tumor necrosis factor-alpha concentrations rose. Unfortunately, parallel data in humans are unavailable.

TABLE 3-3 Magnesium Depletion and the Skeleton in Rats

Study	Diet, % Nutrient Requirement	Outcome
Rude et al., 2004	50	↓ Trabecular volume, ↑ osteoclast number
Rude et al., 2005	25	also ↑ TNF-alpha
Rude et al., 2006	10	also ↑ TNF-alpha

NOTE: TNF-alpha = tumor necrosis factor alpha

Two large epidemiologic studies involving large numbers of subjects with long follow-up periods are of special interest. In these studies, the subjects in the highest versus lowest quintile of magnesium intake had a decreased risk of developing both type II diabetes (Lopez-Ridaura et al., 2004) and stroke (Ascherio et al., 1998). Although these studies can help develop hypotheses that may be promising, the approach does not really isolate a nutrient—especially since the magnesium from food tends to be accompanied by other important nutrients.

Little has changed with regard to the indicators of magnesium status, but a report by Barbagallo and colleagues (2000) merits consideration. This study makes use of the facts that 99 percent of magnesium in the body is intracellular and that, of this, approximately 10 to 15 percent is in the ionized metabolically active form. These investigators found no differences in the free intracellular magnesium concentrations of normal subjects compared to hypertensive individuals or compared to persons with diabetes.

Fluoride

Key research recommendations for fluoride include (1) conducting epidemiologic research on habitual exposure to fluoride, particularly focusing on the prevention of dental caries, quality of bone, and the risk of developing fluorosis; and (2) identifying the factors that influence fluoride absorption and retention.

Two very comprehensive and important reports on fluoride were published over the last seven years—an epidemiologic study by Sowers et al. (2005) and another from the National Health and Nutrition Examination Survey (NHANES) by Beltran-Aguilar et al. (2005). The study by Sowers and colleagues had 4 years of follow-up and looked at fractures and changes in bone density in 1,300 women in three different communities in Iowa. The control community had a fluoride content of 1 ppm in the water supply (within the recommended level), and mean calcium intake was 754 mg/day. For comparison, one of the communities had the same fluoride content in the water but a high mean calcium intake (1,001 mg/day). The other test community had a high fluoride content (4 ppm) of the water, but calcium intake was comparable to that of the control community.

The bottom line is that the fracture rates were similar in the three communities. Bone densities were similar, with the exception of a minor

increase in the forearm in the high fluoride community. These results do not raise much concern about fluoride in these communities.

Using NHANES data, Beltran-Aguilar et al. (2005) compared certain dental outcomes from the period of 1988–1994 with the same outcomes from 1999–2002. The investigators found that caries in permanent teeth in the United States have declined, as has tooth loss in older adults. The prevalence of enamel fluorosis has increased, however, starting in 1980. Racial and ethnic disparities persist in the prevalence of caries and enamel fluorosis.

THE DIETARY REQUIREMENT FOR VITAMIN D: LOOKING BEYOND BONE

Presenter: Bruce Hollis

This presentation focused on vitamin D. Dr. Hollis took the position that vitamin D₂ (the form of vitamin D obtained by the irradiation of ergocalciferol from plants) should be removed from use in food supplements and that vitamin D₃ (the form of the vitamin that is produced by the action of ultraviolet light on vitamin D precursors in the skin) should be used instead. The biggest reason given for this position is that vitamin D₂ is less biologically active than vitamin D₃ (Armas et al., 2004). Giving vitamin D₂ elicits more variable responses in 25-(OH)D values than does giving vitamin D₃.

Vitamin D₃ is metabolized fairly rapidly to 25-(OH)D, which is the major circulating form of vitamin D and is a biomarker for vitamin D status. One can influence the concentration of 25-(OH)D through diet or sun exposure, but one can have little influence on the amount of circulating 1,25-(OH)₂D—the active form of the vitamin. Vitamin D metabolism differs depending on the amount of vitamin D₃ supplied to the body. When vitamin D supplies are inadequate, the flow of 25(OH)D through other potential pathways, including its utilization by peripheral tissues for paracrine regulation, is compromised. Dr. Hollis indicated that a chronic depleted status may be common today and that a depleted vitamin D status poses risks for within-tissue processing.

Research Questions on Vitamin D Requirements

Research on vitamin D requirements needs to consider the cutaneous generation of vitamin D and differences in vitamin D₃ generation that relate to skin color, latitude, the use of sun screen, and the amount of skin actually exposed to the sun. In view of the relative lack of vitamin D₃ in the food supply and public health recommendations to use sun screen to reduce the risk of skin cancer, what amount of oral vitamin D supplementation should be provided? Heaney et al. (2003) examined the response of 25-(OH)D as a function of oral vitamin D₃ intake over a 5-month period. Circulating 25-(OH)D values at the highest intakes (5,000 to 10,000 IU/day) were comparable to those resulting from extensive solar exposure, and those at the lowest intakes (400 to 1,000 IU/day) tended to decrease over time.

According to Dr. Hollis, the 400 IU (10 µg) dose of vitamin D supplied to subjects in the Women's Health Initiative studies was too small to increase the 25-(OH)D values at all. He based his position on the 2003 Heaney et al. study, including a regression of the equilibrium increment in serum 25-(OH)D concentration on the labeled dose for the mean intake by each treatment group.

Another important question involves the identification of optimal circulating concentrations of 25-(OH)D. Different types of evidence suggest that concentrations previously identified as normal are too low. In particular, Dr. Hollis noted problems with the interpretation of the study by Haddad and Chyu (1971) that identified a low 25-(OH)D level as normal. A biomarker would be especially useful if it would respond to both increases and decreases in the 25-(OH)D concentration.

Dr. Hollis indicated that effects of vitamin D on PTH are well known, as are vitamin D interactions in bone. He considers insulin sensitivity, beta cell function, immune function, and circulating cytokines (both proinflammatory and anti-inflammatory), and cardiac health to be promising research areas.

There are several examples of relationships of vitamin D with infection. A recent study by Liu and colleagues (2006) provides an explanation for findings by Finsen (Rochietta, 1960) that ultraviolet light benefits patients with lupus vulgaris and for the demonstrated beneficial effects of ultraviolet light exposure on tuberculosis patients in sanatoria. In particular, the study found that human toll-like receptor triggers a vitamin D receptor-dependent antimicrobial response. This response in-

volves the innate immune system, which provides the first response to pathogens when they invade the body.

In Caucasians and African Americans, Liu and coworkers (2006) showed that nutritional vitamin D status controlled cathelicidin, an antimicrobial peptide made by monocytes and macrophages. At low concentrations (20 or 30 nmol/L) of serum 25-(OH)D, subjects could not mount a response to kill the internalized tuberculosis in the macrophages. After supplementation with vitamin D in amounts sufficient to achieve serum 25-(OH)D concentrations of 80 nmol/L, the subjects could mount this attack, produce cathelicidin, and attack the tuberculosis.

A brief study conducted in India (Rehman, 1994) shows that vitamin D supplements of 60,000 IU/week for 6 weeks could help children with elevated alkaline phosphatase concentrations to overcome respiratory infections.

A study by Giovannucci and colleagues (2006) estimates the effects of a 25-nmol/L increase in serum 25-(OH)D in a population on the odds ratios of developing various forms of cancer. For 11 of the 14 forms of cancer, the odds ratios of developing those cancers are less than one. Additional work is in progress.

A small pilot vitamin D supplementation study (Wagner et al., 2006) looked at periodic changes in maternal and infant blood concentrations of 25-(OH)D and of vitamin D activity in mother's milk. The study found that the higher level of maternal vitamin D₃ supplementation (6,400 IU compared with 400 IU) resulted in substantial increases in vitamin D₃ and 25-(OH)D in the mother and in milk vitamin D activity. The serum 25-(OH)D concentration in the infants of the highly supplemented women was comparable to that of infants who were supplemented directly.

Dr. Hollis challenged the current Adequate Intake (AI) of 10 µg of vitamin D/day for adults and probably for adolescents as not supportive of health. He stated that it is almost certain that chronic deficiency of vitamin D puts populations at risk for debilitating chronic diseases. Moreover, he posited that a serum concentration of 80 nmol/L of 25-(OH)D, which has been proposed to protect the skeleton, is too low to reduce the risk of muscular sclerosis or cancer or to promote immune function.

Tolerable Upper Intake Level for Vitamin D

Dr. Hollis challenged the Tolerable Upper Intake Level (UL) that the Institute of Medicine set for vitamin D. In studies by Hollis and Wagner (2004), Wagner et al. (2006), and Heaney et al. (2003) involving 10,000 IU/day of vitamin D (or more), no instances of hypercalciuria or hypercalcemia occurred. Furthermore, Dr. Hollis indicated that the low UL is an impediment to human health because it sets too low a limit on what the vitamin manufacturers can add to their vitamin supplements.

DISCUSSION

Questions and comments by Drs. Dennis Bier, Irwin Rosenberg, Larry Appel, Paul Pencharz, and Robert Russell are summarized below, along with responses from the presenters.

Optimal Parathyroid Hormone level

Dr. Bier asked how one defines the optimal PTH level in the context of adequacy. Drs. Hollis and Dawson-Hughes responded that many sound studies with reliable PTH assays have looked at this relationship. They all indicate that one can suppress PTH serially only until the 25-(OH)D concentration reaches approximately 80 nmol/L. Lower rates of bone loss provide the basis for the suppression. Neither calcium nor vitamin D (nor both) can suppress PTH more than a maximum of 80 to 90 percent. In such studies, the calcium range is defined in the usual way, with a group of healthy individuals.

Circular Argument

Dr. Bier expressed concern about issues related to PTH suppression and about a circular argument relating to the reference range for calcium. In particular, the reference range for calcium was said to be defined by values obtained from a group of “healthy individuals,” but presentations pointed out the high prevalence of inadequate vitamin D in the population, which presumably is not healthy. Dr. Hollis responded that some recent studies address the points of having an adequate calcium index

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and setting the PTH reference ranges in relation to adequate vitamin D status.

Measures of Early Adverse Effects

Dr. Rosenberg asked what are potential measures of early adverse effects. Dr. Dawson-Hughes responded that probably the easiest and least expensive measure to obtain would be a random spot-urine calcium, which correlates very highly with the 24-hour urine calcium-to-creatinine ratio. That correlation is so strong that one would not need to conduct a 24-hour urine collection to assess safety. Basically, the random spot-urine calcium content would increase before the renal threshold was exceeded and before an increase in the blood calcium concentration occurred. Thus, the random spot-urine calcium would be an early point for detection, and it would be easier to interpret than a PTH value. Such a measurement would not be used to set a requirement but to monitor for safety. Dr. Hollis agreed that the random spot-urine calcium could be measured easily in a research setting or a clinical setting. He added that, because PTH is potentially cardiotoxic, it probably is in the interest of cardiovascular health to have lower PTH concentration as well. Dr. Dawson-Hughes stated that there is no evidence of any extension of the curve of the inverse relationship between insufficient levels of either calcium or vitamin D intake and PTH concentration.

Vitamin D and Chronic Illness

Dr. Hollis suggested that lack of vitamin D might be related to the high prevalence of lupus, multiple sclerosis, and diabetes among a population of African Americans called the Gullah in South Carolina. He reported that their vitamin D concentrations were the lowest he had ever seen.

Clarification of Units

Dr. Bier asked about the dramatic increase in maternal vitamin D intake that had a relatively small effect on circulating 25-(OH)D concentrations for the mother and the baby—the circulating levels for the mother

and the infant were still below 80. Dr. Hollis clarified that the units are nanograms. To convert nanograms to nanomoles, one multiplies that number times 2.5.

Maternal-Infant Findings

Dr. Pencharz referred to a Muslim woman who bore a baby with neonatal rickets. These investigators in Saudi Arabia were able to treat the neonatal rickets by having the mother exposed to sunlight. That proved in a biological way what Dr. Hollis has shown biochemically. It appears that the evidence now supports recommendations to supplement the mother. Dr. Hollis responded that blinded maternal studies are underway but the increased blood concentrations of 25-(OH)D clearly indicate the individuals who are receiving the vitamin D. Data still are needed.

A model of the transfer of vitamin D during lactation is evolving. In response to questions from Dr. Allen, Dr. Hollis indicated that about 0.5 percent of the circulating 25-(OH)D passes into breast milk but that 30 to 80 of the parent compound (vitamin D₃) passes into the milk.

Persistence of Circulating 25-(OH)D

Dr. Russell asked how long very high blood concentrations of 25-(OH)D persist after extensive sun exposure, as in a life guard working outside. Dr. Dawson-Hughes was unaware of such data but referred to data from healthy older subjects in Boston. In the summer, their concentration of 25-(OH)D is approximately 80, and it decreases to about 60 in the winter. Dr. Hollis stated that the half life of 25-(OH)D is approximately 3 weeks.

Vitamin D₂ Versus D₃

Dr. Whiting asked Dr. Dawson-Hughes to elaborate on the relative value of vitamin D₂ and D₃. Dr. Dawson-Hughes responded that, from the current available evidence, D₃ is preferred. Although the evidence base is not extensive, it is consistent in suggesting that, for a given dose of D₂ or D₃, over at least a 2-week period, a considerably larger increment in the

total 25-(OH)D concentration occurs with repeated dosing with vitamin D₃.

The study by Armas and colleagues (2004), which used a single dose of 50,000 units of vitamin D₂ or D₃, found similar increments in 25-(OH)D over the first 3 days; but subsequently the total 25-(OH)D concentration decreased in the D₂-treated subjects. In fact, it was lower than baseline at the end of 30 days. In contrast, the concentration in the D₃-treated subjects remained well above the baseline level at 30 days. Thus, the staying power of the single dose of vitamin D₃ is greater, and the increment over 2 weeks is greater. Vitamin D₂ can be effective, but it takes a much larger dose on a frequently repeated dosing regimen.

