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Original Communication
Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it

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Objective: We asked whether women self-reporting the recommended consumption of vitamin D from milk and multivitamins would be less likely to have low wintertime 25-hydroxyvitamin D (25(OH)D) levels.
Methods: This cross-sectional study enlisted at least 42 young women each month (age 18–35 y, 796 women total) through one year. We measured serum 25(OH)D and administered a lifestyle and diet questionnaire.
Results: Over the whole year, prevalence of low 25(OH)D (<40 nmol/l) was higher in non-white, non-black subjects (25.6% of 82 women) than in the white women (14.8% of 702 white women, $P<0.05$). Of the 435 women tested during the winter half of the year (November–April), prevalence of low 25(OH)D was not affected by vitamin D intake: low 25(OH)D occurred in 21% of the 146 consuming no vitamin D, in 26% of the 140 reporting some vitamin D intake, up to 5 μg/day (median, 2.5 μg/day), and in 20% of the 149 women reporting vitamin D consumption over 5 μg/day (median, 10 μg/day).
Interpretation: The self-reported vitamin D intake from milk and/or multivitamins does not relate to prevention of low vitamin D nutritional status of young women in winter. Recommended vitamin D intakes are too small to prevent insufficiency. Vitamin D nutrition can only be assessed by measuring serum 25(OH)D concentration.
Descriptors: cholecalciferol; dietary intake; RDA; AI; osteoporosis; deficiency
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Introduction

Recommended nutrient intakes are intended to prevent development of nutrient insufficiencies in virtually all healthy persons (Yates, 1998; Dwyer, 2000). Vitamin D recommendations have posed a problem because, except for some fish, vitamin D is not naturally present the foods humans normally eat. The intake for adults was arbitrarily recommended as half the amount of vitamin D in a teaspoon full of cod-liver oil (Blumberg *et al*, 1963)—a centuries old folk remedy, used to help infants thrive (Vieth, 1999).

Serum 25-hydroxyvitamin D (25(OH)D) is the objective measure of vitamin D nutritional status. The vitamin D *deficiency* that causes rickets in infants, or osteomalacia in adults, is diagnosed by a serum 25(OH)D level <25 nmol/l (10 ng/ml; Parfitt *et al*, 1982). Diagnosis of less severe vitamin D malnutrition is based on what is now a well-documented inverse relationship between serum 25(OH)D levels and parathyroid hormone (PTH; Gallagher *et al*, 1998; Harris & Dawson-Hughes, 1998). The same criteria for interpreting serum 25(OH)D apply to all adults, regardless of age. Serum 25(OH)D $<40-50$ nmol/l is regarded as low (Liu *et al*, 1997: McKenna & Freaney, 1998; Thomas *et al*, 1998; Need *et al*, 2000). Desirable 25(OH)D levels may exceed 73 nmol/l, based on a large French study, of 1500 normal adults tested in winter. An apparent low plateau in serum PTH was sustained so long as serum 25(OH)D levels exceeded 73–82 nmol/l (Chapuy *et al*, 1997; Guillemant *et al*, 1999; Heaney, 2000). This

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Contributors: RV conceived this nutritional component to the larger study initiated by GAH and LAR, carried out statistical analysis, and wrote the manuscript. GAH and LAR initiated the survey of young women, supervised its completion, assembled the data, and contributed to the intellectual development of the work presented here. DEC supervised assembly of the data, ensured appropriate handling and retrieval of sample material, and joined in the intellectual development of the present manuscript. HMT ensured appropriate handling and retrieval of sample material, carried out vitamin D assays, and joined in the intellectual development of the present manuscript.
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implies that the basal 25(OH)D level of 'healthy' subjects should be at least 73 nmol/l, since below this level the body attempts to compensate and re-establish equilibrium by a physiological mechanism — namely, there is a compensatory increase in serum PTH.

The excess PTH associated with vitamin D insufficiency probably promotes mineral loss (Dawson-Hughes *et al*, 1991). One cause of osteoporosis is a nutritional deficiency disorder, a pattern not unlike scurvy, which is a long-term process contributed to by years of a subclinical, marginal lack of vitamin D (Heaney, 1999; Peacock, 1998).

The recommended vitamin D intake for adults under 50 y is 5 μg/day (Blumberg *et al*, 1963). It is generally assumed that children and the elderly require more vitamin D than younger adults (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997; Gloth *et al*, 1991; Kinyamu *et al*, 1997). Therefore, the AI for adults under 50 y remains at the old RDA level of 5 μg (200 IU)/day. The AI (adequate intake) is the recommended intake target in situations where there is not enough evidence to set an RDA. For adults over 50 y, the AI level was doubled in 1997, and for those over 70 y, it was tripled to 15 μg/day (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997). To justify the young adult AI, the Food and Nutrition Board (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997) presented only one report that was never intended to address the issue of how much vitamin D might be necessary to prevent vitamin D deficiency (Kinyamu *et al*, 1997). Milk is fortified with vitamin D (10 μg/0.95 l) in Canada and the United States, and many women take multivitamins (10 μg/day), but vitamin D is essentially absent from other foods normally consumed (Takeuchi *et al*, 1995).

Since it is well known that, at northern latitudes, serum 25(OH)D changes with season (Scharla, 1998), we asked whether it is reasonable to assume that serum 25(OH)D will exceed a conservative nutritional target of 40 nmol/l in young women who report consuming vitamin D in the amounts currently recommended for them (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997).

Methods

Subjects

Subjects were volunteers from the community who responded to advertisements seeking healthy female subjects for osteoporosis research, between ages 18–35 y. Determinants of the bone mass in most of these women, including the effect of calcium intake, have been reported previously (Rubin *et al*, 1999). Exclusion criteria were conditions known to be associated with secondary bone loss (Crohn's disease, symptom addict hyperthyroidism, rheumatoid arthritis, bilateral oophrectomy, or use of systemic corticosteroids therapy for more than 3 months' duration at any time past). Women previously diagnosed with, or investigated for osteopenia were excluded. Mean age was 27.3 y. There were 796 women in this cross-sectional study, assessed once, and different women were tested over the 16 month period. White women comprised 89% of subjects; black women, 2%; Asian women, 6%; Indo-Asian women, 3%. This study was reviewed and approved by an ethics committee of the University of Toronto, and subjects provided written consent to participate.

Study design

From October, 1995 to March, 1997, at 43°N latitude, women were tested at a rate of at least 42 per month. The women took part in an interview and questionnaire process to investigate the relative contribution of clinical and environmental variables in the attainment of peak bone density. This covered exercise, lifestyle factors, menstrual and reproductive history, medical conditions, and family history of osteoporosis. Dietary intakes focused on milk and calcium sources, vitamin supplements and medication use. The approach to assessing vitamin D consumption was essentially the same as that used in previous, validated food frequency questionnaires (Friis *et al*, 1997; New *et al*, 1997). Vitamin D intake was derived from the question, 'How many glasses of milk (8 ounces) do you drink on average, each day?', and the question, 'Please list all the medications, vitamins, over-the-counter products, health food store preparations, and prescribed medicines you are currently taking'. Based on the vitamin D3 added to these products, we assumed that one glass of milk contained 2.5 μg (100 IU) vitamin D, and one multivitamin contained 10 μg (400 IU) vitamin D. In Canada milk is the only dairy product fortified with vitamin D, 10 μg vitamin D per quart; the only other vitamin D fortified food is margarine, 1.2 μg/10 g, which we did not assess.

Measurements and statistical analysis

Serum 25(OH)D was measured with the DiaSorin radioimmunoassay (Stillwater, MN, USA), which detects 25(OH)D2 and 25(OH)D3 equally. In our hands, the assay performs with a between-assay CV of $< 16\%$, and a within-run CV of $< 10\%$, and consistently reports results within the central $\pm$ 1s.d. of the mean of all laboratories in the External Quality Assurance Survey (Northwest Thames, England). Samples were assayed in batches after all had been collected. To minimize the chance of fluctuation due to batch variation, the samples were not analyzed in order, but in two passes of assays through the sample set. November and May were used to divide winter and summer because human insolation to UVB radiation is negligible between November and April (Webb *et al*, 1990); the monthly 25(OH)D levels agree with this; lastly, since 25(OH)D has a 1–2 month half-life, its levels should fluctuate in a cycle 1–2 months later than the cycle of solar radiation. For this study we followed our established practice, classifying low 25(OH)D levels as those < 40 nmol/l (Liu *et al*, 1997). Some serum samples were selected to measure PTH using a solid-phase, two-epitope,

chemiluminescent enzyme immunometric assay, Immulite 2000 automated immunoassay system (DPC Cirrus Inc, Randolph NJ). To confirm that a relationship exists between 25(OH)D and PTH in our sample group, we selected the samples with 10 highest 25(OH)D, 10 samples with 25(OH)D at approximately 80 nmol/l, and 10 samples with 25(OH)D < 40 nmol/l.

Results were analyzed with SPSS statistical software, version 8 (Chicago, IL, USA). Prevalence of low 25(OH)D was calculated by dividing the number of women with 25(OH)D < 40 nmol/l by the total number tested in the group context (we variously grouped according to race, vitamin D intake, or timeframe). The 95% confidence limits for prevalence were calculated based on the binomial heorem, true population proportion $= P \pm 1.96 \sqrt{(P(1-P)/n)}$, where P is the observed proportion, and n is the number of individuals tested in the group (Dixon & Massey, 1969). The relationships between dietary vitamin D intake and serum 25(OH)D were analyzed both as continuous variables and by classification of them into groups specified by ranges of each, as indicated in the results.

Results

Figure 1 shows the monthly mean 25(OH)D and prevalence of insufficiency in the white women. 25(OH)D levels were highest in August, and lowest in February. Each month from December to April, 20–28% of white women were classified as having a low 25(OH)D level. Between April and May, there was an abrupt decline in the prevalence of low 25(OH)D, coinciding with pleasant weather, and insolation (sun exposure, and higher UV index). In contrast, the deterioration phase of vitamin D nutritional status required more time, from September to December.

Figure 2 shows serum PTH levels in samples selected to represent certain 25(OH)D concentrations. The correlation was significant ($r = -0.33$, $P < 0.04$, $n = 42$), and the line shown is a 'lowess' regression line, which is a best fit weighted according to localized data clusters.

Table 1 summarizes racial characteristics and seasonal effects on 25(OH)D and the prevalence of low 25(OH)D levels. In winter, the mean serum 25(OH)D level was not significantly different among racial groups. However, both summertime and full-year prevalence of low 25(OH)D in non-white women was significantly greater than in white women.

Of all the 796 subjects, 272 (34% of them) obtained essentially no vitamin D from milk or multivitamins; 196 (25%) took supplements that contained vitamin D; 366 (46%) did not drink milk; 251 (32%) reported consumption of the recommended intake for their age, 5 μg/day or greater. The overall mean vitamin D consumption was 4.6 μg/day, and the median was 2.5 μg/day. Only four women consumed the 25 μg (1000 IU)/day vitamin D3 pills that are the highest dose available without prescription ('over the counter').

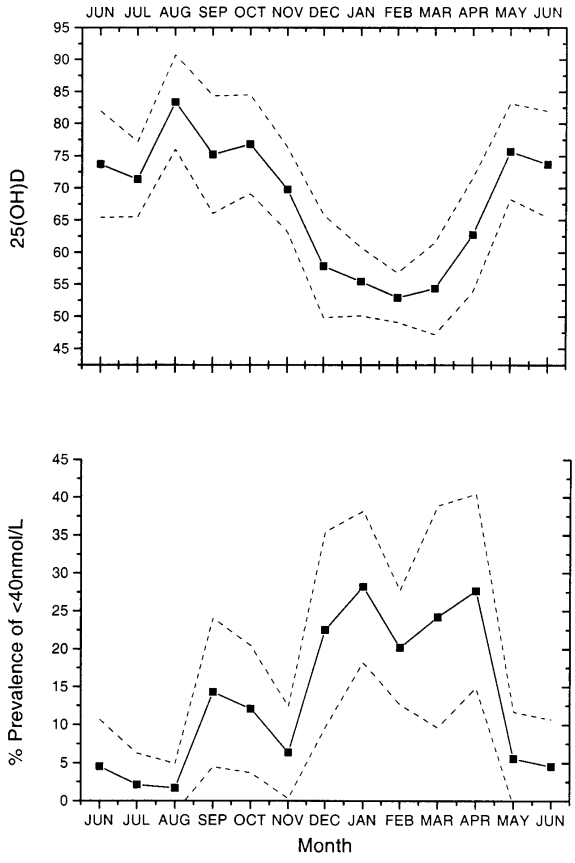

Figure 1 Effect of month of year on mean 25(OH)D levels (upper panel), and the percentage prevalence of vitamin D insufficiency (serum 25(OH)D < 40 nmol/l (< 16 ng/ml), lower panel). Dotted lines connect the 95% confidence limits for mean 25(OH)D and prevalence of insufficiency each month. These results are for the sample subgroup, white women ($n = 702$).

The effect of vitamin D intake from milk and vitamin supplements on serum 25(OH)D in young women is shown in Figure 3, separately for summer and winter. There was no evidence that vitamin D consumption was related to the 25(OH)D level during winter. The box plots for winter (Figure 3) show that the 25th percentile for serum 25(OH)D hovered near 40 nmol/l for each grouping of vitamin D intake. During winter, of the women who consumed essentially no vitamin D from milk or supplements, 31 of 146 (21%) had low 25(OH)D levels. Of those consuming up to two glasses of milk (vitamin D to 5 μg/day, median, 2.5 μg/day), 37 of 140 (26%) had low 25(OH)D. Of the women consuming more than the recommended intake for vitamin D (> 5.0, median, 10 μg/day) 30 of 149 (20%) had low 25(OH)D. No approach to classification according to the vitamin D intake reported by the young women revealed any effect of intake on the prevalence of low 25(OH)D during winter (Spearman correlation = 0.011, $P = 0.81$; or chi-square, $P = 0.39$). The number of glasses of milk consumed per day did not correlate with serum

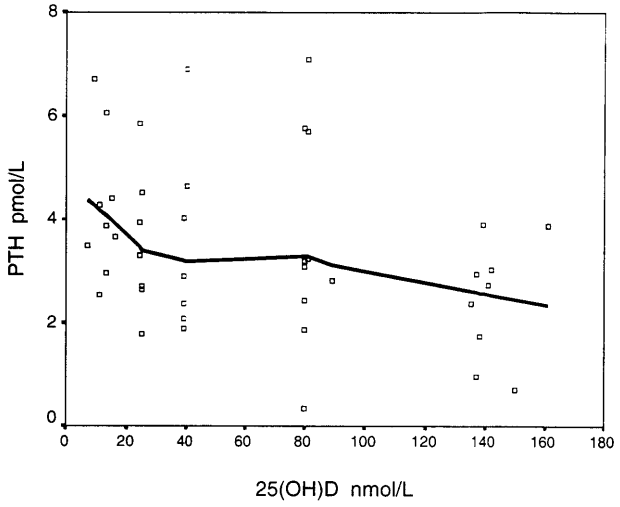

Figure 2 Relationship between serum PTH and 25(OH)D concentrations in healthy young women selected on the basis of serum 25(OH)D. The line through the data is a lowess regression plot, a regression weighted to local data clusters. Conventional linear regression analysis indicated a significant negative relationship ($r = 0.33$, $P < 0.04$, $n = 42$).

25(OH)D in winter or summer (correlation, $P > 0.3$ in each season), and there was no association between milk intake and the prevalence of vitamin D insufficiency (chi-square, $P > 0.30$ in each season).

During summer, there was a modest association between vitamin D intake and 25(OH)D levels (Figure 3) which by logistic regression reflected a significant relationship between multivitamin use and serum 25(OH)D ($r = 0.0866$, $P = 0.0265$) in summer only. For the full year, the exercise variable, 'do you perform any activity to work up a sweat', correlated with the greater use of multivitamins we anticipated (Fisher's exact test, $P = 0.056$, one-tail $P = 0.033$). During summer, but not winter the exercise variables, 'how much recreational exercise do you do (hours/week)?' ($r = 0.151$, $P = 0.008$), and 'do you engage in regular activity long enough to work up a sweat (yes/no)?' ($r = 0.142$, $P = 0.013$), correlated with 25(OH)D levels, as had been expected if their effects were mediated through sun exposure.

Discussion

Serum 25(OH)D changed substantially with season. We divided the year into summer and winter halves based on the seasonal cycle of 25(OH)D concentrations (Figure 1), which lags the earth's solar cycle by 2 months. This lag is because the half-life of 25(OH)D in the circulation is about 2 months (Vieth, 1999). The prevalence of a low 25(OH)D concentrations was greatest during the winter half of the annual vitamin D cycle (November to April). However, it was only during summer phase (May–October) that there was a significant relationship between multivitamin use and serum 25(OH)D. This was because women who were more physically active were also more likely to take multivitamins. Surrogate indices of sun exposure, including activity to sweat and exercise, were significantly related to serum 25(OH)D during the summer, but not in winter when sun intensity is not enough to generate vitamin D. Contrary to what nutritional recommendations should lead one to expect, we could not detect a relationship between vitamin D intake from milk and multivitamins. and serum 25(OH)D during winter (Figure 3).

We divided subjects according to skin color to characterize its effect (Table 1). Since there were only 12 black women in our study, we focused on the other 87 non-white women. We asked whether North American non-white women who are not black are, like black women, at greater risk of vitamin D deficiency. The mean serum 25(OH)D levels in the non-white women were not significantly lower in either season than in white women, indicating that their vitamin D status is not as severely compromized as American black women (Harris & Dawson-Hughes, 1998), but prevalence of vitamin D insufficiency in non-white women was greater during summer than in white women. This agrees with findings in Europe (Serhan *et al*, 1999) and the US (Awumey *et al*, 1998). The explanation for this, given anecdotally by several non-white women, was that women whose skin is naturally darker in color do not intentionally spend time in the sun to deepen its color further. Often, they actively avoid the sun because of this.

After many decades of laws requiring the fortification of milk in North America with vitamin D, there is still no report showing that vitamin D intakes at recommended levels achieve the goal of preventing nutritional insuffi-

Table 1 Seasonal breakdown of race, serum 25(OH)D, and prevalence of vitamin D insufficiency

	Winter			*Summer*		
	Number of volunteers	*25(OH)D (nmol/l) mean*	*Percentage of women < 40 nmol/l (< 16 ng/ml)*	*Number of volunteers*	*25(OH)D (nmol/l) mean*	*Percentage of women < 40 nmol/l (< 16 ng/ml)*
White women	380	58±24	21.3	322	76±28	7.1
Non-white women[a]	47	51±22	31.9	35	68±33	17.1[b]
Black women	8	68±40	25	4	68±15	0

[a]These were Asian (including two Native North American women), and Indo-Asian women, grouped together because of skin color.
[b]Summertime prevalence of vitamin D insufficiency in non-white women was greater than in white women, by a ratio of 2.41 (95% confidence limits, 1.05–5.59). Over the full year, prevalence of vitamin D insufficiency was 25.6% in the 82 non-white women, compared to 14.8% in the 702 white women (odds ratio 1.73, 95% confidence limits 1.15–2.60).

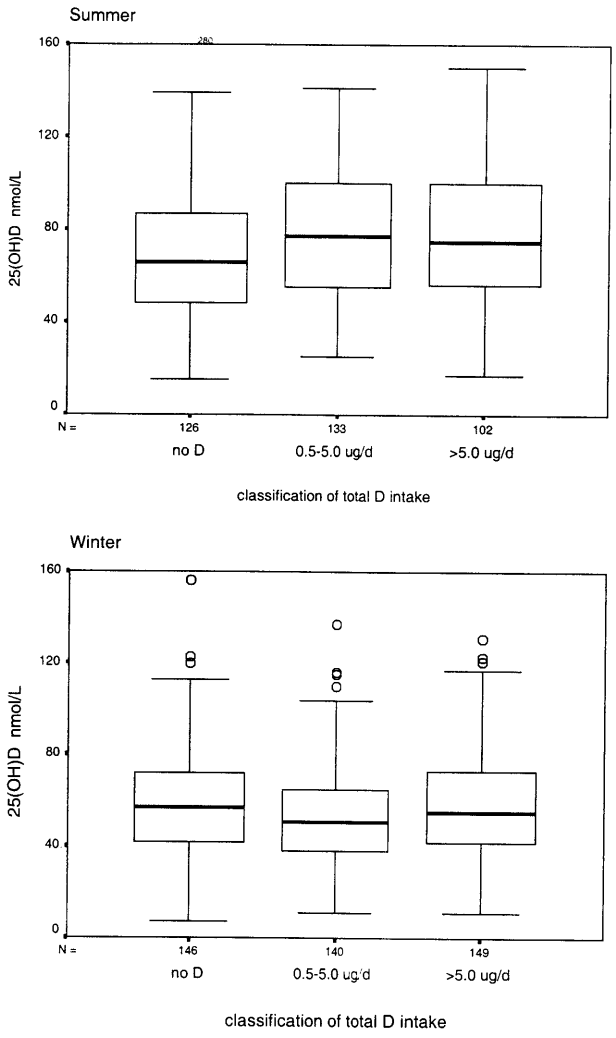

Figure 3 Effect of artificially added vitamin D intake on serum 25(OH)D level. The data are presented separately for season due to the differences in UV exposure: summer (May to October) and winter (November to April). Median vitamin D intakes for each category are, from left to right: essentially no vitamin D from milk or multivitamins, 2.5 μg (100 IU)/day, and 10 μg (400 IU)/day. Note that misclassification of subjects is possible, because there are sources of vitamin D other than milk and multivitamins, and those would mainly include sun exposure and certain kinds of fish. The categories in this figure reflect the vitamin D artificially added to milk and/or multivitamins, presumably for the purpose of providing a measurable benefit in terms of a higher serum 25(OH)D. Box plots present the results in a percentile format: the bottom of the box is at the 25th percentile; the line in the box shows the 50th percentile; and the top of the box is at the 75th percentile; the whiskers indicate the highest and lowest values not classed as outliers. The points above or below these limits are levels of 25(OH)D for outliers. The number of women reporting each level of vitamin D consumption is indicated by *N*, which here incorporates all women studied. Only four women consumed the highest dose, over-the-counter, vitamin D pills, 25 μg (1000 IU)/day, and these were included in the analysis.

ciency. Some reports show that mean 25(OH)D concentrations increase slightly with vitamin D intake (Takeuchi *et al*, 1995; Thomas *et al*, 1998), but data about mean 25(OH)D do not address the nutritional goal of preventing insufficiency. Other reports show that officially adequate intakes of vitamin D have little or no effect. Children, given twice the recommended adult dose of vitamin D (10 μg/day), were not protected from low wintertime 25(OH)D levels (Lehtonen-Veromaa *et al*, 1999). Younger adults, 18–55 y, given 34 μg/day vitamin D2 for 8 weeks showed an 8 nmol/l increase in 25(OH)D concentration, compared to placebo (Jones *et al*, 1991). In relatively depleted, postmenopausal women, 7.5 μg/day vitamin D increased the 25(OH)D level by only 9 nmol/l (Heikkinen *et al*, 1998). Vitamin D-deficient adults given 5 μg of vitamin D2 in winter showed no change (Poskitt *et al*, 1979). Even elderly patients supplemented with 20 μg/day, serum 25(OH)D levels sometimes showed no response (Prestwood *et al*, 1999). (The two studies showing the largest effects with 20 μg/day (Chapuy *et al*, 1992; Dawson-Hughes *et al*, 1997) used an inaccurate 25(OH)D methodology (Heaney, 2000; Vieth, 2000; Lips *et al*, 1999).) Gloth *et al*, studied the homebound elderly, and in those with 25(OH)D < 25 nmol/l, mean vitamin D intake was 12 μg/day (Gloth *et al*, 1991). Kinyamu *et al* presented a weak correlation between vitamin D intake and serum 25(OH)D, but women of all ages were pooled to show this, and from the figure in their paper, it is unlikely that the correlation would have been significant in young women as a separate group (Kinyamu *et al*, 1997). Irish adults given vitamin D-fortified milk had average winter 25(OH)D levels only 8 nmol/l higher than adults not given fortified milk (McKenna *et al*, 1995). This effect of vitamin D added to milk, fortified to current North American standards, is clearly not enough if the goal is to prevent low 25(OH)D concentrations in adults. More recent work indicates that, to ensure that virtually all adults maintain serum 25(OH)D above 40 nmol/l, an intake of 25 μg/day of vitamin D3 (as distinct from vitamin D2) is needed (Vieth *et al*, 2001).

The present results, and other data from our laboratory (Liu *et al*, 1997) indicate that average serum 25(OH)D concentrations of Canadians are similar to those of Europeans, whose diets are not fortified with vitamin D (van der Wielen RP *et al*, 1995; Poskitt *et al*, 1979; Scharla, 1998). Comparisons among 25(OH)D levels from different places are reasonable now, so long as the same methods are used for measuring 25(OH)D, and the laboratories take part in the DEQAS proficiency survey (Vieth & Carter, 2001). We do take part in the survey and our 25(OH)D results for shared samples match those of laboratories elsewhere. The similarities in Canadian and European 'normal' ranges for 25(OH)D made us suspect that the vitamin D added to milk in Canada, but not in Europe, might be of marginal value to adults, and this is confirmed by the present results.

Osteoporosis is now regarded in one sense as the final stage of a long-term process that should be addressed and prevented long before it manifests itself (Heaney, 1999). Bone-mineral density reaches its peak at around 30 y of age, after which it declines progressively. The definition of low 25(OH)D is supported here by the relationship with PTH which becomes steeper when 25(OH)D levels are < 40 nmol/l (Figure 2). This increase in PTH reflects a

physiological compromise that could contribute to development of osteoporosis.

Originally, a vitamin D intake of 10 μg/day was intended to prevent rickets in infants and children. The dose was halved for adults because it was not clear in 1963 whether adults actually needed any vitamin D. An adult recommendation was made simply because of 'the hypothesis for a small requirement for vitamin D in adults' (Blumberg *et al*, 1963). The recent AI was not changed from previous RDA values because the Food and Nutrition Board of the Institute of Medicine (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997) considered current intakes for the young women studied by Kinyamu *et al* to be adequate. This was because, with a mean wintertime vitamin D intake of 3.3 μg/day (Kinyamu *et al*, 1997), 'most of the women had serum 25(OH)D concentrations greater than 12 ng/ml (30 nmol/l)' (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997). In comparison, if we apply our decision cut-off for insufficiency to the data shown by Kinyamu *et al*, their findings are consistent with ours, in that nine of the 52 young women had low 25(OH)D levels (Kinyamu *et al*, 1997). Thus, our subjects are not unique, and our findings should apply to others at similar latitudes.

We conclude that the adequacy of vitamin D nutritional status cannot be inferred from the vitamin D intake reported by subjects. The weight of evidence indicates that currently recommended vitamin D intakes for young adults are of marginal benefit. The only reliable way to address the question of vitamin D nutrition is to measure the 25(OH)D level.

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