Table of Contents

	Page No.
I. Introduction	4
II. Vitamin D: Measurement of "Intake"	5-7
III. Evidence of need for improving Vitamin D intake	8-10
IV. Benefits of proposed Vitamin D enrichment to reduce risk of osteoporosis	11
V. Benefits of proposed Vitamin D enrichment to reduce risk of colon cancer	12-18
VI. Changes specifically requested in this petition	19-26
VII. Impact statement	27-29
VIII. Summary	30
IX. References	31-40
X. List of Appendicies	41-47
XI. Appendicies	48-367

I. Introduction

Vitamin D is a critical essential nutrient to maintain the health and function of many key systems in men and women of all ages, including skeletal, immune, muscular, and some cancer prevention systems. Vitamin D's major biological function is to maintain serum calcium and phosphate concentrations within the normal range by enhancing the efficiency of the small intestine to absorb these minerals from the diet. In the U.S. there is a current high prevalence of Vitamin D inadequacy, and re-emergence of rickets. In addition, the importance of adequate Vitamin D intake in the prevention of chronic diseases is further strengthened by several studies that demonstrate a significant association between increased Vitamin D intake and reduction of risk of osteoporosis (1, 2, Appendicies 1 & 2), and colon cancer (3-5, Appendicies 3-5). While the development of a relation of Vitamin D inadequacy or deficiency with other chronic diseases has also been documented, the available data, although strongly suggestive, is not currently strong. These include diabetes (6, 7), multiple sclerosis (8) and rheumatiod arthritis (9).

II. Vitamin D: Measurement of "Intake"

VI. Sources

1. Analytical Methodology. Cholecalciferol or Vitamin D3 is supplied in the diet and also is synthesized in the skin upon exposure to sufficient sunlight or other sources of UV blue light (UVB) to cleave the B steroid ring of 7-dehydrocholesterol, a normal skin constituent (10, Appendix 6). Vitamin D3 must undergo 2 separate hydroxylation steps to become functional in its primary biological role in calcium and phosphorus homeostasis. After dietary intake or synthesis in the skin, it is transported to the liver, where it is metabolized to 25(OH)D and released into the circulation. This intermediary metabolite is the major circulating and storage form that is delivered to tissues for further metabolic activation. The serum concentration level of 25(OH)Vitamin D has a fairly long half-life of 2-3 weeks and correlates approximately with the total liver Vitamin D storage (11-13, see Appendicies 7 & 8). It is readily available for reliable quantitative measurement in individual subjects. It has therefore, become the best clinical indicator of individual Vitamin D status currently available. It must be noted that neither Vitamin D, or 25(OH) Vitamin D produced by the liver are biologically active. The availability of serum 25(OH) Vitamin D in a normal range of concentration is necessary for adequate conversion to the active 1,25$(OH)_2$ Vitamin D in the kidney and some specific tissues. However, unlike 25(OH) Vitamin D, circulating 1,25 $(OH)_2$ Vitamin D is not a good indicator of Vitamin D status, partially due to its short serum half life (4-6 hours).

2. Sunlight exposure

In most individuals in the U.S., the majority of the circulating 25(OH)D originates from cholecalciferol or Vitamin D3, which is synthesized in the skin upon exposure to sufficient sunlight, or other sources of UV blue light (UVB) to cleave the B steroid ring of 7-dehydrocholesterol, as described above. However, adequate sun exposure for production of Vitamin D in sufficient amounts poses problems of seasonal non-availability, especially in northern states of the U.S. outside of a few summer months. In addition, adequate sun skin exposure may increase the risk of skin cancers. Some dermatologists, concerned about an anticipated over 50,000 annual cases of melanoma, a deadly form of skin cancer, have voiced strong objection to suggestions or recommendations of increasing skin exposure to sunlight to increase Vitamin D intake (14).

3. Diet

In nature, very few foods generally consumed by the U.S. population contain appreciable amounts of Vitamin D. These includes some fish oils, the flesh of fatty fish, and liver and fat from aquatic mammals such as seals and polar bears (12, 13 see Appendix 8). To reduce the risk of rickets in the U.S., fortification of a few selected foods with moderate levels of Vitamin D (e.g. fluid milk, butter, margarine, infant formulas, breakfast cereals) has been practiced for about 70 years, largely eliminating rickets in the U.S. However, a conference in October 2003 that was organized by the U.S. National Institutes of Health found an "alarming prevalence of low circulating levels of Vitamin D," with an apparent resurgence of rickets in the United States (15, Appendix 9).

Dietary intake of Vitamin D represents a far safer means of increasing daily intake, as compared to increasing sun exposure. Also, properly controlled and planned addition or fortification of the diet with pre-formed (i.e. synthetic) Vitamin D can supply a fairly steady year-round (and non-seasonal) regular intake. However, a potential problem may occur with excess intake, such as regular daily intake above the upper limit (UL) currently established for Vitamin D of 2000 IU (50 mcg) per day for children above 1 year and healthy adults (13). Therefore we believe that high levels of Vitamin D added to any one food or group of foods may pose nutritional risks as potential for exceeding the UL in some segments of the population. In contrast, adding low levels of Vitamin D as fortification to several well distributed and consumed foods would serve as a safe, conservative, distribution means of achieving Vitamin D nutritional adequacy with minimum risk of excess intake. The U.S. Food and Drug Administration has apparently recognized this concept and has approved the addition (e.g. fortification) of some fruit juices with 400 I.U. (10 mcg) of Vitamin D per quart (946 ml) (16), similar to the long established level used for fluid milk of 42 I.U. per 100 g (about 98 ml) of milk. We propose, in this petition, to further broaden modest fortification of foods with Vitamin D, by including Vitamin D as a mandatory component of cereal grain enrichment to be used when enrichment (or fortification) is utilized.

III. Evidence of need for improving Vitamin D intake.

A. Scientific estimates of the requirements for Vitamin D have been established by the Nutrition Board, Institute of Medicine, National Academy of Science, Institute of Nutrition (13), quoted as follows (page 259 of reference (13):

ESTIMATING REQUIREMENTS FOR VITAMIN D

Selection of Indicators for Estimating the Vitamin D Requirement

"The serum 25(OH)D concentration is the best indicator for determining adequacy of vitamin D intake of an individual since it represents a summation of the total cutaneous production of vitamin D and the oral ingestion of either Vitamin D_2 or Vitamin D_3 (17, 18)]. Thus, serum 25(OH)D will be used as the primary indicator of Vitamin D adequacy.

The normal range of serum 25(OH)D concentration is the mean serum 25(OH)D ± 2 standard deviations (SD) from a group of healthy individuals. The lower limit of the normal range can be as low as 20 nmol/liter (8 ng/ml) and as high as 37.5 nmol/liter (15 ng/ml) depending on the geographic location where the blood samples were obtained. For example, the lower and upper limits of the normal range of 25(OH)D in California will be higher than those limits in Boston (19). Two pathologic indicators, radiologic evidence of rickets (20) and biochemical abnormalities associated with metabolic bone disease, including elevations in alkaline phosphatase and PTH concentrations in the circulation (20), have been correlated with serum 25(OH)D. A 25(OH)D concentration below 27.5 nmol/liter (11 ng/ml) is considered to be consistent with Vitamin D deficiency in infants, neonates, and young children (21, Appendix 10) and is therefore used as the key indicator for determining the Vitamin D reference value."

B. Reports of Vitamin D Inadequacy in North America

Vitamin D is largely supplied by synthesis in the skin with sunlight exposure, and this synthesis may be adequate in white-skinned people who are active outdoors, especially in southern states. Older, less active people, especially those with heavily pigmented skin, are more prone to have vitamin D inadequacy. Thomas et al. (22, Appendix 11) showed that more than one-half the patients admitted to the Massachusetts General Hospital for general medical and surgical conditions had serum 25-hydroxyvitamin D_3 concentrations that were below adequacy. A recent publication from a group at the Centers for Disease Control and Prevention in Atlanta indicated a high prevalence of hypovitaminosis D in African American women (≈40%) and a lower prevalence in white women (≈ 4%) (23, Appendix 12). The study was based on data from the third National Health and Nutrition Examination Survey (NHANES III), which measured circulating 25-hydroxyvitamin D_3 concentrations and used rather conservative criteria for assessment of Vitamin D status. An increased lower reference limit, as suggested by newer data (24-26, Appendicies 13-15), would substantially increase the estimate of the prevalence of Vitamin D insufficiency. In a recent editorial, Hollick (27, Appendix 16) points out that very few foods contain significant amounts of naturally occurring Vitamin D and that the incidence of hypovitaminosis D in males would likely be similar to that found in females. Sunlight skin exposure, a significant source of human Vitamin D, is essentially lacking in the winter in the northern United States, and it has long been known that body stores of Vitamin D from summer skin exposure may be inadequate by the end of the winter. Tangpricha et al. (28, Appendix 17) recently reported on a study in Boston that indicated that >30% of young adults aged 18-29 y were Vitamin D insufficient at the end of the winter. A conference in October 2003 that was organized by the U.S. National Institutes of Health found an "alarming prevalence of low circulating levels

of Vitamin D," with an apparent resurgence of rickets in the United States (15, Appendix 9). Evidence of appreciable incidence of Vitamin D deficiency has been found in both southern and northern states in the U.S. (29, Appendix 18).

Gordon et al. (30, Appendix 19) found a significant prevalence of Vitamin D deficiency among apparently healthy adolescents, suggesting a major fracture risk factor in these young people for eventual prevalence of osteoporosis. A similar finding was made by Vieth et al (31, Appendix 20) who found that Vitamin D insufficiency was common in young Canadian women in the wintertime, and that their current normal Vitamin D intake (i.e. dietary) was inadequate to prevent it. A recent report by Moore, et al. (32, Appendix 21) using the 1999-2000 National Health and Nutrition Examination survey (NHANES 1999-2000) found that while only about half of children and teenagers achieved an adequate Vitamin D intake (Adequate Intake, AI) as set forth in ref. (13) only 4% of adults over 51 years of age met or exceeded the AI from food alone.

C. In summary, based on measurement of serum 25-OH Vitamin D, or estimates of dietary intake, there are large, significant segments of the U.S. (and probably Canadian) population with inadequate or deficient Vitamin D intake. This is a serious nutritional defect that can be an important contributing factor to several chronic diseases. We have chosen two of these diseases to illustrate the effects of inadequacy of Vitamin D, and the potential benefits in health, disease risk reduction, and medical care cost savings to be achieved with low, moderate economic cost, by modestly increasing Vitamin D enrichment (fortification) of foods, such as cereal grain products. The diseases we use as illustrations are:

1. Osteoporosis
2. Colon Cancer

IV. Benefits of proposed Vitamin D enrichment to reduce risk of osteoporosis.

Vitamin D deficiency bone disease was once thought to be confined to rickets and osteomalacia. However Parfitt (33), using histomorphometric criteria, showed that impairment of the canonical function of Vitamin D (i.e., promotion of active intestinal calcium absorption) produces osteoporosis first, because of inadequate absorbed calcium input. Only the most severe or prolonged Vitamin D deficiency results in osteomalacia (rickets in children). Even so, severe Vitamin D deficiency is found in 50% or more of patients with hip fracture (34, Appendix 22). That this deficiency plays a causal role is shown by a ~40% reduction in hip fracture incidence in an 84 year old cohort of women within 18 months of starting Vitamin D and calcium supplementation (35, Appendix 23). The most successful of the clinical trials of calcium have been those that used 700-800 IU of supplemental Vitamin D_3 per day (35-37, Appendicies 23-25). Additionally, Vitamin D_3, by itself, reduced hip, spine, and wrist fractures by 33% in a 4-year British trial (38, Appendix 26). Meta-analyses of studies of Vitamin D using both falls (39, Appendix 27) and fractures (37, Appendix 25) as endpoints show significant reductions in both, so long as a therapeutic blood level of serum 25(OH)D is achieved. Neuromuscular performance in the elderly, important for fall protection, has been shown to be better at higher serum 25(OH)D levels, both in the U.S. NHANES database (40, Appendix 28) and in the Amsterdam Longitudinal Aging Study (41, Appendix 29). Unsuccessful trials [e.g., RECORD (42, Appendix 30)] can be shown to be due to failure to achieve a therapeutic blood level as a result of substantial non-compliance with a daily pill-taking regimen. Such failed trials are, in fact, an argument for fortification of foods with broad population penetrance (e.g., cereal grain products) since, at a population level, adherence is effectively ensured and cost decreases.

V. Benefits of proposed Vitamin D enrichment to reduce risk of colon cancer.

Colon cancer development in the U.S. population at present is believed to be multi-factorial in origin. Some of these factors have been identified as dietary, including inadequate intake of Vitamin D. An aim of this petition is to increase the dietary intake of Vitamin D to reduce the risk of colon cancer. Increasing dietary Vitamin D intake in the U.S. population would be a significant method of colon cancer prevention, particularly if combined with increased dietary calcium intake. These two nutrients, Vitamin D and calcium, are strongly biologically interactive, so that each depends on adequate intake of the other for optimum full biochemical effect. We estimate that the increased dietary intake of the combination of Vitamin D and calcium would reduce risk of colon cancer by 20% or more (43, Appendix 31). However, since colon cancer development is multi-factorial, or results of interactions of several factors, no single agent or modality can be expected to completely prevent all risk. Rather, additional factors, including dietary ones, would need to be separately addressed in order to further the reduction of risk.

We present evidence from some pertinent recent peer reviewed reviews and publications that support the relationship between increased Vitamin D intake and reduction of colon cancer risk:

A. Newmark, H.L., Heaney, R.P. and Lachance, P.A. should calcium and vitamin D be added to the current enrichment program for cereal-grain products? *Am. J. Clin. Nutr.*, 80: 264-270, 2004.

This review (43, Appendix 31) represents our summary of the rationale, data, efficacy, safety, cost and practicality of the addition of both calcium and Vitamin D to reduce the risk of osteoporosis and colon cancer. Based on the data, we estimated (in this review),

a conservative estimate of at least a 20% reduction of disease and death rate of both colon cancer and osteoporosis, resulting in large economic saving in medical care costs, and significant reduction of death from these diseases.

We urge the petition reviewers to read this review in detail carefully, as a summary of relevant studies up to 2004. The review considers both calcium and Vitamin D, and justifies the approval of mandatory addition of both calcium and Vitamin D for cereal grain product enrichment. In our previous petition, assigned by the Food and Drug Administration as Docket Number 2005P-0273, we requested the mandatory addition of calcium to the current mandatory list of five nutrients included in cereal grain product enrichment. This present petition would add Vitamin D to the list of current mandatory nutrients included in cereal grain enrichment.

B. Grau, M.V., Baron, J.A., Sander, R.S., Haile, R.W., Beach, M.L., Church, T.R., Heber, D. Vitamin D, calcium supplementation, and colorectal adenomas: Results of a randomized trial. *J. Natl. Cancer Inst.*, 95: 1765-1771, 2003.

The study by Grau, et al. (44, Appendix 32) clearly demonstrates the important interaction of Vitamin D adequacy, as determined by serum levels of 25-hydroxy (25-OH) Vitamin D, in combination with calcium dietary supplementation to reduce the risk of colorectal adenoma recurrence. These data clearly suggest the potential for a strong chemopreventive effect of combined increases of dietary intake of calcium and Vitamin D to reduce colorectal adenoma risk of recurrence, and eventual cancer risk.

C. Gorham, E.D., Garland, C.F., Garland, F.C., Grant, W.B., Mohr, S.B., Lipkin, M, Newmark, H.L., Giovannucci, E., Wei, M., Holick, M.F. Vitamin D and prevention of colorectal cancer. *J. Steroid Biochem. Mol. Biol.*, 97: 179-194, 2005.

This study by Gorham et al. (45, Appendix 33) reports the dose-response gradients from observational studies of Vitamin D intake, and serum 25-hydroxyviatmin D, plotted as trend lines. The point on each linear trend line corresponding to an odds ratio of 0.50 provided the prediagnostic Vitamin D intake, or 25-hydroxyvitamin D serum concentration, associated with 50% lower risk of colon cancer compared to <100 IU/day Vitamin D, or <13 ng/ml (about 33 nM) serum 25-hydroxyvitamin D. Medians of these values were determined. The authors concluded that serum 25-hydroxyvitamin D of 33 ng/ml (about 87 nM), which is known to be safe, was associated with 50% lower risk of colon cancer. Prompt public health action is needed to increase intake of Vitamin D_3 to raise serum 25-hydroxyvitamin D, by encouraging a modest duration of sunlight exposure, and increasing dietary Vitamin D intake.

D. In the report by Goham, et al. (45, Appendix 33) the authors also summarize four observational studies (46-49, Appendicies 34-37) that have provided dose-response relationship between serum 25-hydroxyvitamin D (25(OH)D and risk of colorectal cancer. This summary is in Table 1, Gorham, et al. (45, Appendix 33), and is shown below:

Table 1 (from ref. (45)
Serum 25-hydroxyvitamin D concentration associated with 50% reduction in risk of colorectal or distal colon and rectal cancer according to observational studies, 1989-2005

Authors, year, reference	Cancer site	Sex	Cases	Controls	25-Hydroxyvitamin D concentration associated with 50% reduction (ng/ml)	p for trend
Garland et al, 1989 (46)	Colon	Both	34	67	25	>0.20
Braun et al, 1995 (47)	Distal colon and rectum	Both	57	114	27	0.13
Tangrea et al, 1997 (48)	Distal colon and rectum	Both	103	204	67	0.03
Feskanich et al., 2004 (49)	Colorectal	Women	193	383	40	0.01
No. of subjects, median serum 25(OH)D			387	768	33	0.01

Note: Good statistical significance for "trend" is shown for the studies of Tangrea, et al. (48, Appendix 36) and Feskanich, et al. (49, Appendix 37). Therefore further details of these studies are shown below in paragraphs E & F.

E. Tangrea, J., Helzlsouer, K., Pietinen, P., Taylor, P., Hollis, B., Virtamo, J., Albanes, D. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men, *Cancer Causes Control*, 8(4): 615-625, 1997.

Tangrea et al. (48, Appendix 36) performed a nested case-control study of prediagnostic serum 25(OH)D based on 146 incident cases of colorectal cancer including 103 of the distal colon and rectum that occurred in a cohort of 29,133 men aged 50-69 years. Cases were obtained as part of routine surveilance of the cohort, which originally was established for the Alpha-Tocopherol and Beta Carotene Cancer Prevention Study. Cases were matched to controls on age, date of baseline blood drawn, and study clinic. The study was performed in Finland during 1985-1993. The boundaries of serum 25(OH)D concentrations for quartiles from lowest to highest were, in ng/ml: 0-9.8, 9.9-13.8, 13.9-19.2, and ≥19.3. Relative risks for distal colon and rectal cancer were 1.0, 0.8, 0.6 and 0.5 (p trend = 0.03). There was a similar but non-significant trend for distal colon cancer alone (see figure below).

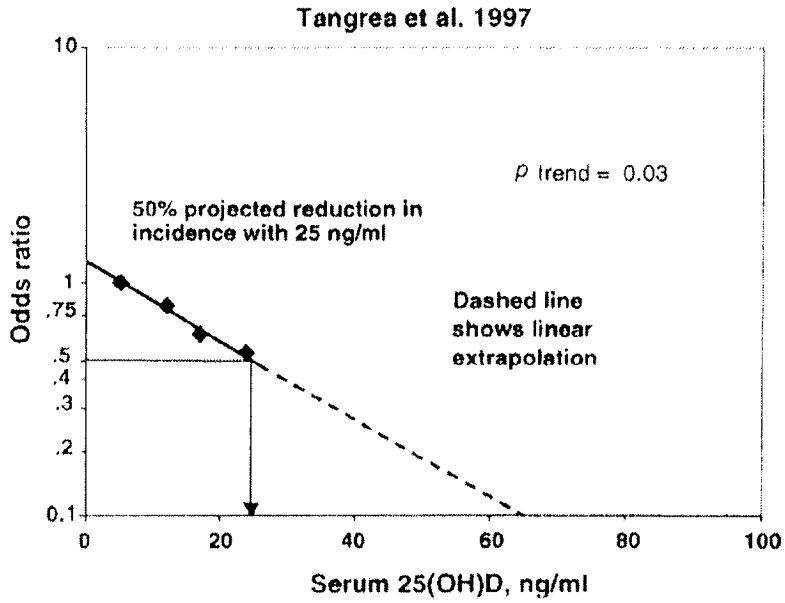

Fig. 1. Dose-response gradient of risk of distal colon and rectal cancer according to quartile serum 25(OH)D, The Alpha Tocopherol and Beta Carotene Cancer Prevention Study Cohort, men, Finland, 1985-1993. Source: Tangrea et al., (48, Appendix 36).

F. Feskanich, D., Ma, J., Fuchs, C.S., Kirkner, G.J., Hankison, S.E., Hollis, B.W., Giovanucci, E.L. Plasma vitamin D metabolites and risk of colorectal cancer in women, *Cancer Epidemiol. Biomarkers Prev.,* 13(9): 1502-1508, 2004.

This large study, performed by Feskanich et al. provided a clear and highly significant dose-response gradient (49, Appendix 37). It was a study of incidence of colorectal cancer in the Nurses Health Study cohort, based on 193 new cases of colorectal cancer that occurred in 32,826 female nurses aged 46-78 years during 1981-1991. Healthy controls from the cohort were matched to the cases on age and month blood was drawn. The median serum 25(OH)D levels for quintiles from lowest to highest were, in ng/ml: 16.2, 22.2, 26.9, 31.2, and 39.9. Odds ratios for colorectal cancer from lowest to highest quintile were: 1.0, 0.87, 0.70, 0.52, and 0.53 (p for trend ≤ 0.01). (See figure below.) Although serum $1,25(OH)_2D$ was determined, it was not associated with risk of colorectal cancer (data not

shown), indicating, as expected, that serum 25(OH)D is the reliable predictor of Vitamin D adequacy, and not the short-lived serum 1,25$(OH)_2$D.

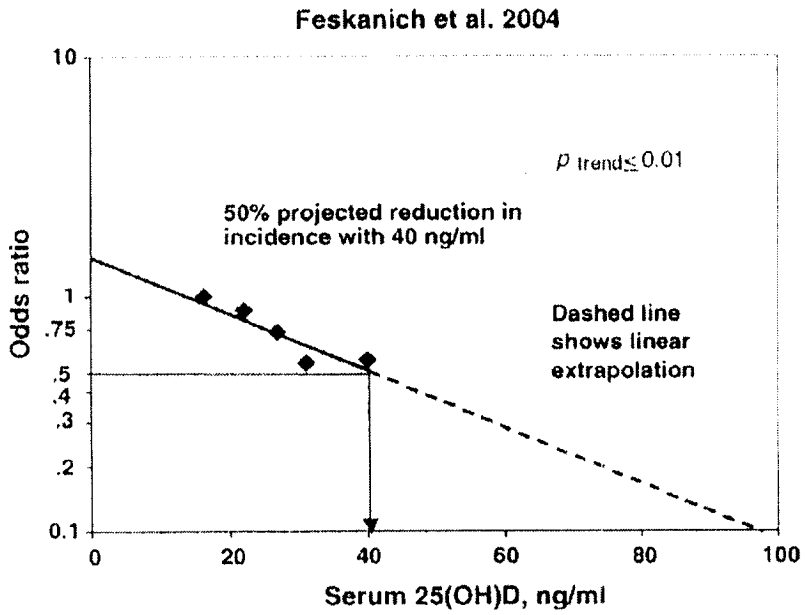

Fig 2. Dose-response gradient of risk of colorectal cancer according to quintile of serum 25(OH)D, Harvard University Nurses Health Study Cohort, women, United States, 1989-1991. Source: Feskanich, et al. (49, Appendix 37).

G. There are a number of observational studies of the association between prediagnostic estimates of oral intake of Vitamin D and risk of colorectal cancer. The following is a Table of 8 of these studies which included estimates of statistical probability (p-value) of an inverse association of oral Vitamin D intake and reduction in risk of colorectal cancer (1985-2005) (45, Appendix 33).

Table 2. Vitamin D oral intake associated with 50% reduction in risk of colorectal cancer, according to observational studies, 1985-2005.

Authors, year, references	Cancer site	Sex	Cases	Controls	Cohort	Person-years	Vitamin D intake associated with 50% reduction (IU/day)	*p* for trend
Garland et al., 1985 (50)	Colorectal	Men	49	1905	1954	31597	480	0.05
Bostick et al., 1993 (51)	Colon	Women	212	35004	35216	167447	1000	0.02
Kearney et al., 1996 (52)	Colon	Men	203	47732	47935	272681	770	0.02
Martinez et al., 1996 (53)	Colorectal	Women	501	88947	89448	1012280	800	0.04
Pritchard et al., 1996 (54)	Colorectal	Both	392	512	N/A	N/A	430	0.08
Marcus et al., 1998 (55)	Colorectal	Women	512	678	N/A	N/A	1000	0.05
McCollough et al., 2003 (4)	Colorectal	Both	683	127,066	127,749	243464	600	0.02
La Vecchia et al., 1997 (56)	Colorectal	Both	1953	4154	N/A	N/A	475	0.01

Abbreviation: N/A, not applicable (Adapted from ref. 45)

The following is a list of some specific references in Table 2 above that are supplied as appendices within this petition.

Authors, year, references (from Table 2)	Appendix Number
Garland et al., 1985 (50)	38
Bostick et al., 1993 (51)	–
Kearney et al., 1996 (52)	–
Martinez et al., 1996 (53)	39
Pritchard et al., 1996 (54)	40
Marcus et al., 1998 (55)	41
McCollough et al., 2003 (4)	–
La Vecchia et al., 1997 (56)	42

H. In the above studies described in C-G, a large range of Vitamin D daily intake, and/or a wide range of serum 25 hydroxy Vitamin D concentration levels were used to establish the reported high statistical probability of colorectal cancer risk reduction with increased Vitamin D intake adequacy. However, small increases in Vitamin D dietary intake, as we propose for enrichment for cereal grain products, would be difficult to extrapolate to quantitative estimates of reduction of colorectal cancer risk. In the U.S. population as a whole, estimated adult Vitamin D requirements for Adequate Intake (AI) for adults ranges from 200 I.U./day (19-50 years of age) to 600 I.U./day (over 70 years of age) (13). Also reliable quantitative relationships between dietary intake and serum 25(OH)D concentrations, in various populations and age groups are not available although some approximate estimates have been made (57). Nevertheless, utilizing the estimates of the best two studies of relation of serum 25(OH)D levels to colorectal cancer risk, as shown in the Figures E and F above (refs. (45, 48, 49, Appendicies 33, 36, 37) we estimate that a 10-20% reduction in colorectal cancer risk would occur with an increased Vitamin D dietary intake of about 100 I.U./day.

VI. Changes specifically requested in this petition.

A. This new petition is to add Vitamin D to the mandatory items in cereal product grain enrichment. Scientific reports, especially in the last 2 decades, have established more extensive data that give strong credibility to the concept that increased dietary intake of Vitamin D can significantly reduce the risk of osteoporosis and colon cancer.

Human sources in the U.S. of Vitamin D include greater sunlight ultraviolet light exposure to increase skin biosynthesis, however such exposure increases the risk of skin cancers, and sufficient sunlight is normally not available during a large part of the year, particularly in northern states, producing seasonal inadequacy. In addition populations with pigmented skin (e.g. individuals of African, Middle Eastern or some Far Eastern descent) have reduced efficiency for skin biosynthesis of Vitamin D from ultraviolet light or direct sunlight.

Natural food sources of Vitamin D are few and not sufficiently diverse. Rickets, formerly a widespread disease, particularly in children, from Vitamin D deficiency, has been largely (but not completely) controlled over the last half century by the enrichment (or fortification) of a few foods (e.g. fluid milk, butter, margarine, infant formula, etc.), known to be consumed generally by children. In recent years rickets has reemerged in some segments of the U.S. population (15, Appendix 9), indicating a need to reevaluate the adequacy of the limited range and extent of food fortification with Vitamin D in the U.S.

B. Vitamin D has been approved by the FDA in recent years as a nutrient supplement in calcium-fortified fruit juice and fruit juice drinks under 21 CFR Part 172.380, and these are also now being used for calcium and Vitamin D nutrient enrichment to increase intake in the U.S. population. We applaud such increased diversity of food enrichment. However, we strongly suggest that cereal grain products be added to the list, as a group of very reliable nutrient carriers, little prone to permit excess intake, safe and simple to use, low cost, very broadly consumed and well distributed in the general population. This group of foods is widely consumed across wide variations of ethnic, cultural and age differences in the U.S.

C. Our choice of cereal grain products for enrichment as a vehicle to increase calcium intake in the U.S. is guided by the following:

1. Approximately one fourth of the daily calorie intake in the U.S. is from cereal grain products, and the intake of total cereal grain products does not vary greatly by income or geographic region in the U.S. (58-60).

2. Cereal grain product enrichment has been historically credited for (essentially) the eradication of pellagra by niacin enrichment in the U.S., as illustrated by Fig. 2 in Ref. (43, Appendix 31). More recently, addition of folic acid (i.e. folate) to cereal grain enrichment has significantly reduced neural tube defects in newborns in the U.S. (61-66, see Appendicies 43-45).

D. Specifically, we request that selected parts of the U.S. Code of Federal Regulations (CFR), in Section 136, 137 and 139 to be amended as follows in the column labeled "Proposal shall contain Vitamin D," in Table 3 below:

Table 3. Selected parts of the U.S. Code of Federal Regulations (2001), Title 21, Sections 136, 137 and 139, that relate to calcium and Vitamin D

Section and paragraph nos.	Title	Current statement on vitamin D	Proposal: shall contain vitamin D
136.115	Enriched bread, rolls, and buns	None	90 I.U/100g
137.160	Enriched bromated flour	None	90 I.U/100g
137.165	Enriched flour	None	90 I.U/100g
137.185	Enriched self-rising flour	None	90 I.U/100g
137.260	Enriched corn meals	May contain 250-1000 IU vitamin D/lb	90 I.U/100g
137.305	Enriched farina	May contain ≥250 IU vitamin D/lb	90 I.U/100g
137.350	Enriched rice	May contain 250-1000 IU vitamin D/lb	90 I.U/100g
137.115	Enriched macaroni products	May contain 250-1000 IU vitamin D/lb	90 I.U/100g
139.117	Enriched macaroni products with fortified protein	None	90 I.U/100g
139.122	Enriched nonfat milk macaroni products	None	90 I.U/100g
139.135	Enriched vegetable products	May contain 250-1000 IU vitamin D/lb	90 I.U/100g
139.155	Enriched noodle products	None	90 I.U/100g
139.165	Enriched vegetable noodle products	May contain 250-1000 IU vitamin D/lb	90 I.U/100g

Note: 1 lb or 1 pound = 0.45 kg. This list includes the majority of enriched cereal grain products.

Note: Federal Register/Vol. 70, No. 124/Wednesday, June 29, 2005/Rules and Regulations, page 37255, which includes the following:

Table 4. [1]Vitamin D, including vitamin D3, also is affirmed as generally recognized as safe (GRAS) for use in food under § 184.1950 (21 CFR 184.1950) with the following limitations:

Category of Food	Maximum levels in food (as served)
Grain products and pasta	90 I.U/100g

[1]Vitamin D comprises a group of fat-soluble secosterols and comes in many forms. The two major physiologically relevant forms are vitamin D_2 and Vitamin D_3. Vitamin D without a subscript represents either D_2 or D_3. Section 184.1950 includes crytalline vitamin D_2, crystalline vitamin D_3, vitamin D_2 resin, and vitamin D_3 resin.

Note: The above newly approved maximum level of Vitamin D in cereal grain products and pasta (as served) of 90 I.U./100g as described in 21 CFR 184.1950 above, is equivalent to about 400 I.U. per pound (lb) of food. This is almost midway in the range of 250-1000 I.U. per lb (pound) previously stated in several specific cereal grain product description as indicated in the table above. Thus a practical uniform Vitamin D addition regulation for cereal grain products would be aimed at 90 I.U./100g (400 I.U. per pound of food).

E. Sources of Vitamin D suitable for cereal grain product enrichment are commercially available as described in 21 CFR 184.1950:

[1]Vitamin D comprises a group of fat-soluble secosterols and comes in many forms. The two major physiologically relevant forms are vitamin D_2 and Vitamin D_3. Vitamin D without a subscript represents either D_2 or D_3. Section 184.1950 includes crystalline vitamin D_2, crystalline vitamin D_3, vitamin D_2 resin, and vitamin D_3 resin.

F. For some cereal grain products, enrichment inclusion with Vitamin D may require formulated, stabilized compositions, currently manufactured and marketed for use in food and pharmaceutical preparations. These are all based on food approved components. They are designed to supply a variety of physical properties for use in specific food or pharmaceutical applications and also supply optimum chemical stability of the Vitamin D in the final product. One such commercial supplier is:

DSM Nutritional Products (Formerly "Roche Vitamins, Inc.")

USA	Canada
DSM Nutritional Products, Inc. 45 Waterview Blvd. Parsippany, NJ 07054-1298 Tel. 800-526-0189 Fax 973-257-8413	DSM Nutritional Products, Inc. 395 Waydom Drive Ayr, Ontario N0B 1E0 Tel. 800-526-0189 Fax 973-257-8413

A few of their products designed for pharmaceutical and food preparations as sources of Vitamin D are shown below (as taken from their Product Data Sheets).

1. **Dry Vitamin D3, Type 100 CWS**

Description

Dry Vitamin D3, Type 100 CWS consists of off-white to yellowish, free-flowing particles (beadlets). They contain vitamin D_3 (cholecalciferol) in edible fats finely

dispersed in a cornstarch-coated matrix of gelatin and sucrose. *dl*-α-Tocopherol (Vitamin E) is added as an antioxidant.

Vitamin D3 content: not less than 100% and not more than 110% of the declared value (100,000 I.U./g)

Dispersibility

Dry Vitamin D3, Type 100 CWS disperses quickly and completely in cold water, fruit juices, milk and other liquids. High concentrations may give cloudy dispersions which, however, remain uniform for relatively long periods.

2. **Dry Vitamin D3 100 CWS/AM**

Description

Dry Vitamin D3 100 CWS/AM consists of off-white to yellowish, free-flowing particles (beadlets). They contain vitamin D3 (cholecalciferol) in medium-chain triglycerides finely dispersed in a cornstarch-coated matrix of acacia and sucrose. *dl*-α-Tocopherol (Vitamin E) is added as an antioxidant.

Vitamin D3 content (Ph. Eur.) 90,000-110,000 I.U./g

Dispersibility

Dry Vitamin D3 100 CWS/AM disperses quickly and completely in cold water, fruit juices, milk and other liquids. High concentrations may give cloudy dispersions which, however, remain uniform for relatively long periods.

3. **Dry Vitamin D3 Type 100-SD**

Description

Dry Vitamin D3 Type 100-SD is a free-flowing form of cholecalciferol compounded with dicalcium phosphate, lactose, acacia, coconut oil, BHT and silicon dioxide. Sodium benzoate and sorbic acid are added as preservatives.

Assay: Minimum of 100,000 I.U./g Vitamin D

G. Cost Estimates

The above 3 product forms commercially available from DSM Nutritional Products, each containing 100,000 I.U. Vitamin D per gram of formulated dry product, are currently priced at less than $40.00 per kilo, or 4 cents per gram, containing 100,000 I.U. Vitamin D. (Information from Dr. Leonard Johnson, DSM Nutritional Products, Inc., communication of 12/1/05).

We estimate that adding Vitamin D to cereal grain product enrichment, as indicated in Table 3 would supply about 100 I.U. daily to most individuals in the U.S. For the 300 million approximate total population of the U.S., for the entire year, this would require about 100,000 kg of one of the DSM formulated products costing about $4 million per year for suitable formulated stabilized Vitamin D forms to enrich or fortify all of the cereal grain products at the levels listed in Table 3. The technical, or mechanical addition costs of incorporating a suitable Vitamin D dry powder form to the current multivitamin and mineral powder mixtures used in enrichment of these food products would be very small, since technology, equipment and control systems for current enrichment of these cereal grain products have been in regular use for many decades. Thus the essential total cost of adding Vitamin D to the enrichment programs for cereal grain products can be conservatively estimated at less than $5

million annually for the total U.S. population, at less than 2 cents per person per year.

H. Stability in bread baking.

Dr. Christel H. Lamberg-Allardt studied the stability and human bioavailability of Vitamin D in bread fortification, in the enclosed manuscript (in press, J. Nutrition) (67, Appendix 46).

Natri, A-M., Solo-Väänänen, P., Vikstedt, T., Palssa, A., Huttunen, M., Käkkäinen, M.U.M., Salovaara, H., Pironen, V., Jakobesen, J., and Lamberg-Allardt, C.J.

The abstract states:

"**Design**: Three batches of fortified low-fiber wheat and high-fiber rye breads were baked, and from each batch, three samples of dough and bread were analyzed for their Vitamin D_3 content. In a single blinded bioavailability study, forty-one healthy women, aged 25-45 years and mean serum 25-hydroxyvitamin D concentration 29 nmol/L (range 12-45 nmol/L), were randomized into four study groups receiving either fortified wheat bread (FWheat), fortified rye bread (FRye), regular wheat bread (control) or regular wheat bread and a Vitamin D_3 supplement of 10 µg (Vitamin D control) daily for three weeks.

Results: Vitamin dispersed evenly in the breads and was stable. Both fortified breads increased serum 25-hydroxyvitamin D concentration as effectively as did the Vitamin D_3 supplement. Supplementation or fortification had no effect on serum iPTH concentration or urinary calcium excretion.

Conclusions: Fortified bread is a safe and feasible means to improve Vitamin D nutrition of all population groups and in many countries all over the world."

It should be noted that the Vitamin D source used successfully in these studies was "Dry Vitamin D-3, Type 100 CWS" obtained from Roche Ltd. (Espoo, Finland) as an "imported" product. It is identical to the DSM Nutritional Products (formerly Roche Vitamins, Inc.) described in Section E above.

I. Stability of Vitamin D in other cereal grain product applications can be achieved by use of a suitable dry powder or other formulation of Vitamin D. The Technical Service aid of supply companies such as DSM Nutritional Products, or others, will be of great benefit, and help to reduce potential developmental costs.

VII. Impact Statement

The potential effect on the U.S. diet of the mandatory addition of Vitamin D to enrichment of cereal-grain products as indicated (Table 3) can be approximately estimated. Some ready-to-eat breakfast cereals are currently marketed with added Vitamin D, and regulated by 21 CFR 184.1950 to be 350 I.U. per 100 g of food as served. However, Vitamin D addition to other cereal grain products is neither uniform nor universal and would benefit by standardization of enrichment (or fortification). Estimates of increased Vitamin D intake can be made on the assumptions that mandatory enrichment would be practiced on all the products listed, and in the amounts listed, as we propose in Table 3. Data on year 2000 per capita annual consumption of cereal products were obtained from the U.S. Department of Agriculture, Economic Research Service (68). In Table 5 the total proposed additions of Vitamin D to the average U.S. daily diet may be estimated at about 204 I.U., if the additions described in Table 3 were to become mandatory.

Table 5. Estimated increased daily dietary intake of Vitamin D from major consumed cereal grain product foods if the current optional enrichment regulation were to become mandatory[1], based on USDA per capita consumption data for 1997.

	Year 2000 per capita		Vitamin D enrichment	
	Annual	Daily	Amount	Daily intake
	lb	lb	I.U./lb	I.U.
Item				
Wheat flour & products	137	0.375	400 I.U.	150
Corn flour and meal	16.1	0.044	400 I.U.	18
Pastas (macaroni, noodles	13	0.036	400 I.U.	14
etc: semolina and durum				
durum flour)				
Rice	20	0.055	400 I.U.	22
Total	–	0.51	–	204 I.U.

Notes for Table 5:

1. Numbers of Vitamin D enrichment represent the approximate effects of using the value 90 I.U. per 100 g food, or 400 I.U. per pound (lb) of food for enrichment for Vitamin D. 1 lb = 0.45 kg.

2. From U.S. Department of Agriculture, Economic Research Service. Food consumption (per capita) data system. Washington, D.C.: U.S. Government Printing Office, 2005 (Ref. 68).

However, the estimate in the above Table 5 are probably higher than reality, since the data on "per capita consumption" from the USDA data system actually is based largely on cereal grain production data, rather than actual consumption, and does not allow for spoilage, waste, etc. in distribution and losses in home use. The amount of such losses is difficult to ascertain with accuracy. This is shown by the estimate total daily per capita consumption in Table 5 of 0.51 lb (0.23 Kg), representing about an 900 Kcal intake of cereal grain products, which is overly high for mean U.S. dietary intake of cereal grain products, normally estimated at about 500 Kcal intake, based on 25% of a 2000 Kcal diet. Calculated on a 500 Kcal daily intake, cereal grain products the Vitamin D intake from fortified cereal grain products would more closely approximate about 113 I.U. per day per person.

Another method of estimating potential impact of Vitamin D enrichment of cereal grain products is based on the estimate by the National Research Council (58-60, 69) that approximately one-fourth (25%) of daily caloric intake in the U.S. is from cereal-grain products, mostly from wheat products. In Table 6, estimates are given for a low (2,000 Kcal) diet and a higher (2,700 Kcal) diet for Vitamin D dietary impact, using the proposed addition of 400 I.U. per Kg Vitamin D per pound of wheat flour and other cereal grain products (about 900 I.U. per Kg flour, or about 0.9 I.U. Vitamin D per gm of flour).

Table 6. Estimated increased daily dietary intake of Vitamin D from major consumed cereal grain product foods, based on current optional enrichment regulations, if made mandatory, based on estimated intake in a range of total caloric intake.

Kcal	Kcal 25% cereal	gm/day cereal	Vitamin D/day added
2000	500	125 g	113 mg
2700	675	169 g	152 mg

Notes for Table 6:

1. As discussed above, estimates of U.S. diet (Refs. (58, 59, 69) indicates that about one fourth (25%) is from cereal grain products, which are essentially 4 Kcal per gram, dry weight.

2. Vitamin D addition is calculated based on 90 I.U. per 100 g cereal grain product, in accordance with 21 CFR 184.1958.

By this method of impact estimate, Vitamin D increase in the U.S. diet resulting from cereal grain proposed enrichment, would be about 113-152 I.U. per day, which we believe may be approximated to 100-150 I.U./day increase.

VIII. Summary

In summary, we estimate that an increase of about 100-150 I.U./day of Vitamin D in the U.S. mean diet would occur with mandatory Vitamin D fortification of cereal grain products, a modest increase of at least one-half of the A.I. for adults (200 I.U./day), but a smaller fraction of the A.I. for older adults (400 to 600 I.U./day depending on age). This would be accomplished at a modest cost of $5 million annually for the entire U.S., or less than 2 cents per person per year.

IX. References:

1. Feskanich, D., Willett, W. C., and Colditz, G. A. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. American Journal of Clinical Nutrition, *77:* 504-511, 2003. (Appendix 1)
2. Meier, C., Woitge, H. W., Witte, K., Lemmer, B., and Seibel, M. J. Supplementation with oral vitamin D3 and calcium during winter prevents seasonal bone loss: a randomized controlled open-label prospective trial. Journal of Bone & Mineral Research, *19:* 1221-1230, 2004. (Appendix 2)
3. Lieberman, D. A., Prindiville, S., Weiss, D. G., Willett, W., and Group, V. A. C. S. Risk factors for advanced colonic neoplasia and hyperplastic polyps in asymptomatic individuals. JAMA, *290:* 2959-2967, 2003. (Appendix 3)
4. McCullough, M. L., Robertson, A. S., Rodriguez, C., Jacobs, E. J., Chao, A., Carolyn, J., Calle, E. E., Willett, W. C., and Thun, M. J. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). Cancer Causes & Control, *14:* 1-12, 2003. (Appendix 4)
5. Slattery, M. L., Neuhausen, S. L., Hoffman, M., Caan, B., Curtin, K., Ma, K. N., and Samowitz, W. Dietary calcium, vitamin D, VDR genotypes and colorectal cancer. International Journal of Cancer, *111:* 750-756, 2004. (Appendix 5)
6. Fronczak, C. M., Baron, A. E., Chase, H. P., Ross, C., Brady, H. L., Hoffman, M., Eisenbarth, G. S., Rewers, M., and Norris, J. M. In utero dietary exposures and risk of islet autoimmunity in children. Diabetes Care, *26:* 3237-3242, 2003.
7. Chiu, K., Chu, A., Go, V., and Soad, M. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. American Journal of Clinical Nutrition, *79:* 820-825, 2004.

8. Munger, K. L., Zhang, S. M., O'Reilly, E., Hernan, M. A., Olek, M. J., Willett, W. C., and Ascherio, A. Vitamin D intake and incidence of multiple sclerosis. Neurology, *62:* 60-65, 2004.

9. Merlino, L. A., Curtis, J., Mikuls, T. R., Cerhan, J. R., Criswell, L. A., Saag, K. G., and Iowa Women's Health, S. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis & Rheumatism, *50:* 72-77, 2004.

10. Norman, A. W. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. American Journal of Clinical Nutrition, *67:* 1108-1110, 1998. (Appendix 6)

11. Adams, J. S., Clemens, T. L., Parrish, J. A., and Holick, M. F. Vitamin-D synthesis and metabolism after ultraviolet irradiation of normal and vitamin-D-deficient subjects. New England Journal of Medicine, *306:* 722-725, 1982. (Appendix 7)

12. Holick, M. F. Vitamin D: New horizons for the 21st century. American Journal of Clinical Nutrition, *60:* 619-630, 1994. (Appendix 8)

13. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride. Food and Nutrition Board, Institute of Medicine, Chapter 7, pp. 250-287. Washington, DC: National Academy Press, 1997.

14. Allen, S. BU advocate of sunligh draws ire. The Boston Globe http://www.boston.com/news/local/massachusetts/articles/2004/04/13//bu_advocate [accessed April 15, 2004]. 2004.

15. Stokstad, E. The Vitamin D Deficit. Science, *302:* 1886-1888, 2003. (Appendix 9)

16. Code of Federal Regulation, Title 21, Food and Drug Administration, Par. 172.380 (21CFR 172.380, Vitamin D).

17. Haddad, J. G., Jr. and Hahn, T. J. Natural and synthetic sources of circulating 25-hydroxyvitamin D in man. Nature, *244:* 515-517, 1973.

18. Holick, M. F. Vitamin D: Photobiology, metabolism, and clinical applications. *In:* L. J. DeGroot, H. G. Burger, J. L. Jameson, D. L. Loriaux, J. C. Marshall, W. D. O'Dell, and J. T. Potts (eds.), Endocrinology, 3rd Edition. Philadelphia, PA: W.B. Saunders, 1995.

19. Clemens, T. L. and Adams, J. S. Vitamin D metabolites. *In:* M. J. Favus and S. Christakos (eds.), Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 3rd Edition, pp. 109-114. Philadelphia, PA: Lippincott-Raven, 1996.

20. Demay, M. B. Hereditary defects in vitamin D metabolism and vitamin D receptor defects. *In:* L. J. DeGroot, M. Besser, H. G. Burger, J. L. Jameson, D. L. Loriaux, J. C. Marshall, W. D. O'Dell, J. T. Potts, and A. H. Rubenstein (eds.), Endocrinology, Third edition, Vol. 2, pp. 1173-1178. Philadelphi, PA: W.B. Saunders, 1995.

21. Specker, B. L., Ho, M. L., Oestreich, A., Yin, T. A., Shui, Q. M., Chen, X. C., and Tsang, R. C. Prospective study of vitamin D supplementation and rickets in China. Journal of Pediatrics, *120:* 733-739, 1992. (Appendix 10)

22. Thomas, M. K., Lloyd-Jones, D. M., Thadhani, R. I., Shaw, A. C., Deraska. D. J., Kitch, B. T., Vamvakas, E. C., Dick, I. M., Prince, R. L., and Finkelstein, J. S. Hypovitaminosis D in medical inpatients. New England Journal of Medicine, *338:* 777-783, 1998. (Appendix 11)

23. Nesby-O'Dell, S., Scanlon, K. S., Cogswell, M. E., Gillespie, C., Hollis, B. W., Looker, A. C., Allen, C., Doughertly, C., Gunter, E. W., and Bowman, B. A. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. American Journal of Clinical Nutrition, *76:* 187-192, 2002. (Appendix 12)

24. Vieth, R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety.[see comment]. American Journal of Clinical Nutrition, *69:* 842-856, 1999. (Appendix 13)

25. Glerup, H., Mikkelsen, K., Poulsen, L., Hass, E., Overbeck, S., Thomsen, J., Charles, P., and Eriksen, E. F. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. Journal of Internal Medicine, *247:* 260-268, 2000. (Appendix 14)

26. Heaney, R. P., Davies, K. M., Chen, T. C., Holick, M. F., and Barger-Lux, M. J. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol.[see comment][erratum appears in Am J Clin Nutr. 2003 Nov;78(5):1047]. American Journal of Clinical Nutrition, *77:* 204-210, 2003. (Appendix 15)

27. Holick, M. F. Too little vitamin D in premenopausal women: why should we care? American Journal of Clinical Nutrition, *76:* 3-4, 2002. (Appendix 16)

28. Tangpricha, V., Pearce, E. N., Chen, T. C., and Holick, M. F. Vitamin D insufficiency among free-living healthy young adults. American Journal of Medicine, *112:* 659-662, 2002. (Appendix 17)

29. Holick, M. F., Siris, E. S., Binkley, N., Beard, M. K., Khan, A., Katzer, J. T., Petruschke, R. A., Chen, E., and de Papp, A. E. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. Journal of Clinical Endocrinology & Metabolism, *90:* 3215-3224, 2005. (Appendix 18)

30. Gordon, C. M., DePeter, K. C., Feldman, H. A., Grace, E., and Emans, S. J. Prevalence of vitamin D deficiency among healthy adolescents. Archives of Pediatrics & Adolescent Medicine, *158:* 531-537, 2004. (Appendix 19)

31. Vieth, R., Cole, D. E., Hawker, G. A., Trang, H. M., and Rubin, L. A. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. European Journal of Clinical Nutrition, *55:* 1091-1097, 2001. (Appendix 20)

32. Moore, C. E., Murphy, M. M., and Holick, M. F. Vitamin D intakes by children and adults in the United States differ among ethnic groups. Journal of Nutrition. *135:* 2478-2385, 2005. (Appendix 21)

33. Parfitt, A. M. Osteomalacia and related disorders. *In:* L. V. Avioli and S. M. Krane (eds.), Metabolic Bone Disease and Clinically Related Disorders, 2nd Edition, pp. 329-396. Philadelphia: W.B. Saunders, 1990.

34. Nurmi, I., Kaukonen, J. P., Lüthje, P., Naboulsi, H., Tanninen, S., Kataja, M., Kallio, M. L., and Leppilampi, M. Half of the patients with an acute hip fracture suffer from hypovitaminosis D: a prospective study in southeastern Finland. Osteoporos. Int., *16:* 2018-2024, 2005. (Appendix 22)

35. Chapuy, M. C., Arlot, M. E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P. D., and Meunier, P. J. Vitamin D3 and calcium to prevent hip fractures in the elderly women. New England Journal of Medicine, *327:* 1637-1642, 1992. (Appendix 23)

36. Dawson-Hughes, B., Harris, S. S., Krall, E. A., and Dallal, G. E. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. New England Journal of Medicine, *337:* 670-676, 1997. (Appendix 24)

37. Bischoff-Ferrari, H. A., Willett, W. C., Wong, J. B., Giovannucci, E., Dietrich, T., and Dawson-Hughes, B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA, *293:* 2257-2264, 2005. (Appendix 25)

38. Trivedi, D. P., Doll, R., and Khaw, K. T. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ, *326:* 469-474, 2003. (Appendix 26)

39. Bischoff-Ferrari, H. A., Dawson-Hughes, B., Willett, W. C., Staehelin, H. B., Bazemore, M. G., Zee, R. Y., and Wong, J. B. Effect of Vitamin D on falls: a meta-analysis. JAMA, *291:* 1999-2006, 2004. (Appendix 27)

40. Bischoff-Ferrari, H. A., Dietrich, T., Orav, E. J., Hu, F. B., Zhang, Y., Karlson, E. W., and Dawson-Hughes, B. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. American Journal of Clinical Nutrition, *80:* 752-758, 2004. (Appendix 28)

41. Wicherts, I. D., van Schoor, N. M., Boeke, A. J. P., and Lips, P. Vitamin D deficienty and neuromuscular performance in the Longitudinal Aging Study Amsterdam. Journal of Bone and Mineral Research, *20(Suppl 1):* S35, 2005. (Appendix 29)

42. Grant, A. M., Avenell, A., Campbell, M. K., McDonald, A. M., MacLennan, G. S., McPherson, G. C., Anderson, F. H., Cooper, C., Francis, R. M., Donaldson, C., Gillespie, W. J., Robinson, C. M., Torgerson, D. J., Wallace, W. A., and Group, R. T. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet, *365:* 1621-1628, 2005. (Appendix 30)

43. Newmark, H. L., Heaney, R. P., and Lachance, P. A. Should calcium and vitamin D be added to the current enrichment program for cereal-grain products? American Journal of Clinical Nutrition, *80:* 264-270, 2004. (Appendix 31)

44. Grau, M. V., Baron, J. A., Sandler, R. S., Haile, R. W., Beach, M. L., Church, T. R., and Heber, D. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. Journal of the National Cancer Institute, *95:* 1765-1771, 2003. (Appendix 32)

45. Gorham, E. D., Garland, C. F., Garland, F. C., Grant, W. B., Mohr, S. B., Lipkin, M., Newmark, H. L., Giovannucci, E., Wei, M., and Holick, M. F. Vitamin D and

prevention of colorectal cancer. Journal of Steroid Biochemistry & Molecular Biology, *97:* 179-194, 2005. (Appendix 33)

46. Garland, C. F., Comstock, G. W., Garland, F. C., Helsing, K. J., Shaw, E. K., and Gorham, E. D. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study.[see comment]. Lancet, *2:* 1176-1178, 1989. (Appendix 34)

47. Braun, M. M., Helzlsouer, K. J., Hollis, B. W., and Comstock, G. W. Colon cancer and serum vitamin D metabolite levels 10-17 years prior to diagnosis. American Journal of Epidemiology, *142:* 608-611, 1995. (Appendix 35)

48. Tangrea, J., Helzlsouer, K., Pietinen, P., Taylor, P., Hollis, B., Virtamo, J., and Albanes, D. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes & Control, *8:* 615-625, 1997. (Appendix 36)

49. Feskanich, D., Ma, J., Fuchs, C. S., Kirkner, G. J., Hankinson, S. E., Hollis, B. W., and Giovannucci, E. L. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiology, Biomarkers & Prevention, *13:* 1502-1508, 2004. (Appendix 37)

50. Garland, C., Shekelle, R. B., Barrett-Connor, E., Criqui, M. H., Rossof, A. H., and Paul, O. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. Lancet, *1:* 307-309, 1985. (Appendix 38)

51. Bostick, R. M., Potter, J. D., Sellers, T. A., McKenzie, D. R., Kushi, L. H., and Folsom, A. R. Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. American Journal of Epidemiology, *137:* 1302-1317, 1993.

52. Kearney, J., Giovannucci, E., Rimm, E. B., Ascherio, A., Stampfer, M. J., Colditz, G. A., Wing, A., Kampman, E., and Willett, W. C. Calcium, vitamin D, and dairy foods

and the occurrence of colon cancer in men. American Journal of Epidemiology, *143:* 907-917, 1996.

53. Martinez, M. E., Giovannucci, E. L., Colditz, G. A., Stampfer, M. J., Hunter, D. J., Speizer, F. E., Wing, A., and Willett, W. C. Calcium, vitamin D, and the occurrence of colorectal cancer among women. Journal of the National Cancer Institute, *88:* 1375-1382, 1996. (Appendix 39)

54. Pritchard, R. S., Baron, J. A., and Gerhardsson de Verdier, M. Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. Cancer Epidemiology, Biomarkers & Prevention, *5:* 897-900, 1996. (Appendix 40)

55. Marcus, P. M. and Newcomb, P. A. The association of calcium and vitamin D, and colon and rectal cancer in Wisconsin women. International Journal of Epidemiology, *27:* 788-793, 1998. (Appendix 41)

56. La Vecchia, C., Braga, C., Negri, E., Franceschi, S., Russo, A., Conti, E., Falcini, F., Giacosa, A., Montella, M., and Decarli, A. Intake of selected micronutrients and risk of colorectal cancer. International Journal of Cancer, *73:* 525-530, 1997. (Appendix 42)

57. Heaney, R. P. Long-latency deficiency disease: insights from calcium and vitamin D. American Journal of Clinical Nutrition, *78:* 912-919, 2003.

58. Baurenfiend, J. C. Nutrition of foods. *In:* M. E. Shils, S. J. Olsen, and M. Shike (eds.), Modern Nutrition in Health and Disease, 8th Edition, Vol. 2. Philadelphia, PA: Lea and Febiger, 1994.

59. Baurenfiend, J. C. and Lachance, P. A. Nutrient Additions to Food: Nutritional Technological and Regulatory Aspects: Food and Nutrition Press, Inc., 1991.

60. Rubin, S. H., Emodi, A., and Scialpi, L. Micronutrient additions to cereal grain products. Cereal Chemistry, *54:* 895-904, 1977.

61. Honein, M. A., Paulozzi, L. J., Mathews, T. J., Erickson, J. D., and Wong, L. Y. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA, *285:* 2981-2986, 2001. (Appendix 43)

62. Williams, L. J., Mai, C. T., Edmonds, L. D., Shaw, G. M., Kirby, R. S., Hobbs, C. A., Sever, L. E., Miller, L. A., Meaney, F. J., and Levitt, M. Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. Teratology, *66:* 33-39, 2002. (Appendix 44)

63. Gucciardi, E., Pietrusiak, M. A., Reynolds, D. L., and Rouleau, J. Incidence of neural tube defects in Ontario, 1986-1999.[see comment]. CMAJ Canadian Medical Association Journal, *167:* 237-240, 2002. (Appendix 45)

64. Persad, V. L., Van den Hof, M. C., Dube, J. M., and Zimmer, P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification.[see comment]. CMAJ Canadian Medical Association Journal, *167:* 241-245, 2002.

65. Bower, C., Ryan, A., Rudy, E., and Miller, M. Trends in neural tube defects in Western Australia. Australian & New Zealand Journal of Public Health, *26:* 150-151, 2002.

66. De Wals, P., Rusen, I. D., Lee, N. S., Morin, P., and Niyonsenga, T. Trend in prevalence of neural tube defects in Quebec. Birth Defects Research, *67:* 919-923, 2003.

67. Natri, A.-M., Salo-Väänäen, P., Vikstedt, T., Palssa, A., Huttunen, M., Kärkkäinen, M. U. M., Salovaara, H., Piironen, V., Jakobsen, J., and Lamberg-Allardt, C. J. Bread fortified with Vitamin D3 increases serum 25-Hydroxyvitamin D concentration as effectively as a Vitamin D3 supplement. Journal of Nutrition, *136:* 123-127, 2006. (Appendix 46)

68. U.S. Department of Agriculture, Econimic Research Service (ERS). Food consumption (per capita) data system. Washington, D.C.: U.S. Government Printing Office. Data for 1970-1997., 2005.

69. National Research Council (NRC), Proposed cereal-grain products. National Academy of Sciences. Washington, D.C., 1974.

XI. List of Appendicies

1. Feskanich, D., Willett, W. C., and Colditz, G. A. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. American Journal of Clinical Nutrition, *77:* 504-511, 2003.
2. Meier, C., Woitge, H. W., Witte, K., Lemmer, B., and Seibel, M. J. Supplementation with oral vitamin D3 and calcium during winter prevents seasonal bone loss: a randomized controlled open-label prospective trial. Journal of Bone & Mineral Research, *19:* 1221-1230, 2004.
3. Lieberman, D. A., Prindiville, S., Weiss, D. G., Willett, W., and Group, V. A. C. S. Risk factors for advanced colonic neoplasia and hyperplastic polyps in asymptomatic individuals. JAMA, *290:* 2959-2967, 2003.
4. McCullough, M. L., Robertson, A. S., Rodriguez, C., Jacobs, E. J., Chao, A., Carolyn, J., Calle, E. E., Willett, W. C., and Thun, M. J. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). Cancer Causes & Control, *14:* 1-12, 2003.
5. Slattery, M. L., Neuhausen, S. L., Hoffman, M., Caan, B., Curtin, K., Ma, K. N., and Samowitz, W. Dietary calcium, vitamin D, VDR genotypes and colorectal cancer. International Journal of Cancer, *111:* 750-756, 2004.
6. Norman, A. W. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. American Journal of Clinical Nutrition, *67:* 1108-1110, 1998.
7. Adams, J. S., Clemens, T. L., Parrish, J. A., and Holick, M. F. Vitamin-D synthesis and metabolism after ultraviolet irradiation of normal and vitamin-D-deficient subjects. New England Journal of Medicine, *306:* 722-725, 1982.
8. Holick, M. F. Vitamin D: New horizons for the 21st century. American Journal of Clinical Nutrition, *60:* 619-630, 1994.

9. Stokstad, E. The Vitamin D Deficit. Science, *302:* 1886-1888, 2003.

10. Specker, B. L., Ho, M. L., Oestreich, A., Yin, T. A., Shui, Q. M., Chen, X. C., and Tsang, R. C. Prospective study of vitamin D supplementation and rickets in China. Journal of Pediatrics, *120:* 733-739, 1992.

11. Thomas, M. K., Lloyd-Jones, D. M., Thadhani, R. I., Shaw, A. C., Deraska, D. J., Kitch, B. T., Vamvakas, E. C., Dick, I. M., Prince, R. L., and Finkelstein, J. S. Hypovitaminosis D in medical inpatients. New England Journal of Medicine, *338:* 777-783, 1998.

12. Nesby-O'Dell, S., Scanlon, K. S., Cogswell, M. E., Gillespie, C., Hollis, B. W., Looker, A. C., Allen, C., Doughertly, C., Gunter, E. W., and Bowman, B. A. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. American Journal of Clinical Nutrition, *76:* 187-192, 2002.

13. Vieth, R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety.[see comment]. American Journal of Clinical Nutrition, *69:* 842-856, 1999.

14. Glerup, H., Mikkelsen, K., Poulsen, L., Hass, E., Overbeck, S., Thomsen, J., Charles, P., and Eriksen, E. F. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. Journal of Internal Medicine, *247:* 260-268, 2000.

15. Heaney, R. P., Davies, K. M., Chen, T. C., Holick, M. F., and Barger-Lux, M. J. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol.[see comment][erratum appears in Am J Clin Nutr. 2003 Nov;78(5):1047]. American Journal of Clinical Nutrition, *77:* 204-210, 2003.

16. Holick, M. F. Too little vitamin D in premenopausal women: why should we care? American Journal of Clinical Nutrition, *76:* 3-4, 2002.

17. Tangpricha, V., Pearce, E. N., Chen, T. C., and Holick, M. F. Vitamin D insufficiency among free-living healthy young adults. American Journal of Medicine, *112:* 659-662, 2002.

18. Holick, M. F., Siris, E. S., Binkley, N., Beard, M. K., Khan, A., Katzer, J. T., Petruschke, R. A., Chen, E., and de Papp, A. E. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. Journal of Clinical Endocrinology & Metabolism, *90:* 3215-3224, 2005.

19. Gordon, C. M., DePeter, K. C., Feldman, H. A., Grace, E., and Emans, S. J. Prevalence of vitamin D deficiency among healthy adolescents. Archives of Pediatrics & Adolescent Medicine, *158:* 531-537, 2004.

20. Vieth, R., Cole, D. E., Hawker, G. A., Trang, H. M., and Rubin, L. A. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. European Journal of Clinical Nutrition, *55:* 1091-1097, 2001.

21. Moore, C. E., Murphy, M. M., and Holick, M. F. Vitamin D intakes by children and adults in the United States differ among ethnic groups. Journal of Nutrition, *135:* 2478-2385, 2005.

22. Nurmi, I., Kaukonen, J. P., Lüthje, P., Naboulsi, H., Tanninen, S., Kataja, M., Kallio, M. L., and Leppilampi, M. Half of the patients with an acute hip fracture suffer from hypovitaminosis D: a prospective study in southeastern Finland. Osteoporos. Int., *16:* 2018-2024, 2005.

23. Chapuy, M. C., Arlot, M. E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P. D., and Meunier, P. J. Vitamin D3 and calcium to prevent hip fractures in the elderly women. New England Journal of Medicine, *327:* 1637-1642, 1992.

24. Dawson-Hughes, B., Harris, S. S., Krall, E. A., and Dallal, G. E. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. New England Journal of Medicine, *337:* 670-676, 1997.

25. Bischoff-Ferrari, H. A., Willett, W. C., Wong, J. B., Giovannucci, E., Dietrich, T., and Dawson-Hughes, B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA, *293:* 2257-2264, 2005.

26. Trivedi, D. P., Doll, R., and Khaw, K. T. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ, *326:* 469-474, 2003.

27. Bischoff-Ferrari, H. A., Dawson-Hughes, B., Willett, W. C., Staehelin, H. B., Bazemore, M. G., Zee, R. Y., and Wong, J. B. Effect of Vitamin D on falls: a meta-analysis. JAMA, *291:* 1999-2006, 2004.

28. Bischoff-Ferrari, H. A., Dietrich, T., Orav, E. J., Hu, F. B., Zhang, Y., Karlson, E. W., and Dawson-Hughes, B. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. American Journal of Clinical Nutrition, *80:* 752-758, 2004.

29. Wicherts, I. D., van Schoor, N. M., Boeke, A. J. P., and Lips, P. Vitamin D deficienty and neuromuscular performance in the Longitudinal Aging Study Amsterdam. Journal of Bone and Mineral Research, *20(Suppl 1):* S35, 2005.

30. Grant, A. M., Avenell, A., Campbell, M. K., McDonald, A. M., MacLennan, G. S., McPherson, G. C., Anderson, F. H., Cooper, C., Francis, R. M., Donaldson, C., Gillespie, W. J., Robinson, C. M., Torgerson, D. J., Wallace, W. A., and Group, R. T. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet, *365:* 1621-1628, 2005.

31. Newmark, H. L., Heaney, R. P., and Lachance, P. A. Should calcium and vitamin D be added to the current enrichment program for cereal-grain products? American Journal of Clinical Nutrition, *80:* 264-270, 2004.

32. Grau, M. V., Baron, J. A., Sandler, R. S., Haile, R. W., Beach, M. L., Church, T. R., and Heber, D. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. Journal of the National Cancer Institute, *95:* 1765-1771, 2003.

33. Gorham, E. D., Garland, C. F., Garland, F. C., Grant, W. B., Mohr, S. B., Lipkin, M., Newmark, H. L., Giovannucci, E., Wei, M., and Holick, M. F. Vitamin D and prevention of colorectal cancer. Journal of Steroid Biochemistry & Molecular Biology, *97:* 179-194, 2005.

34. Garland, C. F., Comstock, G. W., Garland, F. C., Helsing, K. J., Shaw, E. K., and Gorham, E. D. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study.[see comment]. Lancet, *2:* 1176-1178, 1989.

35. Braun, M. M., Helzlsouer, K. J., Hollis, B. W., and Comstock, G. W. Colon cancer and serum vitamin D metabolite levels 10-17 years prior to diagnosis. American Journal of Epidemiology, *142:* 608-611, 1995.

36. Tangrea, J., Helzlsouer, K., Pietinen, P., Taylor, P., Hollis, B., Virtamo, J., and Albanes, D. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes & Control, *8:* 615-625, 1997.

37. Feskanich, D., Ma, J., Fuchs, C. S., Kirkner, G. J., Hankinson, S. E., Hollis, B. W., and Giovannucci, E. L. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiology, Biomarkers & Prevention, *13:* 1502-1508, 2004.

38. Garland, C., Shekelle, R. B., Barrett-Connor, E., Criqui, M. H., Rossof, A. H., and Paul, O. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. Lancet, *1:* 307-309, 1985.

39. Martinez, M. E., Giovannucci, E. L., Colditz, G. A., Stampfer, M. J., Hunter, D. J., Speizer, F. E., Wing, A., and Willett, W. C. Calcium, vitamin D, and the occurrence of colorectal cancer among women. Journal of the National Cancer Institute, *88:* 1375-1382, 1996.

40. Pritchard, R. S., Baron, J. A., and Gerhardsson de Verdier, M. Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. Cancer Epidemiology, Biomarkers & Prevention, *5:* 897-900, 1996.

41. Marcus, P. M. and Newcomb, P. A. The association of calcium and vitamin D, and colon and rectal cancer in Wisconsin women. International Journal of Epidemiology, *27:* 788-793, 1998.

42. La Vecchia, C., Braga, C., Negri, E., Franceschi, S., Russo, A., Conti, E., Falcini, F., Giacosa, A., Montella, M., and Decarli, A. Intake of selected micronutrients and risk of colorectal cancer. International Journal of Cancer, *73:* 525-530, 1997.

43. Honein, M. A., Paulozzi, L. J., Mathews, T. J., Erickson, J. D., and Wong, L. Y. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA, *285:* 2981-2986, 2001.

44. Williams, L. J., Mai, C. T., Edmonds, L. D., Shaw, G. M., Kirby, R. S., Hobbs, C. A., Sever, L. E., Miller, L. A., Meaney, F. J., and Levitt, M. Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. Teratology, *66:* 33-39, 2002.

45. Gucciardi, E., Pietrusiak, M. A., Reynolds, D. L., and Rouleau, J. Incidence of neural tube defects in Ontario, 1986-1999.[see comment]. CMAJ Canadian Medical Association Journal, *167:* 237-240, 2002.

46. Natri, A.-M., Salo-Väänäen, P., Vikstedt, T., Palssa, A., Huttunen, M., Kärkkäinen, M. U. M., Salovaara, H., Piironen, V., Jakobsen, J., and Lamberg-Allardt, C. J. Bread

fortified with Vitamin D3 increases serum 25-Hydroxyvitamin D concentration as effectively as a Vitamin D3 supplement. Journal of Nutrition, *136:* 123-127, 2006.