THE RELATIONSHIP BETWEEN VITAMIN D STATUS OF ADULT WOMEN AND
DIET, SUN EXPOSURE, SKIN REFLECTANCE, BODY COMPOSITION,
AND INSULIN SENSITIVITY

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ABSTRACT

The Relationship Between Vitamin D Status of Adult Women and Diet, Sun Exposure, Skin Reflectance, Body Composition, and Insulin Sensitivity

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As the prevalence of vitamin D deficiency continues to grow, mounting evidence supporting its link with chronic disease strengthens suggesting vitamin D's candidacy in the prevention and treatment of multiple disease states and their complications. Dietary guidelines, however, do not take sun exposure into account. The present study sought to explore the impact of sun exposure on vitamin D status (serum 25(OH)D), and identify other significant determinants of serum levels which may have the greatest effects on overall health. Participants ($n = 34$) were pre-menopausal women aged 18 to 50 years (mean age 39 ± 6 years), who had their blood drawn at a local pathology lab and a follow-up appointment at a health assessment lab for the collection of other measurements. Mean serum 25(OH)D level was 64 ± 18 nmol/L, and mean dietary vitamin D intake was approximately 327 ± 229 IU/day. Although 82% of participants were below the RDA guidelines (600 IU/day for females ages 9-50 years) for dietary vitamin D intake, only 32% had serum 25(OH)D levels < 50 nmol/L (the recommended level of sufficiency for bone health) reflecting deficiency. While serum 25(OH)D levels were significantly correlated to dietary vitamin D intake ($r = 0.42$, $p = 0.0139$), it is reasonable to assume that participants obtained adequate vitamin D from sun exposure. Fasting serum insulin levels were significantly, positively correlated with BMI ($r = 0.83$, $p < 0.0001$), and sun exposure index (Body Surface Area x Minutes of Direct Sunlight) was significantly, positively correlated with serum 25(OH)D levels (fall weekend SEI: $r = 0.47$, $p = 0.0059$; spring weekend SEI: $r = 0.43$, $p = 0.0135$; average weekend SEI: $r = 0.43$, $p = 0.013$; and average overall SEI: $r = 0.39$, $p = 0.0247$). Reported sun exposure appeared to be least during winter weekdays and the most during summer weekends. Regression analysis was used to determine the strongest predictors of serum 25(OH)D levels, which were found to be sun exposure, dietary vitamin D intake, skin reflectance, age, BMI, and ethnicity ($R^2 = 0.58$, $p = 0.0031$), demonstrating that simple questionnaires, such as those employed in this study, can help to predict serum 25(OH)D status and thus be considered in the future treatment of vitamin D deficiency.

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VITAMIN D: A REVIEW OF LITERATURE

Background on Vitamin D

Classifications

Vitamin D is a fat-soluble vitamin and secosteroid. Its primary natural source is derived from the photolysis of the skin's 7-dehydrocholesterol (pro-vitamin D₃) under the influence of UV-B light. In this aspect, vitamin D can be considered to be more of a pro-hormone than a "vitamin" provided by dietary sources (Takiishi *et al.*, 2010).

Vitamin D has two major forms: vitamin D₂, or ergocalciferol, and vitamin D₃, or cholecalciferol. They are collectively known as calciferol. Vitamin D₂ is obtained from the ultraviolet irradiation of a yeast sterol, ergosterol, and is naturally occurring in sun-exposed mushrooms. Contrastingly, vitamin D₃ is synthesized from the skin's cholesterol precursor 7-dehydrocholesterol by UV-B light between 290 and 315 nm (MacLaughlin *et al.*, 1982). This form is also found in fatty fish and commercially available supplements (Holick, 2007).

Production and Absorption

Despite their structural similarity, vitamin D₂ and D₃ undergo different pathways of metabolism initially before joining pathways to become fully activated and thus more biologically available. There are few factors that affect dietary vitamin D₂ and D₃ absorption, such as food quality, source, and the gut's absorption capacity (Holick, 2007). There are multiple factors, however, that influence the absorption and metabolism of vitamin D₃ by impacting subcutaneous vitamin D synthesis, including solar UV intensity, age, clothing type, and sunscreen (Holick, 1987; Webb *et al.*, 1988; Matsuoka *et al.*, 1992; Matsuoka *et al.*, 1987; Clemens *et al.*, 1982). And lastly, melanin, the principal pigment present in the skin, has a significant effect on reducing cholecalciferol synthesis. Consequently, longer periods of sun exposure are required for

equivalent vitamin D synthesis in people of African ancestry (AA) compared to those of European ancestry (EA) (Clemens *et al.*, 1982; Holick *et al.*, 1981).

Metabolism and Activation

Following ingestion via dietary sources or through production from sun exposure, both forms of vitamin D are biologically inert. To become biologically available and thus “activated,” dietary vitamin D gets incorporated into chylomicrons following ingestion and sun-produced vitamin D binds to the vitamin D binding protein (DBP) once leaving the skin. Both molecules then travel via the lymphatic system and subsequently enter circulation (Holick *et al.*, 2011), where they are first transported to the liver. In the liver, vitamin D is hydroxylated by vitamin D-25-hydroxylase (25-OHase), creating 25-hydroxyvitamin D (25(OH)D), alternatively known as calcidiol (Holick, 2007; DeLuca, 2004). This partially activated form of vitamin D is easily detected in the blood and is used to determine vitamin D status because it reflects both intake and production.

Next, the newly created 25(OH)D travels to the kidneys where it is hydroxylated once more by 25(OH)D-1 α -OHase (CYP27B1) to become the most biologically active form of vitamin D, 1,25(OH)₂D, or calcitriol (Holick, 2007; DeLuca, 2004). Up until only recently, this pathway of activation was believed to be the only method of creating the biologically available form of vitamin D. It has been found, however, that not only do most cells in the body contain vitamin D receptors, but that there are also several tissues and cells which possess 1 α -OHase activity to activate inert forms of vitamin D at a local level (Holick, 2007; Moan *et al.*, 2008; Adams and Hewison, 2010; Liu *et al.*, 2006). The local production of 1,25(OH)₂D may be involved in regulating up to 200 genes (Nagpal *et al.*, 2005) which facilitate many of the pleiotropic health benefits that have been reported for vitamin D (Holick, 2007a; Holick, 2008; Holick and Chen, 2008; Holick *et al.*, 2007; Moan *et al.*, 2008; Adams and Hewison, 2010).

Functions

While vitamin D is most commonly known for its' role in bone metabolism, it is also critical for many other biochemical functions. Additionally, most of the enzymes and proteins essential for the action of vitamin D are also present in numerous tissues unrelated to bone and calcium metabolism, including those of the immune and cardiovascular system, as well as those involved in glycemic control, renal function and cognitive performance (Takiishi *et al.*, 2010).

The biologically active form of vitamin D, 1,25(OH)₂D, interacts with vitamin D nuclear receptors, which are present in most tissues and cells in the human body, such as that of the intestine, kidneys, and heart (Holick, 2007; DeLuca, 2004; Adams and Hewison, 2010). Additionally, 1,25(OH)₂D helps to stimulate intestinal calcium absorption, which contributes to one of vitamin D's most critical roles in bone metabolism. Without vitamin D, a mere 10 to 15% of dietary calcium and 60% of dietary phosphorus would be absorbed (Christakos *et al.*, 2003). When sufficient plasma levels of vitamin D are present, 1,25(OH)₂D enhances calcium and phosphorus absorption from the intestines by approximately 40 and 80%, respectively (Heaney, 2004). Furthermore, 1,25(OH)₂D interacts with the vitamin D receptor in the osteoblast to mobilize calcium (among other minerals) from the skeleton, and it also stimulates calcium reabsorption from the kidney's glomerular filtrate (Holick, 2007; Dusso *et al.*, 2005). Thus, there are multiple processes involved in increasing and sustaining bone density.

Active vitamin D has a wide spectrum of biological responsibilities, including but not limited to inhibiting cellular proliferation and inducing terminal differentiation, inhibiting angiogenesis, stimulating insulin production, inhibiting renin production, and stimulating macrophage cathelicidin production (Holick, 2007; Adams and Hewison, 2010; Liu *et al.*, 2006; Bouillon *et al.*, 2008). Furthermore, 1,25(OH)₂D can regulate its own destruction by enhancing the expression of 25-hydroxyvitamin D-24-hydroxylase (CYP24R), which metabolizes both 25(OH)D and 1,25(OH)₂D into water soluble compounds which are excreted from the body (Holick, 2007).

Sources

Sun Exposure:

The major source of vitamin D for most humans comes from production in the skin from exposure to sunlight, or more specifically, solar ultraviolet B (UV-B) radiation during the spring, summer, and fall seasons (Holick *et al.*, 2007; Holick, 2008; Holick and Chen, 2008; Moan *et al.*, 2008). For those individuals who avoid sunlight exposure, reside in high-latitude areas where solar intensity is lessened, or have darker skin pigmentation, dietary and/or supplemental vitamin D becomes critical for maintaining optimal 25(OH)D plasma concentrations (Sun *et al.*, 2011).

Dietary Sources:

Meaningful amounts of vitamin D are present in a limited number of foods including fatty fish and fortified dairy products (**Table 1**) (Holick, 2007). In the US and Canada, milk is fortified with vitamin D, as are some breads, juices, cereals and other dairy products (Holick, 2007). On the other hand, most countries do not fortify milk with vitamin D due to an outbreak of vitamin D intoxication among young children in the 1950s that resulted in a law forbidding the fortification of foods with vitamin D (Holick, 2007). Since that time, Sweden and Finland have reinstated milk fortification due to the growing prevalence of vitamin D deficiency, and many other European countries have followed suit with cereals, breads, and margarine (Holick, 2007).

Supplemental to food, both vitamin D₂ and D₃ can be found in isolated capsules (often prepared with glycerol due to their solubility in fat) and multivitamin preparations containing anywhere from 400 to 5,000 IU/capsule. American pharmaceutical preparations, which often contain mega doses, contain only the D₂ form and are used to treat deficiency (Holick, 2007).

Table 1: Dietary Sources of Vitamin D

Food item	IUs per serving*	Percent DV**
Cod liver oil, 1 tablespoon	1,360	227
Salmon (sockeye), cooked, 3 ounces	794	132
Mushrooms	400	67
Mackerel, cooked, 3 ounces	388	65
Tuna fish, canned in water, drained, 3 ounces	154	26
Milk, nonfat, reduced fat, and whole, vitamin D-fortified, 1 cup	115-124	19-21
Orange juice fortified with vitamin D, 1 cup	100	17
Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces	80	13
Margarine, fortified, 1 tablespoon	60	10
Sardines, canned in oil, drained, 2 sardines	46	8
Liver, beef, cooked, 3.5 ounces	46	8
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 1 cup	40	7
Egg, 1 whole (vitamin D is found in yolk)	25	4
Cheese, Swiss, 1 ounce	6	1

Taken from ODS 2010

* An international unit (IU) is an internationally accepted amount of a substance and often used when standardizing measurements. Note: 40 IU = 1 mcg.

** Daily Value (DV) is associated with the RDA (Recommended Dietary Allowance) and is based on a 2000 kcal/day intake. The DV for vitamin D is 600 IU for adults and children age 4 and older (ODS 2010).

Food Fortification:

Because natural food sources of vitamin D are limited, vitamin D fortification policies in North America serve to increase the amount of vitamin D in the diet. Data from the NHANES III indicate that fortified milk and cereals contribute 63 and 42% of intake in young Caucasian men and women, respectively, compared to 30 and 40% from supplements (Park *et al.*, 2001). These findings also suggest that food fortification policies contribute substantially to vitamin D intake, and although supplemental intake is recommended in North America and by the revised Canada's Food Guide for men and women over 50 years (Health Canada, 2009), it is not the major source of vitamin D in most reports. Enhanced food fortification with vitamin D, in addition to recommendations for supplement consumption, may help the population's majority achieve a more optimal vitamin D status.

Sun Exposure

Dermal Synthesis

The scientific consensus remains that sun exposure offers the greatest and most bioavailable source of vitamin D. Compared to dietary sources, vitamin D produced in the skin from sunlight offers a much greater dose without compromising safety (Haddad *et al.*, 1993). For instance, when a Caucasian wearing a bathing suit is exposed to one erythemal dose of UV-B radiation which produces a slight coloring of the skin 24 hours following his/her exposure, the amount of vitamin D synthesized is equivalent to ingesting between 10,000 and 25,000 IU (Holick and Chen, 2008).

When solar UV-B photons with energies between 290 and 315 nm from sunlight strike the skin, photolysis of 7-dehydrocholesterol (provitamin D₃) to previtamin D₃ occurs in the plasma membrane of skin cells (MacLaughlin, Anderson and Holick, 1982). Approximately 15% of the initial provitamin D₃ concentration becomes previtamin D₃ (Holick, 1987), and nearly 50% of previtamin D₃, in its cis,cis isomer form, is thermodynamically unstable and rapidly transforms

to vitamin D₃ within two hours by the rearrangement of its double bonds (Holick, Tian and Allen, 1995). Once vitamin D₃ enters the extracellular space, it is attracted to the vitamin D binding protein (DBP) in the circulation and together they enter the dermal capillary bed (Feldman *et al.*, 2005). Vitamin D can be continuously synthesized from the skin for three days after a single exposure to sunlight (Holick *et al.*, 1980).

Loomis (1967) had first speculated that human skin evolved with increased melanin production in those living near the equator to prevent excessive production of vitamin D₃. However, we now know that previtamin D₃ and vitamin D₃ can be broken down with further UV-B radiation to inert photoproducts (Holick, MacLaughlin and Doppelt, 1981; Webb, DeCosta and Holick, 1989). This prevents intoxication of vitamin D₃ from the sun. Once previtamin D₃ is produced in the skin, it can be converted to vitamin D₃ or into biologically inert photoisomers, lumisterol and tachysterol, upon further UV-B exposure (Holick, MacLaughlin and Doppelt, 1981). Once vitamin D₃ is formed, it can either enter circulation or isomerize to at least 3 photoproducts, suprasterol I, suprasterol II, and/or 5,6-trans-vitamin D₃ (Webb, DeCosta and Holick, 1989). Therefore, sunlight can act as a regulator of vitamin D₃ production and prevent against intoxication.

Individual Factors That Influence Production

A variety of factors affect the skin's ability to synthesize the vitamin, including skin pigmentation, aging, and sunscreen use (Holick *et al.*, 2007; Matsuoka *et al.*, 1992; Clemens *et al.*, 1982).

Recent work by Armas *et al.* (2007) demonstrated a dose-response relationship between UV-B exposure from an artificial source and vitamin D status depending on the level of skin pigmentation, as assessed by skin reflectance. While the researchers acknowledged that dietary intake can be an important contributor to vitamin D status, it was overall and consistently less significant as a predictor of status than both sun exposure and skin pigmentation. Other studies

have also shown that vitamin D intake correlates poorly with serum 25(OH)D (Takeuchi *et al.*, 1995, Thomas *et al.*, 1998). In a study conducted by Hall *et al.* (2010), sun exposure adjusted for body surface area (BSA) exposed to the sun and skin reflectance (i.e. skin pigmentation) were the principal predictors of vitamin D status in participating subjects. Ninety-three percent of participants (67 of 72) had a low mean serum 25(OH)D₂ concentration (<50 nmol/L) for the mean of three blood draws. When dietary vitamin D intake, UV-B dose and forehead skin reflectance were examined, 55% of the variance in serum 25(OH)D could be explained. Consistent with the results of numerous other studies, Hall *et al.* (2010) found that sun exposure (adjusted for ambient levels and clothing) had the greatest impact on serum 25(OH)D levels, followed by skin reflectance and lastly, dietary intake. Positive predictors of serum 25(OH)D status also included athlete status, oral contraceptive use, and European ancestry, while North Asian ancestry was a negative predictor of status.

Increased use of sunscreen must also be considered as a participating factor of insufficient vitamin D status. In fact, sunscreen containing a protection factor (SPF) of 30 reduces the skin's vitamin D synthesis by more than 95% (Matsuoka *et al.*, 1987), and individuals with darker skin pigmentation require sun exposure of at least three to five times longer to synthesize the same amount of vitamin D when compared to an individual with a lighter skin pigmentation (Clemens *et al.*, 1982, Hintzpeter *et al.*, 2008). Furthermore, very few dietary sources contain sufficient vitamin D to compensate for inadequate sunlight exposure.

Other studies have shown that additional factors, such as increasing age (MacLaughlin, 1985) and greater BMI (Worstman *et al.*, 2000; Lee *et al.*, 2009), are also associated with lower serum 25(OH)D and could affect subsequent recommendations of sun exposure and dietary intake to achieve optimal status.

Environmental Factors That Influence Production

In addition to numerous personal factors, any alteration of the sun caused by changes in latitude, season, or even time of day influences the skin's production of vitamin D (Holick *et al.*, 2007; Holick and Chen, 2008). For instance, vitamin D₃ synthesis in the skin is greatly limited above and below latitudes of approximately 33 degrees (Holick *et al.*, 2011). Furthermore, sun exposure intensity significantly varies by season. Webb *et al.* (1988) reported that a minimum exposure intensity of 200 J/m² is needed to initiate cutaneous vitamin D synthesis. The time needed to achieve 200 J/m² ranges from nearly a full day in December to less than 20 minutes on some days in the spring and summer (Hall *et al.*, 2010). Individual sun exposure doses followed the same seasonal pattern as for exposure intensity, and dietary recommendations may vary depending upon both individual and seasonal exposure.

Methods for Measuring Sun Exposure

Individual UV-B exposure can be measured in a variety of ways, including by use of daily sun exposure logs, recall questionnaires, dosimeter badges, and a sun exposure index (SEI). Participants in a study by Hall *et al.* (2010) wore polysulphone (PS) dosimeter badges, a material comprised of a photosensitive polymer that is used an objective way to measure broadband UV-B exposure. This material is sensitive to the same wavelengths that affect human skin (Kimlin, Parisi and Wong, 1998). The exposure captured by the badges was quantified and used to validate daily sun exposure logs that recorded all time outside between 7am and 7pm, as well as a SEI which incorporated exposed time and body surface area (BSA) exposed. The average sun exposure logs kept on the same day as the PS badge was worn were significantly correlated ($r = 0.94$, $p < 0.0001$), demonstrating the ability of the sun exposure log and SEI to accurately measure personal UV-B exposure (Hall *et al.*, 2008).

Recommendations

Recommendations of vitamin D intake required to maintain target serum 25(OH)D concentrations remain controversial due to the abundant yet inconsistent research. It is also believed that sun exposure and vitamin D intake could vary by ancestry and risk of deficiency due to differences in behavior, as well as food habits and supplement use (Hall *et al.*, 2010), all of which may impact the recommended daily dietary requirements in different groups of people. Currently, recommendations are only based on age and gender and do not take sun exposure into account.

In efforts to create clinical guidelines for the evaluation, treatment, and prevention of vitamin and mineral deficiencies, including that of vitamin D, given the controversy and wide spectrum of recommendations, the U.S. Institute of Medicine's Food and Nutrition Board developed a system of nutrition recommendations called the Dietary Reference Intakes (DRI). Prior to the most recent update in 2012, the DRIs for vitamin D and calcium were last published in 1997. This system is employed by both the United States and Canada and is intended for the general public and health professionals as a means to broaden existing guidelines known as Recommended Dietary Allowances (RDA's) (USDA). Prior to the development of a vitamin D-specific RDA for each age group, insufficient evidence was available to create concrete dietary guidelines and thus the Food and Nutrition Board had established an Adequate Intake (AI) value. The AI for vitamin D was based on values known to affect bone health and calcium absorption devoid of sun exposure. As the awareness about vitamin D deficiency became more widespread and research questioned the adequacy of the AI level, the Institute of Medicine reexamined these guidelines. Using a systematic review and two conferences on vitamin D and health, US and Canadian government researchers found significant and relevant scientific evidence to develop a new RDA value for each age group (**Table 2**) (Yetley *et al.*, 2009) with the exception of the 0-12 month age group, which remains an AI due to the lack of sufficient evidence warranting further modification. All RDA values have been modified from AI values as of January 2012.

Table 2: Dietary Reference Intakes for Vitamin D

Life Stage Group	**Adequate Intake (AI)	*Recommended Daily Allowance (RDA)
Infants 0-12 months	400 IU/day (previously 200 IU/day)	Insufficient evidence to create RDA.
Children 1-8 years		600 IU/day (previously AI of 200 IU/day)
Ages 9-50 years		600 IU/day (previously AI of 200 IU/day)
Ages 51-70 years		600 IU/day (previously AI of 400 IU/day)
Ages 70+ years		800 IU/day (previously AI of 600 IU/day)
Pregnant & Lactating Women (14-50 years)		600 IU/day (previously AI of 400 IU/day)

USDA National Agricultural Library: DRI Tables (last updated October 2012)

Note: All dietary reference intakes are created under the assumption of minimal sunlight.

*The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in an age group. It is calculated from an Estimated Average Requirement (EAR), which specifies the median requirement for vitamin D for each age group.

**An AI is developed when there exists insufficient scientific evidence to establish an EAR. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, however it lacks quantity or certainty of data to be specified with confidence the percentage of individuals covered by this intake.

Individualizing Dietary Recommendations Based on Risk of Deficiency

At present, it is important to note that the USDA's dietary recommendations do not take sun exposure into account. As a result of the many variables affecting the amount of vitamin D produced through UV-B radiation, recommendations should identify sun exposure as a factor in achieving optimal vitamin D status. For instance, vitamin D intake needed to maintain target serum 25(OH)D concentrations in individuals with low sun exposure and dark skin pigmentation is substantially higher than current recommendations (Hall *et al.*, 2010).

As Hall *et al.* (2010) concluded, a shortcoming of current recommendations for vitamin D intake is that they are not individualized to reflect the risk of deficiency which can be determined by level of sun exposure and skin reflectance. To illustrate how recommendations might vary when these factors are considered, the researchers estimated the amount of additional vitamin D intake that would be required to achieve and maintain a serum 25(OH)D greater than or equal to

50 to 75 nmol/L, and found that individuals with low skin reflectance and low sun exposure would require the highest intakes to reach sufficient levels (Hall *et al.*, 2010). For instance, the estimated dietary intake of African American (AA) participants with low sun exposure ranged from 650 to 1700 IU/day of supplemental vitamin D, depending on the season, to meet the 50 nmol/L threshold and 2100-3100 IU/day to meet the 75 nmol/L threshold. The corresponding values for AA participants with high sun exposure were 0-850 IU/d for the 50 nmol/L threshold and 1000-2250 IU/d for the 75 nmol/L threshold. On the contrary, European American (EA) participants with high sun exposure required no additional vitamin D intake to meet the 50 nmol/L threshold, whereas considerable supplemental intake of 1300 IU/day would be needed in the winter to meet the 75 nmol/L threshold (Hall *et al.*, 2010). However, EA participants with low sun exposure needed considerable supplemental intake, 850-1100 IU/day, to meet the 50 nmol/L threshold in the winter and spring and year-round intake ranging from 1000 to 2550 IU/d to meet the 75 nmol/L threshold (Table 3, 4) (Hall *et al.*, 2010).

Table 3: Estimated vitamin D intake needed to achieve serum 25(OH)D 50 nmol/L in participants with median skin reflectance for African and European ancestries with low (20th percentile) and high (80th percentile) sun exposure for each season

	AA	AA	EA	EA
Cohort	Low sun (IU/day)	High sun (IU/day)	Low sun (IU/day)	High sun (IU/day)
Fall	1050	0	200	0
Winter	1700	850	1100	0
Spring	1500	0	850	0
Summer	650	0	0	0

¹ Estimated using regression model to predict serum 25(OH)D based on skin reflectance, sun exposure and 200 IU/d current vitamin D intake, and using expected serum 25(OH)D response to additional vitamin D supplementation based on a dose- response study using 0.7 nmol/L for every additional 1 mg of vitamin D intake from supplements (Heaney 2003).

² Values include the baseline intake of 200 IU/d.

³ 40 IU=1 ug.

Hall *et al.*, 2010

Table 4: Estimated vitamin D intake needed to achieve serum 25(OH)D >75 nmol/L in participants with median skin reflectance for African and European ancestries with low (20th percentile) and high (80th percentile) sun exposure for each season

	AA	AA	EA	EA
Cohort	Low sun (IU/day)	High sun (IU/day)	Low sun (IU/day)	High sun (IU/day)
Fall	2500	1250	1650	0
Winter	3100	2250	2550	1300
Spring	2900	1400	2250	50
Summer	2100	1000	1000	0

¹ 40 IU = 1 ug

² Estimated as described in footnote to Table 3

Hall *et al.*, 2010

Contrastingly, a recent study in the southwestern US showed that both sun exposure and vitamin D intake significantly predicted vitamin D status, however this effect was more pronounced in Whites than in Blacks or Hispanics (Jacobs *et al.*, 2008). This may be explained by the fact that EA participants commonly consume higher levels of fortified dairy products and have higher total vitamin D intake than other ethnic groups (Calvo *et al.*, 2005). Overall and regardless of ethnicity, this study confirmed that relatively high intakes of vitamin D would be needed to achieve serum 25(OH)D concentrations of 50 or 75 nmol/L for participants with low sun exposure, especially during the winter months, suggesting that dietary vitamin D intake should be customized for those individuals who are at greater risk of deficiency (Calvo *et al.*, 2005).

Toxicity

Etiology and Symptoms of Hypervitaminosis D

While significant attention has been paid to deficiency, vitamin D toxicity is also becoming more common due to the rising popularity of supplementation (Wallace *et al.*, 2011). In particular, calcium plus vitamin D supplements have been recommended for the prevention of osteoporotic fractures in postmenopausal women (National Osteoporosis Foundation, 2008), and millions are relying on supplements to improve health, however, increasing the possibility of toxicity and making it an important clinical issue.

The Women's Health Initiative found a 17% increase in the risk of developing kidney disease when high supplementation of calcium (1,000 mg/day) and vitamin D (400 IU/day) were taken regularly (Jackson *et al.*, 2006). Vitamin D toxicity, which is identified as serum 25(OH)D levels consistently greater than 500 nmol/L (>200 ng/ml), can also clinically manifest in the form of hyperkalemia, hypercalciuria, and retarded growth in infants (Jones, 2008). Additionally, new evidence is emerging for increased risk of all-cause mortality, cancer, cardiovascular disease, falls and fractures at elevated exposures (Melamed *et al.*, 2009; Helzlsouer, 2010; Fiscella and Franks, 2010).

Initial toxicity often manifests itself with non-specific symptoms including anorexia, weight loss, polyuria, and heart arrhythmias (Institute of Medicine (IOM) Food & Nutrition Board, 2010). Symptoms may become more severe with worsening toxicity, in part related to increased serum calcium levels, leading to vascular and tissue calcification with subsequent damage to the heart, blood vessels, and kidneys (IOM Food & Nutrition Board, 2010).

Upper Level Recommendations

Scientific evidence examining the effect of higher exposures remains extremely limited. Thus, the safety of long-term, high dose supplementation and elevated serum levels is yet to be fully and distinctly determined, however, the Food and Nutrition Board (FNB) updated the Upper Level (UL) for intake based off of the most recent evidence.

While symptoms of toxicity are unlikely at daily average intakes below 10,000 IU/day, the FNB reviewed emerging evidence from national survey data, observational studies, and clinical trials finding that even lower levels of vitamin D intakes and serum 25(OH)D levels may have adverse health effects over time. The FNB concluded that serum 25(OH)D levels above approximately 125–150 nmol/L (50–60 ng/mL) should be avoided, while even lower serum levels (approximately 75–120 nmol/L or 30–48 ng/mL) are associated with an increased risk of all-cause mortality, cancer, cardiovascular disease, falls and fractures (National Institute of Health,

2011). The FNB also highlighted research which found that consuming 5,000 IU/day of vitamin D achieved serum 25(OH)D concentrations between 100–150 nmol/L (40–60 ng/mL). This committee then applied an uncertainty factor of 20% to this intake value to derive an UL of 4,000 IU for children ages 9 years and older with corresponding lower amounts for younger children (Table 5) (National Institute of Health, 2011).

Table 5: Upper Level of Vitamin D for Each Age Group

Life Stage Group	Upper Level (UL)*
Infants 0-6 months	1000 IU/day
Infants 6-12 months	1500 IU/day (previously 1000 IU/day)
Children 1-3 years	2500 IU/day (previously 2000 IU/day)
Children 4-8 years	3000 IU/day (previously 2000 IU/day)
Ages 9-50 years	4000 IU/day (previously 2000 IU/day)
Adults 50+ years	4000 IU/day (previously 2000 IU/day)
Pregnant & Lactating Women (14-50 years)	4000 IU/day (previously 2000 IU/day)

*Note: The Upper Level is the highest level of intake from food and supplements that does not pose a health risk.

USDA National Agricultural Library: DRI Tables (last updated October 2012)

Deficiency

Prevalence of Deficiency

Vitamin D insufficiency and deficiency has reached astounding prevalence of up to 63% and has been identified as a worldwide health issue in recent years (National Institute of Health, 2011). Predominantly the result of suboptimal sun exposure in addition to inadequate dietary intake, the true “boundaries” of insufficiency and deficiency remain somewhat controversial. At present, vitamin D deficiency is defined by the Institute of Medicine to be <12 ng/ml (<30 nmol/L), and insufficiency as 12-20 ng/ml (30-50 nmol/L) (National Institute of Health, 2011). Comparatively, >20 ng/ml (>50 nmol/L) is considered to be adequate for bone and overall health in healthy individuals.

In the National Health and Nutrition Examination Survey (NHANES) 2005-2006, approximately 24% of 1-21 year olds (n = 3136) were found to be vitamin D deficient (<15 ng/ml), and 63% were insufficient (15-29 ng/ml). Similarly, 26% of adults (n = 3454), ages >21 years, were found to be deficient and 60% were insufficient (Sharief *et al.*, 2011). Mean serum 25(OH)D concentrations appear to have decreased over time relative to decreased sun exposure and milk consumption and an increased prevalence of obesity (Looker *et al.*, 2008). Parallel to the rising prevalence of deficiency, there also exists an increasing occurrence of multiple conditions beyond bone health and obesity of which vitamin D is thought to be involved, including cancer, diabetes, hypertension, and infections (Holick, 2007; Pittas 2010, Pittas *et al.*, 2007; Forman *et al.*, 2007).

Risk Factors for Deficiency

Undoubtedly, there are some conditions that place individuals at a higher risk for vitamin D deficiency. These abnormalities include chronic kidney disease, hepatic failure, malabsorption syndromes (cystic fibrosis, inflammatory bowel disease, Crohn's disease, bariatric surgery, radiation enteritis), hyperparathyroidism, certain medications (anti-seizure medications, glucocorticoids, medications to treat HIV and AIDS, antifungals, cholestyramine); African-American and Hispanic ancestry; pregnancy and lactation; older age (>70 years) with a history of falls and nontraumatic fractures; obesity (BMI >30); granuloma-forming disorders (sarcoidosis, tuberculosis, histoplasmosis, coccidiomycosis, berylliosis); and some lymphomas (Holick, 2007).

Other factors contributing to deficiency in many otherwise healthy adults are found to include seasonal effects such as decreased sun exposure, a lack of supplemental and dietary vitamin D intake, darker skin pigmentation, and living at higher latitudes (Gozdzik *et al.*, 2010).

Obesity:

Serum 25(OH)D levels have been found to be low in obese children and adults (Liel *et al.*, 1988; Bell *et al.*, 1985; Worstman *et al.*, 2000; Yanoff *et al.*, 2006). While this association

has yet to be fully understood, the hypothesis has been that there is sequestration of vitamin D in the subcutaneous body fat reducing the vitamin's bioavailability (Worstman *et al.*, 2000). This idea is explored and confirmed by Lagunova *et al.* (2010), whose results suggested that despite matched dietary intake and sun exposure, the obese subgroup had significantly lower serum 25(OH)D levels.

Sedentary lifestyles that are often linked to obesity could also be a factor, as they may be associated with less outdoor activities and thus reduced sunlight (Dewey & Heuberger, 2011).

This indirect factor may further contribute to reduced endogenous vitamin D production and compromised vitamin D status. Additionally, some studies have shown significant differences in mean dietary vitamin D intake among obese individuals, suggesting a lower overall consumption of vitamin D-containing foods when compared with non-obese individuals (Rajakumar *et al.*, 2008; Rajakumar *et al.*, 2005).

In a study by Rajakumar *et al.* (2008), researchers found that vitamin D deficiency was common among obese and non-obese preadolescent (ages 6-10 years) African American children, and that 400 IU vitamin D₃ daily (200 IU below the updated RDA as of January 2012 for this age group) for one month was inadequate to raise both group's blood levels of 25(OH)D to a level greater than 30 ng/ml (Rajakumar *et al.*, 2008). This study also showed that bone metabolism, bone turnover, and bone mineral content were altered in severe obesity.

Rajakumar *et al.* (2008) found that the anticipated compensatory increase in parathyroid hormone (PTH) in response to vitamin D deficiency and insufficiency was observed only among the non-obese participants, suggesting that the innate PTH response to vitamin D deficiency may be blunted in the obese state.

Due to the notable potential differences in vitamin D bioavailability and metabolism in obese individuals, it can be argued that adequacy of vitamin D replacement in hypovitaminosis D should be dependent upon body mass index (Lee *et al.*, 2009). Overweight and obese patients

with vitamin D deficiency likely require higher doses of vitamin D to achieve repletion compared with individuals of normal body weight.

In addition to its link with suboptimal serum 25(OH)D levels, obesity is associated with insulin resistance and hyperinsulinemia, which have been implicated in the rising incidence of cardiovascular disease, metabolic syndrome, and type II diabetes mellitus (Osei, 2010). The current “epidemic” of obesity is likely increasing the frequency and severity of vitamin D insufficiency and deficiency encountered in clinical practice. Thus, achieving vitamin D sufficiency in obese patients may improve general well-being and further reduce the risk of commonly related chronic diseases.

Consequences of Deficiency

Calcemic Effects:

Historically, vitamin D has been well known for its importance in bone health due to its numerous roles in maintaining calcium and phosphorus homeostasis and promoting bone mineralization. Vitamin D’s impact on bone integrity is linked to multiple factors. Firstly, the body corrects suboptimal calcium plasma levels by causing an increase in bone resorption to quickly normalize calcium status (Pietschman *et al.*, 2010). When plasma levels of vitamin D become suboptimal, however, there exists an up-regulation of parathyroid hormone with a potential for the development of secondary hyperparathyroidism which leads to increased bone turnover and loss (Frost *et al.*, 2010). The resulting “remodeling space” may subsequently increase fracture risk (Lips, 2001). In an investigation by Frost *et al.* (2010), they found a reduced level of bone mineral density at serum 25(OH)D levels below 50 nmol/L (Frost *et al.*, 2010). At the cellular level, 1,25(OH)₂D has been found to interact with its vitamin D receptor in the osteoblast. This binding facilitates the development of immature monocytes into mature osteoclasts, which dissolve the bone matrix and mobilize calcium and other minerals from the skeleton (Holick, 2007; Dusso *et al.*, 2005).

Vitamin D deficiency also triggers a reduced efficiency in the intestinal absorption of dietary calcium and phosphorus, thereby resulting in increased parathyroid hormone (PTH) secretion (Holick, 2007; Heaney, 2004; Holick *et al.*, 2005; Lips *et al.*, 2006). Interestingly, PTH secretion is inhibited by the active form of vitamin D (Feldman 2005). The form of secondary hyperparathyroidism that results from deficiency helps to maintain serum calcium in the normal range at the expense of mobilizing calcium from the skeleton and increasing phosphorus wasting in the kidneys, a condition known as phosphaturia (Holick, 2007). The PTH-mediated increase in osteoclastic activity creates local foci of bone fragility and causes a generalized decrease in bone mineral density (BMD), resulting in osteopenia and osteoporosis. Phosphaturia often leads to a low-normal or low serum phosphorus level, thereby causing an inadequate calcium-phosphorus product and eventual demineralization of the skeleton (Holick, 2007; Aaron *et al.*, 1974). In young children who have insufficient bone mineral content, this defect results in a variety of skeletal deformities classically known as rickets (Holick, 2006b; Gordon *et al.*, 2008). In adults, the epiphyseal plates are closer, and there is sufficient mineral in the skeleton to prevent skeletal deformities so that this mineralization defect, otherwise known as osteomalacia, often goes undetected. Osteomalacia is characterized by decreased bone mineral density and is associated with isolated or generalized aches and pains in the bones and muscles (Malabanan *et al.*, 1998a; Plotnikoff and Quigley, 2003).

Throughout the life cycle, bone is constantly remodeled; a process that results from a concerted action of osteoclasts and osteoblasts. The process of bone remodeling is regulated by a significant number of growth factors, cytokines, and hormones (including estrogen and PTH) (Pietschmann *et al.*, 2010). When this process becomes impaired, metabolic bone diseases develop.

Studies have shown positive associations between serum levels of vitamin D and bone mass and an inverse relationship with the risk of osteoporotic fractures (Valimaki *et al.*, 2004). Moreover, some (Frost *et al.*, 2010; Abrahamsen *et al.*, 2010) but not all studies (Grant *et al.*,

2005) show fracture reduction on treatment with vitamin D and calcium. Rickets in children and osteomalacia or osteoporosis in adults were the first recognized diseases of a vitamin D deficiency. These conditions will lead to long-term outcomes including bone and muscle pain as well as a greater fracture risk with increased age (Holick, 2004).

Rickets and Osteomalacia

Rickets, or “soft bones,” is defined by a T-score between -1 and -2.5 (International Osteoporosis Foundation, 2010) and is the most common consequence of vitamin D deficiency in children due to its impact on calcium and phosphorus absorption from the diet. It is often characterized by skeletal deformities including bowed legs, bone tenderness, dental problems, and muscle weakness (Bueno and Czepielewski, 2008). While rickets was an epidemic in the 19th century prior to food fortification and increased availability of dietary supplementations, it is still commonly seen in many developing countries (Bueno and Czepielewski, 2008). This condition has nearly disappeared in the US due to the fortification of milk (Bueno and Czepielewski, 2008). The adult form of this disease is termed osteomalacia, which is also most commonly attributed to vitamin D deficiency. Similar to rickets, the condition is often accompanied by diffuse body pains, muscle weakness, bone fragility and an increased risk of osteoporotic fractures (Holick, 2006b).

Osteoporosis

Osteoporosis, literally meaning “porous bone,” is defined by a T-score of -2.5 or lower (International Osteoporosis Founding, 2010) and is distinguished by an imbalance between osteoblast and osteoclast activity. It is considered to be the most common and severe manifestation of metabolic bone diseases (Pietschmann *et al.*, 2010). Principally characterized by compromised bone strength, this skeletal disorder predisposes a person to an increased risk of fracture. Bone strength primarily reflects the integration of bone density and bone quality. While

osteoporosis has a strong genetic component (Obermayer-Pietsch, 2006), it can be differentiated into primary (e.g. lifelong inadequate calcium and vitamin D intake, postmenopausal osteoporosis or idiopathic osteoporosis in men) and secondary (e.g. glucocorticoid-induced osteoporosis) forms. Diabetes mellitus (DM) is among the secondary causes of osteoporosis (Hamdy, 2008).

Diabetes and Bone Health:

Diabetes Mellitus (DM) may further increase one's risk of bone fractures in the setting of vitamin D deficiency. In a large cross-sectional study by Takiishi et al. (2010), investigators found that patients with type II DM experienced bone fractures, predominantly hip fractures, at a significantly increased rate compared to their diabetes-free counterparts (Miazgowski, 2008). The pathophysiology of decreased bone strength in DM is also multifactorial: insulin deficiency, insulin resistance, osteoblast insufficiency, vitamin D deficiency, formation of advanced glycation end-products in bone, and microvascular complications all appear to contribute to its development (Pietschmann *et al.*, 2010). Increased risk of fractures may also be attributed to certain pharmaceutical agents used in type II DM, such as thiazolidinediones, as well as a greater risk of falling as a consequence of hypoglycemia, poor vision, or neuropathy (Pietschmann *et al.*, 2010).

Non-Calceemic Effects:

In addition to bone abnormalities, vitamin D deficiency may result in a multitude of other health problems including muscle weakness. In severe cases, affected children often have difficulty walking and even standing (Gordon *et al.*, 2008; Holick, 2006b), whereas the elderly may experience progressive sway and more frequent falls (Bischoff-Ferrari *et al.*, 2005; Bischoff-Ferrari *et al.*, 2009) thereby further increasing their risk of bone fracture.

It is clear that low levels of 25(OH)D produce numerous, potentially detrimental consequences. Among many of the speculated consequences of vitamin D deficiency, decreased immune function, impaired mental health, and an increased risk of cardiovascular disease have shown some of the strongest links to inadequate levels of 25(OH)D (Holick, 2007).

Cardiovascular Disease

An association between suboptimal serum 25(OH)D concentrations and an increased risk of cardiovascular disease (CVD) are supported by data from both ecological and prospective human studies (Giovannucci *et al.*, 2008; Ginde *et al.*, 2009; Wang *et al.*, 2008; Dobnig *et al.*, 2008; Kilkkinen *et al.*, 2009; Pilz *et al.*, 2008b; Zitterman *et al.*, 2005). Firstly, low levels of the active form, 1,25(OH)₂D, are associated with low HDL-cholesterol levels, while low levels of the storage form, 25(OH)D, are associated with high levels of total cholesterol, LDL-cholesterol and triglycerides increasing risk of CVD (Karhapaa *et al.*, 2010).

This relationship may be explained by numerous factors. For instance, a study by Qi Sun *et al.* (2011) found that a high total vitamin D intake (from both foods and supplements) was associated with a decreased risk of CVD in men (Sun *et al.*, 2011). Their data also suggested that there was a 2.6% reduction in CVD risk for every 2.5 nmol/L increase in 25(OH)D from vitamin D intake (i.e. 143 IU/d).

This association may be more critical in patients who are enduring additional physiologic stresses such as dialysis. For instance, a systematic review identified consistent associations between active vitamin D treatment (either orally or intravenously administered) and a reduced risk of CVD in patients receiving dialysis who were vitamin D deficient (Wang *et al.*, 2010). While the exact mechanism of this relationship has yet to be fully understood, several potential ideas may explain vitamin D's effect, including its impact on the renin-angiotensin system, endothelial dysfunction, vascular smooth muscle proliferation, insulin resistance, and systemic inflammation, all of which support the beneficial effects of vitamin D in the prevention of CVD (Bassuk and Manson, 2009).

In addition to CVD, vitamin D deficiency may also increase risk for hypertension. Schmitz *et al.* (2009) found that severe vitamin D deficiency triggered hypertension in animal models. Also, mild vitamin D deficiency (mean 14.8 ng/ml) was associated with higher blood

pressure in Caucasians, Hispanics, and African Americans. Pilz et al. (2008) demonstrated a clear association between low levels of serum 25(OH)D, as well as of 1,25(OH)₂D, with prevalent myocardial dysfunction, deaths due to heart failure, and sudden cardiac death. In the Multi-Ethnic Study of Atherosclerosis, low 25(OH)D levels were linked to increased risk for developing incident coronary artery calcification (de Boer *et al.*, 2009). These effects, among potentially a number of others, may be related to vitamin D deficiency due to the presence of the vitamin D receptor (VDR) on various cardiac tissues, including cardiomyocytes (Tishkoff *et al.*, 2008), vascular smooth muscle cells (Wu-Wong *et al.*, 2007), and endothelial cells (Takiishi *et al.*, 2010). Since vitamin D affects inflammation as well as cellular proliferation and differentiation, adequate vitamin D may thereby lower the risk of developing cardiovascular disease.

Vitamin D and Glucose Metabolism

Diabetes mellitus (DM) is a disorder that results from defects in insulin production, secretion and sensitivity (Takiishi *et al.*, 2010). Characterized by inappropriate increases in blood glucose concentrations due to inadequate insulin action in the body, DM can be due to a primary loss of insulin secretion (as seen in type I DM), or increased insulin resistance rendering insulin ineffective (as seen in type II DM) (Boucher, 2011). Over time, insulin resistance leads to insulin insufficiency and eventual, permanent damage of the pancreas's insulin-producing beta cells (Boucher, 2011). With more than one million new cases per year of type II DM alone diagnosed in the United States and a major cause of significant morbidity, prevention of the disease is critical (Pittas *et al.*, 2009), as the life-long burden imposed on individuals by DM is heavy.

Over time, associations between vitamin D status and DM have been identified. As early as the 1980s, it was shown that vitamin D deficiency in rodents and rabbits inhibited pancreatic insulin secretion, indicating that vitamin D is essential for the function of the endocrine pancreas (Nyomba *et al.*, 1986). Later, the association between vitamin D and diabetes was reinforced by

the discovery of the vitamin D receptor (VDR) and vitamin D binding protein (DBP) in pancreatic tissue (more specifically, in the insulin-producing beta cells) and also in various cell types of the immune system (Palomer *et al.*, 2008). It has since been shown that vitamin D is essential for normal insulin release in response to glucose for maintenance of glucose tolerance (Palomer *et al.*, 2008).

Vitamin D and Insulin Secretion

In a study by Osei *et al.* (2010), serum 25(OH)D was positively associated with insulin sensitivity and negatively associated with first- and second-phase insulin secretion. More importantly, subjects with hypovitaminosis D (serum levels <20 ng/ml) had a greater risk of insulin resistance and metabolic syndrome, implicating the importance of vitamin D status in glucose hemostasis. It has since been found that vitamin D insufficiency (20-29 ng/ml) and deficiency (<20 ng/ml) have both direct and indirect effects on insulin secretion and insulin action. In this regard, there is mounting evidence that supports the association of low vitamin D with the development of both types I and II DM (Osei, 2010).

Insulin insensitivity and beta-cell secretory defects are critical to the development of impaired glucose tolerance and type II DM. Although there is a genetic basis for this endocrinological dysfunction, there are also environmental factors that play a major role in the development of DM. In addition to low serum 25(OH)D, obesity plays a significant role in the pathogenesis of glucose intolerance and type II DM (Osei, 2010; Pittas *et al.*, 2009).

As previously discussed, increasing body fat percentage may compromise the body's ability to utilize vitamin D due to the body's storage of 25(OH)D in the adipose tissue, however it may also impact an individual's insulin sensitivity. Two other important factors in low serum 25(OH)D levels are race and ethnicity. Consequently, there is higher prevalence of hypovitaminosis vitamin D in minority populations such as African-Americans and Hispanics,

who are also at greater risk of insulin resistance, type II DM, obesity and cardiovascular diseases compared to Caucasians.

Insulin Resistance

Hypovitaminosis D has long been suspected to be a risk factor for insulin resistance. Vitamin D may have a beneficial effect on insulin action either directly, by stimulating the expression of insulin receptor and thus enhancing insulin responsiveness for glucose transport (Maestro *et al.*, 2000), or indirectly via its role in regulating extracellular calcium and ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic calcium pool (Pittas *et al.*, 2007a). Calcium is critical for insulin-mediated intracellular processes in insulin-responsive tissues, such as skeletal muscle and adipose tissue (Ojuka, 2004; Wright *et al.*, 2004; Williams *et al.*, 1990), with a very narrow range of calcium required for optimal insulin-mediated functions (Draznin *et al.*, 1987). Changes in the body's calcium levels may thus contribute to peripheral insulin resistance (Draznin *et al.*, 1989; Segal *et al.*, 1990; Byyny *et al.*, 1992; Ohno *et al.*, 1993; Zemel, 1998).

Associations between low vitamin D levels and decreased insulin sensitivity have been reported in numerous cross-sectional studies (Baynes *et al.*, 1997; Chiu *et al.*, 2004; Gedik and Akalin, 1996; Borissova *et al.*, 2003; Lind *et al.*, 1989; Orwoll *et al.*, 1994; Lind *et al.*, 1995; Scragg *et al.*, 2004). Some (Chiu *et al.*, 2004; Scragg *et al.*, 2004), but not all (Orwoll *et al.*, 1994), observational studies have shown an inverse association between vitamin D or calcium status and insulin resistance. Results from randomized trials on the effect of vitamin D and/or calcium supplementation on insulin resistance show either no effect (Orwoll *et al.*, 1994; Ljunghall *et al.*, 1987; Fliser *et al.*, 1997; Barr *et al.*, 2000; Bowen *et al.*, 2005; Thompson *et al.*, 2005) or improvement (Sanchez *et al.*, 1997; Zemel *et al.*, 2004; Pittas *et al.*, 2007b) in insulin action with supplementation.

Sun Exposure and Incidence of Diabetes

Geographic location may also be associated with vitamin D status and DM. For instance, the incidence of type I DM is greater in countries of northern latitude (Karvonen *et al.*, 2000; Sloka *et al.*, 2010), while this trend is reversed in the southern hemisphere (Staples *et al.*, 2003). UV-B irradiation, which follows a north-south gradient, also has protective properties against immune dysfunction such as that displayed in type I DM (Cantorna and Mahon, 2004). Mohr *et al.* (2008) reviewed the type I DM incidence data for children younger than 13 years during 1990 and 1994 in 51 regions worldwide. They found that global incidence rates of type I DM approached zero in regions providing high UV-B exposure. Furthermore, seasonality of type I DM onset is well known (Karvonen *et al.*, 1998; Neu *et al.*, 1997). Kahn *et al.* (2009) reported that spring births were associated with a greater likelihood of type I DM, which might reflect insufficient maternal (and thus neonatal) vitamin D levels during critical stages of fetal development.

Conclusion

As mounting evidence supporting the link between vitamin D and chronic diseases continues to strengthen, an overall trend is surfacing which reflects an inverse correlation between levels of 25(OH)D and metabolic dysfunction, whether cardiovascular, immune, bone, or inflammatory in nature. The overwhelming strength of clinical data and of numerous observational studies make vitamin D or its analogues strong candidates for the prevention and treatment of multiple disease states and their complications (Takiishi *et al.*, 2010). In 2010, the DRI guidelines for vitamin D tripled from 200 IU to 600 IU/day (1-70 years old) from the original guidelines set in 1997 without a consideration of sun exposure. It is crucial to better understand the impact of sun exposure on vitamin D status, and identify other significant determinants of vitamin D status which may have the greatest effects on overall health. To contribute to this crucial development of research and clinical understanding, the present study

sought to explore major contributors to vitamin D status (serum 25(OH)D) in adult women would require consideration when devising and evaluating successful vitamin D therapies.

MATERIALS AND METHODS

Study Hypotheses and Objectives

This study had four primary hypotheses and related objectives:

Hypothesis 1:

There is a high prevalence of women on the central coast region of California who are vitamin D deficient. Objectives were to determine the vitamin D status of women living near the central coast region of CA; and to determine potential contributors of vitamin D deficiency in women, including ethnicity, skin pigmentation/reflectance and inadequate dietary vitamin D intake.

Hypothesis 2:

Obesity and percent body fat negatively influence vitamin D status. Objectives were to examine the vitamin D status in normal weight women versus overweight women versus obese women; and to measure percent body fat using DXA analysis and determine the relationship with vitamin D status.

Hypothesis 3:

Blood pressure, waist circumference, fasting insulin and glucose levels, parathyroid hormone, glycosylated hemoglobin, and age are negatively associated with vitamin D status, whereas bone density and physical activity are positively associated. Objectives were to measure blood pressure, waist circumference, fasting insulin and glucose levels, parathyroid hormone, and glycosylated hemoglobin, and record age, and determine their association with vitamin D status; and to measure bone density using DXA analysis and determine its relationship with vitamin D status.

Hypothesis 4:

Sun exposure is positively correlated with vitamin D status. Objectives were to use a seasonal questionnaire about typical weekday and weekend sun exposure to capture habitual sun exposure and relate it back to vitamin D status.

Study Design

A cross-sectional study was conducted to examine the factors that influence vitamin D status in a group of pre-menopausal women. This study focused on the baseline data of a larger, intervention-based study, which is not detailed in this paper. Data were gathered on anthropometric measurements, body composition, biochemical data, sun exposure, physical activity, dietary calcium and vitamin D intake and skin reflectance (pigmentation) from 34 participants residing along California's central coast, an area spanning 60 miles from Santa Maria to Paso Robles capturing a population of approximately 300,000. A rolling enrollment was used over all four seasons of the year to better capture changes in sun exposure and acquire a sufficient number of participants for the study. Flyers advertising the study were posted at Cal Poly State University, emailed to faculty and staff, placed in physician and health clinic offices across San Luis Obispo County, as well as in the newsletter of a local women's fitness center. All protocols and procedures in this study were first approved by the university's Human Subjects Research Committee.

Participants

Participants were screened via telephone to determine initial eligibility, which included 18 to 50 years old, non-diabetic, pre-menopausal women. Each prospective participant completed a uniform medical history and screening questionnaire that included items related to the inclusion and exclusion criteria and willingness to have specific tests done to determine their suitability for the study.

Exclusion criteria included: <18 years old, >50 years old, BMI <18.5 or >40, self-reported consistent sun-screen use on all or most exposed skin, frequent use of tanning beds, use of high-dose vitamin supplements (vitamin D > 1,000 IU/day) within the preceding two months, pregnancy, any disease or underlying condition requiring treatment that may affect vitamin D absorption or metabolism, use of medications that affect vitamin D metabolism, fat malabsorption, use of laxatives, liver disease, kidney disease, diabetes, and/or tobacco use within the past one year.

Lab Assessment at Central Coast Pathology Laboratories

After initial eligibility was determined, participants fasted for 12 hours and reported to a local clinical lab to provide blood and urine samples. All blood draws and analyses were performed at a Central Coast Pathology (CCP) Laboratory (www.ccpathology.com). This company provides services to healthcare professionals as well as university studies and has seven locations in the area making it convenient for participants to participate.

During this baseline blood draw, measurements included the following: fasting blood glucose; insulin; glycosylated hemoglobin; parathyroid hormone; and serum 25(OH)D levels. Serum 25(OH)D was measured by an IDS-iSYS Immunodiagnostic System.

If participants were found to have vitamin D levels less than 20 nmol/L (reflecting vitamin D deficiency) and/or a fasting blood glucose greater than 126 mg/dL (reflecting a potential diagnosis of diabetes), they were advised to consult with their personal physician and they were disqualified from the study.

Nutrition and Health Assessment at Cal Poly

The second appointment was scheduled approximately one to two weeks following the initial blood draw so that lab results were available. This appointment included an assessment of: bone density; body composition; blood pressure; anthropometrics; skin reflectance; dietary

vitamin D and calcium intake; sun exposure; and physical activity level. All measurements were performed in a private setting and recorded by one of three designated research team members to achieve maximal consistency. All questionnaires were administered by Registered Dietitians or trained research staff. Assessments took place at one of two locations: the Nutrition and Health Assessment Lab at Cal Poly (n = 30) or at the office of Dr. Mary Oates in Santa Maria (n = 4). Location was dependent upon the participant's convenience.

Body Composition and Bone Mineral Density:

Full-body Dual Energy X-ray Absorptiometry (DXA) was used to determine body fat mass, body fat percentages, lean body mass and bone mineral density of each participant. DXA scans and their analyses were performed using iDXA instruments (Lunar, GE Healthcare) and enCORE software (Lunar, GE Healthcare). Participants were asked to complete a four-hour fast and a pregnancy test (to ensure safety) prior to the DXA scan. Standard procedures were used to complete all DXA scans.

Blood Pressure:

Blood pressure was measured on the left arm of each participant immediately following the DXA scan to insure a resting state. A Life Source UA-787 Digital Blood Pressure Monitor, manufactured by A&D Medical, was used on all participants.

Anthropometric Measurements:

Body weight was recorded to the nearest 0.1 kilogram and height to the nearest 0.1 centimeter. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Waist and hip circumference measurements were recorded to the nearest 0.1 inch for the calculation of waist to hip ratios, which was performed by dividing waist by hip measurement.

Skin Reflectance (Pigmentation) Assessment:

Skin reflectance was measured with a Minolta 2500d spectrophotometer (Konica Minolta Sensing). Measurements were taken on the middle upper inner right arm, the dorsum of the right

hand between the thumb and index finger, and the middle of the forehead. Measurements took approximately 10 seconds each. A small, dime-sized window on the instrument touched the skin, flashed like a camera, and measured the light reflected from each participant's skin. A mean value for each site was obtained by taking three measurements at a given site and moving the instrument slightly between each measurement. The measurements were expressed in the value of lightness (L^*) using the Commission International d'Eclairage System. The L^* value ranges from zero to 100, with zero indicating a lack of light reflectance (pure black) and a value of 100 indicating 100% light reflectance (pure white). This value is highly correlated to the Melanin Index (Matsuoka, 1992) and the L^* value of the forehead was used in the multiple linear regression model because it was previously shown to be more correlated with serum 25(OH)D than the inner arm and hand measurements (Hall *et al.*, 2010).

Dietary Assessment:

Calcium intake was estimated for each participant using an abbreviated Food Frequency Questionnaire (FFQ) known as the Calcium/Vitamin D Block Screener (NutritionQuest). The screener was used to estimate calcium intake in milligrams. The Block screeners have been validated (Cummings *et al.*, 1987) and are well established, resulting in their use at hundreds of research centers and universities. To estimate dietary vitamin D intake, a more detailed, vitamin D-specific FFQ was used, which was previously validated by Dr. Hall (Hall *et al.*, 2010) (see appendix). This questionnaire inquired about typical, cumulative intake of vitamin D by listing the food or beverage consumed which contains vitamin D, the participant's frequency during the past week (seven days), the past one month (28 days), and the typical serving size consumed each time. Items included vitamin D supplements and multivitamins, all dairy products, eggs, fish and seafood, mushrooms, liver, and vitamin D-fortified products including juice, cereals, and breads. Food models were used to help participants identify typical serving sizes consumed. Cumulative totals for weekly and monthly intakes allowed for the calculation of the average international units (IU) per day. Additionally, participants were asked to categorize the frequency of their fish

intake on a monthly or weekly basis. The amount of vitamin D in the foods/beverages were determined using values set forth by the United States Department of Agriculture Database or from food labels.

Sun Exposure Assessment:

A seasonal Sun Exposure Recall questionnaire was completed by each subject (see appendix). This questionnaire examined seasonal exposure by inquiring about the average time spent outdoors during weekdays and weekends as either less than 30 minutes, 30 to 60 minutes, 60 to 90 minutes, or greater than 90 minutes during the Fall, Winter, Spring and Summer months. Additionally, the questionnaire inquired about sunscreen usage and typical attire worn for each season (for both weekdays and weekends) by asking participants to record the parts of their body exposed to sunlight when outdoors, including: face, neck, shoulders, back and chest, upper arms, lower arms, hands, upper thighs, lower legs, and feet (**Figure 1**). This tool was previously validated by Hall *et al.* (2008).

Figure 1: Sun Exposure Clothing Key

The diagram shown was used by participants to document typical attire worn (Hall *et al.*, 2010).

Body surface area (BSA) exposed to the sun was calculated using the participants' height and weight (for total BSA) and then adjusted for clothing worn, which was determined using the "rule of nines" as a basis for calculation, as reflected in **Table 6** (Hall *et al.*, 2010). Average sun exposure for weekday and weekend seasons were then calculated by multiplying the average time out in the sun within the category (minutes/day) by the body surface area (BSA) exposed to determine a sun exposure index (SEI) value for each season (Barger-Lux and Heaney, 2002; Hall *et al.*, 2010).

Table 6: Body Region and Clothing Type with Corresponding BSA Exposed

Body region ²	Clothing type	% BSA exposed
Head	Nothing	4
	Beanie/bike helmet	3
	Baseball cap	2
	Cowboy hat	1
Torso/arms	Nothing	47
	Bikini top/sports bra	42
	Tank top	18
	Tee shirt	10
	Quarter length shirt	3
	Long sleeves	0
Legs	Bikini bottom	38
	Short shorts/ Skirt	24
	Knee-length shorts/skirt	8
	Full-length pants	0
Feet	Nothing	2
	Sandals	1.5
	Shoes	0

¹ From (15).

² Hands contributed 4% of exposed BSA (0% if gloves were worn) and the neck 2% (0% if a scarf was worn).

Physical Activity Assessment:

Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) (see appendix). This questionnaire captures total minutes of exercise per week, categorized as vigorous, moderate or “walking.” The total physical activity is then summarized using the IPAQ Short Form instrument to obtain total MET-minutes/week.

Statistical Methods

The statistical analyses were performed using SAS Version 9.3 (SAS Institute, Cary, NC). All continuous variables were tested for normality using the Kolmogorov-Smirnov test and variables which lacked a normal distribution were transformed using Box-Cox transformations. ANOVAs were used to compare the different variables of interest between the three BMI categories and the three ethnic groups. Pearson correlations were used to identify the strongest correlations between variables.

To predict serum 25(OH)D, multiple linear regression was used with variables capturing sun exposure, vitamin D intake and skin reflectance because they all influence vitamin D status. Measurements used for sun exposure included SEI values for each season's weekday and weekend averages along with an overall average and overall weekday and weekend averages across all seasons. The dietary measurements for vitamin D intake included average vitamin D as expressed from a seven-day and 28-day FFQ (IU/day) and an online nutrition block screener. All measurements were examined in the model, including multiple combinations of variables for sun exposure and vitamin D intake along with forehead skin reflectance. Only forehead skin reflectance was used because the arm and hand skin reflectance were not significant predictors in previous studies (cite). Additionally, multiple covariates were considered including ethnicity, BMI, and age.

The Best Subset Model was employed to help identify the most significant independent variables as well as the strongest models. That is, after all data were examined in the model, final predictor variables and models used were chosen for their overall significance ($p\text{-value} < 0.05$) and the strength of their R-squared value, thereby signifying independent variables and models which serve as the best predictors of serum 25(OH)D status.

RESULTS

Population Characteristics

The study population was comprised of 34 participants with a mean age and standard deviation of 39 ± 6 years, a mean body mass index (BMI) of 27.9 ± 6 kg/m², and a mean serum 25(OH)D of 64 ± 18 nmol/L (sufficiency: >50 nmol/L) (**Table 7**). The prevalence of obesity (BMI ≥ 30 kg/m²) and vitamin D insufficiency (<50 nmol/L) in this population was 35% and 32%, respectively. Mean dietary vitamin D intake was approximately 327 ± 229 IU/day (RDA 600 IU/day), and mean dietary calcium intake was approximately 552 ± 275 mg/day (RDA 1000 mg/day) (**Table 7**).

Sixty-eight percent of this population was Caucasian, 26% were Hispanic, and 6% were Indian, with an average forehead skin reflectance of 62.6 ± 4.2 L*, a mean total body bone density of 1.2 ± 0.1 g/cm² and a mean body fat percentage of $37 \pm 8\%$ (**Table 7**).

Table 7: Population Characteristics

Variable	Mean	Normal Ranges
Serum 25(OH)D (nmol/L) ¹	64 ± 18 (26 ± 7 ng/ml)	>50 ² (>20 ng/ml)
Fasting Insulin (uIU/ml) ¹	8 ± 7	5-15 ⁴
Glycosylated Hemoglobin (%) ¹	5 ± 0.2	4.8-5.9
1-84 Parathyroid Hormone (pg/mL) ¹	25 ± 10	10-55 ⁴
Fasting Blood Glucose (mg/dl) ¹	90 ± 7	70-105
Vitamin D: Monthly FFQ (IU/day)	327 ± 230	RDA 600 ³
Calcium: Block Screener (mg/day)	552 ± 275	RDA 1000 ³
Skin Reflectance of Forehead (L*)	63 ± 4	N/A
Skin Reflectance of Arm (L*)	68 ± 4	N/A
Skin Reflectance of Hand (L*)	62 ± 5	N/A
BMI (kg/m ²)	28 ± 6	18.5-24.9 ⁴
Bone Density (g/cm ²)	1 ± 0.1	N/A
Fat (%)	37 ± 8	25 – 31 ⁵
Systolic BP (mmHg)	115 ± 11	<120
Diastolic BP (mmHg)	76 ± 8	<80
Age (years)	39 ± 6	N/A
Physical Activity (total MET-min/week)	2242 ± 1645	495 ⁵

¹ For serum measures, n= 33; all other measures n= 34

² Serum 25(OH)D level considered to mark deficiency by RDA guidelines

³ RDA guidelines for women aged 9 to 50 years old

⁴ Normal ranges as determined by National Institute of Health

⁵ Acceptable range by American Council on Exercise

Vitamin D Intake

The majority (82%) of participants reported an average dietary vitamin D intake of 327 ± 230 IU/day, which was below the RDA of 600 IU/day. Additionally, serum 25(OH)D levels significantly correlated to dietary vitamin D intake ($r = 0.42$, $p = 0.0139$) (**Figure 2**).

Figure 2. Serum 25(OH)D and Dietary Vitamin D Intake

Comparison by Body Mass Index

Participants were divided into three groups, based on BMI, for further comparison:

Normal: BMI 18.5 – 24.9 kg/m²; Overweight: BMI 25.0 – 29.9 kg/m²; and Obese: BMI ≥ 30 kg/m² (**Table 8**). Fasting serum insulin was significantly higher in the obese group compared to the normal weight group (**Table 8**). There was a positive correlation between fasting insulin levels and BMI ($r = 0.83$, $p < 0.0001$) (**Figure 4**). Forehead skin reflectance was significantly higher (meaning, skin pigment was lighter) in the Normal and Overweight groups compared to the Obese group ($p = 0.0122$). Total body bone density was significantly lower in the Normal group compared to the Overweight and Obese groups ($p = 0.0204$). Systolic blood pressure was significantly higher in the Obese group than in the Normal and Overweight groups ($p = 0.0449$). Percent body fat was significantly higher in the Obese group compared to the normal weight group as expected (**Table 8**) and there was a positive correlation between body fat and BMI ($r = 0.89$, $p < 0.0001$).

No significant differences were found between the BMI groups and weekday or weekend sun exposure or serum 25(OH)D levels (**Table 8; Figure 3**). Although not significantly different, the obese group had the lowest average levels of serum 25(OH)D.

Table 8. Characteristics by BMI Group

Variable	Mean BMI “Normal” (n = 12)	Mean BMI “Overweight” (n = 10)	Mean BMI “Obese” (n = 12)	P value
Serum 25(OH)D (nmol/L) ¹	62 ± 20 ^a	72 ± 12 ^a	57 ± 20 ^a	0.1516
Fasting Insulin (uIU/ml) ¹	2 ± 2 ^a	7 ± 4 ^b	14 ± 6 ^c	<0.0001*
HgbA1c (%) ¹	5 ± 0.1 ^a	5 ± 0.2 ^a	5 ± 0.4 ^a	0.8067
1-84 Parathyroid Hormone (pg/mL) ¹	25 ± 8 ^a	21 ± 9 ^a	27 ± 12 ^a	0.4235
Fasting Blood Glucose (mg/dl) ¹	88 ± 7 ^a	91 ± 6 ^a	92 ± 7 ^a	0.3019
Physical Activity (total MET-min/week)	2517 ± 1551 ^a	2344 ± 2058 ^a	2909 ± 3907 ^a	0.8825
Dietary Vitamin D (IU/day)	247 ± 207 ^a	317 ± 160 ^a	416 ± 281 ^a	0.2007
Dietary Calcium (mg/day)	526 ± 275 ^a	636 ± 258 ^a	505 ± 297 ^a	0.5200
Forehead Skin Reflectance	64 ± 3 ^a	63 ± 2 ^a	60 ± 5 ^b	0.0122*
Age (years)	38 ± 7 ^a	40 ± 5 ^a	40 ± 5 ^a	0.6776
Systolic BP (mmHg)	111 ± 11 ^a	110 ± 12 ^a	121 ± 9 ^b	0.0449*
Diastolic BP (mmHg)	74 ± 9 ^a	73 ± 7 ^a	80 ± 8 ^a	0.0882
Bone Density (g/cm ²)	1.2 ± 0.08 ^a	1.3 ± 0.06 ^b	1.3 ± 0.2 ^b	0.0204*
Body Fat (%)	28 ± 5 ^a	38 ± 5 ^b	44 ± 3 ^c	<0.0001*

¹ n = 9 for blood values only in the Overweight category

* Significance denoted by p-value < 0.05

Note: Values sharing a letter (a, b, c) do not have significantly different means

Figure 3. Body Mass Index and Serum 25(OH)D

*BMI Category 1 = Normal weight; 2 = Overweight; 3 = Obese

Note: Red line shows RDA cut-off for vitamin D sufficiency at 50 nmol/L.

Figure 4. Body Mass Index and Fasting Insulin

*BMI Category 1 = Normal weight; 2 = Overweight; 3 = Obese

Comparison by Ethnicity

Next, participants were subdivided into groups based on ethnicity for further comparison: Caucasian; Hispanic; and Indian (**Table 9**). Skin reflectance was significantly lighter in the Caucasian and Hispanic groups compared to the Indian group ($p = 0.0006$). Fasting blood glucose was significantly lower in the Hispanic group compared to the Indian group ($p = 0.0426$); and glycosylated hemoglobin was significantly greater in the Indian group compared to the Caucasian and Hispanic groups ($p = 0.0163$). No significant differences were noted between groups for serum 25(OH)D (**Figure 5**), although levels were markedly lower in the Indian group. Additionally, there were no significant differences between groups for dietary vitamin D intake, sun exposure, blood pressure, BMI, body fat percentage, or any other serum measurements (data not shown).

Table 9. Characteristics by Ethnicity Group

Variable	Caucasian <i>n</i>=23	Hispanic <i>n</i>=9	Indian <i>n</i>=2	P value
Serum 25(OH)D (nmol/L)	65 ± 18 ^a	62 ± 19 ^a	47 ± 18 ^a	0.3921
Forehead Skin Reflectance (L*)	64 ± 3 ^a	61 ± 3 ^a	54 ± 9 ^b	0.0006*
Fasting Blood Glucose (mg/dl)	96 ± 5 ^{ab}	87 ± 8 ^a	98 ± 1 ^b	0.0426*
HgbA1c (%)	5.4 ± 0.2 ^a	5.3 ± 0.3 ^a	5.8 ± 0.1 ^b	0.0163*

*Significance denoted by p -value < 0.05.

Note: Values sharing a letter (a, b, c) do not have significantly different means

Figure 5. Serum 25(OH)D and Ethnicity

*Ethnicity Group 1 = Caucasian; 2 = Hispanic; 3 = Indian

Note: Red line shows RDA cut-off for vitamin D sufficiency at 50 nmol/L.

Seasonal Sun Exposure

For average weekday sun exposure in the fall, nearly half (47%) of the participants reported that they spend 30 to 60 minutes/day outside, whereas half (50%) of participants decreased their estimated weekday exposure to less than 30 minutes/day during the winter season (**Table 10**). Similarly, 35% of participants spent 30 to 60 minutes/day outdoors during weekdays in the spring season, which was less than the number of participants (41%) who spent 60 to 90 minutes/day outdoors during the summer season. Furthermore, the number of participants who spent more than 90 minutes/day outdoors during weekdays in the summer more than doubled in the summer season from previous seasons (from 6% to 15%).

For weekend exposure, most participants (35%) reported that they spent greater than 90 minutes/day outdoors during the fall season, which was only slightly less than the winter season:

32% estimated that they spent greater than 90 minutes/day and 32% estimated that they spent only 30 to 60 minutes/day outdoors during weekends (**Table 10**). This exposure dramatically increased during the warmer months when the majority of participants reported greater than 90 minutes/day during weekends in the spring (50%) and summer (62%) seasons.

Table 10: Reported Sun Exposure From Study Questionnaire (n = 34)

	Season	< 30 min	30 – 60 min	60 – 90 min	> 90 min
Weekday	Fall	38%	47%	9%	6%
	Winter	50%	35%	9%	6%
	Spring	27%	35%	32%	6%
	Summer	12%	32%	41%	15%
Weekend	Fall	12%	24%	29%	35%
	Winter	18%	32%	18%	32%
	Spring	6%	24%	29%	50%
	Summer	6%	3%	29%	62%

Sun Exposure Index (SEI)

Participants' average SEI (BSA x minutes) was not significantly correlated to vitamin D status on the fall weekdays, winter weekends nor weekdays, spring weekdays, summer weekends nor weekdays, and average weekday. Significant correlations were found, however, for serum 25(OH)D and fall weekends ($r = 0.47$, $p = 0.0059$) (**Figure 6**), spring weekends ($r = 0.43$, $p = 0.0135$) (**Figure 7**), average weekend sun exposure ($r = 0.43$, $p = 0.013$) (**Figure 8**), and average overall sun exposure ($r = 0.39$, $p = 0.0247$) (**Figure 9**).

Table 11: Sun Exposure and Serum 25(OH)D Levels by Season

Variable	Mean SEI ¹	Mean 25(OH)D (nmol/L)
Fall Weekend SEI ²	28 ± 21	56 ± 20 ^a
Fall Weekday SEI	16 ± 17	
Fall Average SEI	22 ± 18	
Winter Weekend SEI ³	15 ± 12	68 ± 19 ^a
Winter Weekday SEI	9 ± 7	
Winter Average SEI	12 ± 9	
Spring Weekend SEI ⁴	44 ± 30	58 ± 16 ^a
Spring Weekday SEI	27 ± 23	
Spring Average SEI	36 ± 25	
Summer Weekend SEI ⁵	62 ± 29	65 ± 17 ^a
Summer Weekday SEI	45 ± 25	
Summer Average SEI	53 ± 26	
Weekday Average SEI	25 ± 15	
Weekend Average SEI	38 ± 19	
Overall Average SEI	31 ± 16	

¹ SEI: Sun Exposure Index

² Fall season: n = 6

³ Winter season: n = 15

⁴ Spring season: n = 5

⁵ Summer season: n = 7

Note: Values sharing a letter (a, b, c) do not have significantly different means

Figure 6. Serum 25(OH)D and Fall Weekend Sun Exposure

Figure 7. Serum 25(OH)D and Spring Weekend Sun Exposure

Figure 8. Serum 25(OH)D and Average Weekend Sun Exposure

Figure 9. Serum 25(OH)D and Average Sun Exposure

Models Predicting Serum 25(OH)D Status

Individually, average dietary vitamin D intake explained 18% of the variation in serum 25(OH)D ($p = 0.0139$). When examining sun exposure alone, the average weekday SEI explained 9% of the variation in serum 25(OH)D ($p = 0.0885$) while average weekend SEI explained 18% ($p = 0.0130$) and the overall SEI explained 15% ($p = 0.0247$). The strongest predictor of vitamin D status was the average weekend SEI in the fall season, which explained 22% of the variation in serum 25(OH)D ($p = 0.0059$) (data not shown).

These different measures of sun exposure were compared in models along with dietary vitamin D intake and forehead skin reflectance to predict serum 25(OH)D status (**Table 12**). When weekday SEI was used in the full model, 29% of the variation in serum 25(OH)D was explained. When weekend SEI was used in the full model, 31% of the variation in serum 25(OH)D was explained, which was the same percentage when an overall average SEI was used

in the model (**Table 12**). And lastly, when the fall weekend SEI was used, 32% of the variation in serum 25(OH)D was explained.

Table 12: Models Comparing Different Measures of Sun Exposure, Along with Dietary Vitamin D Intake and Skin Reflectance to Predict 25(OH)D Status

Sun Exposure Models	p-value	R ²
Model 1	0.0174*	0.29
SEI (Weekday Average) ¹	0.0711	
Vit D Intake (IU/day) ²	0.0131*	
Skin Reflectance (forehead) ³	0.1371	
Model 2	0.0122*	0.31
SEI (Weekend Average)	0.0456*	
Vit D Intake (IU/day)	0.0550	
Skin Reflectance (forehead)	0.1798	
Model 3	0.0116*	0.31
SEI (Overall Average)	0.0428*	
Vit D Intake (IU/day)	0.0309*	
Skin Reflectance (forehead)	0.1404	
Model 4	0.0107*	0.32
SEI (Fall Weekend)	0.0386*	
Vit D Intake (IU/day)	0.0951	
Skin Reflectance (forehead)	0.2165	

* Significant denoted by p-value < 0.05

¹ SEI = Sun exposure index (minutes in direct sun x body surface area exposed)

² Mean dietary vitamin D intake including supplements using 28-day FFQ

³ Mean skin reflectance/pigmentation of forehead in L* (lightness)

Using the strongest individual variables (**Model 4, Table 12**), the covariates of age, BMI, and ethnicity were incorporated into the full model and explained 43% of the variation in serum 25(OH)D (**Table 13**). Furthermore, when the interaction variable (Age x BMI) was added, 58% of variation in serum 25(OH)D was explained, and the variables age and BMI became significant in the model (**Table 13**).

Table 13: Models Predicting 25(OH)D Status with Covariates

Sun Exposure Models	p-value	R ²
Model 1	0.0318*	0.43
SEI (Fall Weekend) ¹	0.0426*	
Vit D Intake (IU/day) ²	0.0542	
Skin Reflectance (forehead) ³	0.4913	
Age (years)	0.1789	
Body Mass Index (kg/m ²)	0.2394	
Ethnicity	0.3155	
Model 2	0.0031*	0.58
SEI (Fall Weekend) ¹	0.0219*	
Vit D Intake (IU/day) ²	0.0250*	
Skin Reflectance (forehead) ³	0.6055	
Age (years)	0.0035*	
BMI (kg/m ²)	0.0138*	
Ethnicity	0.3418	
Age x BMI (interaction) ⁴	0.0071*	

* Significant denoted by p-value < 0.05

¹ SEI = Sun exposure index (minutes in direct sun x body surface area exposed)

² Mean dietary vitamin D intake using 28-day FFQ

³ Mean skin reflectance/pigmentation of forehead in L* (lightness)

⁴ Interaction variable of [Age (years)] x [BMI (kg/m²)]

DISCUSSION

The present study found that 82% of participants did not meet the current RDA for dietary vitamin D intake. In fact, mean dietary vitamin D intake was estimated to be 327 ± 229 IU/day, nearly 300 IU/day below the RDA of 600 IU/day for this age group (1-70 years of age) (FNIC, 2011). Similarly, mean dietary calcium intake was approximately 552 ± 275 mg/day, nearly 450 mg/day below the RDA of 1000 mg/day for this age group (19-70 years of age) (FNIC, 2011). Despite the prevalence of self-reported inadequate dietary intake of vitamin D, only 32% of participants had insufficient serum 25(OH)D levels below 50 nmol/L. Findings in the present study were similar to the NHANES national survey data (2005-2006), which reported that approximately 26% of adults ($n = 3454$) were found to be deficient using the same cut-off value of 50 nmol/L (Sharief *et al.*, 2011). It is reasonable to assume that participants in this study obtained adequate vitamin D not from dietary sources but rather from sun exposure given the location of this study on the central coast of California (35 degrees North), where the weather is moderate year-round.

While it is known that low dietary intake and low sun exposure both contribute to vitamin D deficiency, other studies have shown that additional factors, such as older age due a decreased concentration of the skin's provitamin D₃ in the skin with greater age (MacLaughlin 1985), and greater BMI secondary to the sequestration of vitamin D in adipose tissue (Worstman *et al.*, 2000; Lee *et al.*, 2009), are also associated with lower serum 25(OH)D. This idea was thoroughly explored and confirmed by Lagunova *et al.* (2010), whose results suggested that despite matched dietary intake and sun exposure, the obese subgroup had significantly lower serum 25(OH)D levels. In the present study, however, there was not a significant difference between serum 25(OH)D levels and increasing age or BMI. Additionally, some research has shown that obese individuals have lower dietary vitamin D intake and sun exposure secondary to different dietary habits and sedentary lifestyles (Rajakumar *et al.*, 2008; Rajakumar *et al.*, 2005; Dewey &

Heuberger, 2011), and yet no significant differences were found between the BMI groups for dietary vitamin D intake and sun exposure in this study (**Table 8**). Interestingly, however, bone density was found to be significantly greater in the normal weight group than in the overweight and obese groups ($p = 0.0204$), which could hint of potential differences in behavior. Comparatively, this association could be consistent with the results of a study by Rajakumar *et al.* (2008), which found that bone metabolism, bone turnover, and bone mineral content were altered in the obese state.

Similar to increasing BMI, elevated body fat percentage may compromise the body's ability to utilize vitamin D due to the body's storage of 25(OH)D in the adipose tissue (Holick 2007). While no significant correlation was found between body fat percentage and serum 25(OH)D levels, there was a significant increase in body fat percentage from the Normal group to Obese group (**Table 8**) ($p < 0.0001$) and 25(OH)D was lower in the obese group, although not significant. Thus, with a larger study population, body fat percentage would likely be inversely associated with serum 25(OH)D.

In addition to its link with suboptimal serum 25(OH)D levels, obesity has been shown to be associated with insulin resistance and hyperinsulinemia, which have been implicated in the rising incidence of cardiovascular disease, metabolic syndrome, and type II diabetes mellitus (Osei, 2010). This study showed that fasting serum insulin levels significantly increased from the Normal to Obese groups (**Table 8**), thereby reflecting a positive correlation between fasting insulin levels and BMI ($r = 0.83$, $p < 0.0001$) (**Figure 4**). Although fasting insulin was not significantly correlated to serum 25(OH)D, there was a negative trend as expected.

Another significant difference found between the BMI groups in the present study was skin reflectance/pigmentation, which is associated with ethnicity. Specifically, forehead skin reflectance was found to be significantly greater (meaning, lighter skin pigment) in the Normal and Overweight groups than the Obese group ($p = 0.0122$). Since darker skin pigmentation

reduces vitamin D synthesis by the sun, certain ethnicities (such as those with darker pigmented skin) are at a greater risk for vitamin D deficiency. As the literature supports, two important factors in low serum 25(OH)D levels are in fact race and ethnicity (Osei, 2010). Consequently, there is higher prevalence of hypovitaminosis vitamin D in minority populations such as African-Americans and Hispanics, who are also at greater risk of insulin resistance, type II DM, obesity and cardiovascular diseases compared to Caucasians. While no significant differences were noted between ethnic groups for serum 25(OH)D levels, the mean Indian group's status was markedly lower (**Table 9**). Additionally, fasting blood glucose was found to be significantly lower in the Hispanic group than the Indian group ($p = 0.0426$), and glycosylated hemoglobin was significantly lower in both the Caucasian and Hispanic groups than the Indian group ($p = 0.0163$).

When sun exposure was examined, participants reported that their greatest level of sun exposure occurred during the weekends of summer, when 62% estimated that they receive >90 minutes/day outdoors in direct sunlight (**Table 10**). On the contrary, participants' average SEI (BSA x minutes) had the strongest and most significant correlation to vitamin D status on fall weekends ($r = 0.47$, $p = 0.0059$) over any other season. Due to the higher heat intensity during the summer, participants may be more apt to seek shade in summer months than during the fall season when temperatures are more moderate. Interestingly, while not statistically significant, mean serum 25(OH)D levels appear to be the greatest in the winter season (68 ± 19 nmol/L) over any other season. The elevated serum levels seen during winter may likely be tied to a delayed effect of increased sun exposure during the fall.

As our data supports, numerous studies suggest that sun exposure remains one of the strongest predictors of serum 25(OH)D status (Hall et al., 2010; Jacobs et al., 2008; Calvo et al., 2005; Armas et al. 2007). Armas et al. (2007) demonstrated a dose-response relationship between UV-B exposure from an artificial source and vitamin D status depending on the level of skin pigmentation, as assessed by skin reflectance. While the researchers acknowledged that

dietary intake can be an important contributor to vitamin D status, it was less significant as a predictor of status than both sun exposure and skin pigmentation. In a study conducted by Hall et al. (2010), sun exposure adjusted for body surface area (BSA) exposed to the sun and skin reflectance were the principal predictors of vitamin D status in participating subjects. Ninety-three percent of participants (67 of 72) had a low mean serum 25(OH)D₂ concentration (<50 nmol/L) for the mean of three blood draws. When dietary vitamin D intake, UV-B dose and forehead skin reflectance were examined, 55% of the variance in serum 25(OH)D could be explained. Consistent with the results of numerous other studies, Hall et al. (2010) found that sun exposure (adjusted for ambient levels and clothing) had the greatest impact on serum 25(OH)D levels, followed by skin reflectance and lastly, dietary intake.

Similar to the findings of Armas et al. (2007) and Hall et al. (2010), the strongest individual predictors of vitamin D status in the present study were fall weekend SEI, which explained 22% of the variation in serum 25(OH)D ($p = 0.0059$), followed by average dietary vitamin D intake, which explained 18% of the variation in serum 25(OH)D ($p = 0.0139$). When these two variables were collectively examined with forehead skin reflectance to predict serum 25(OH)D, 32% of the variance was explained ($p = 0.0107$). When the covariates age, BMI, ethnicity, and the interaction variable (Age x BMI) were incorporated into the model, 58% of variation in serum 25(OH)D levels were explained, and the variables age and BMI became significant (0.0031) (**Table 13**). This final and most noteworthy model had a R-squared value that was nearly identical (yet slightly greater) to that which was achieved in the study by Hall et al. (2010), thereby confirming that sun exposure, followed by dietary intake, skin reflectance, age and BMI are all significant predictors of serum 25(OH)D.

Although sun exposure is an important contributor to serum 25(OH)D status, it is very difficult to quantify. McCarthy (2008) found that individual-level sun exposure data was more accurate than environmental measurements of sun exposure because participants' sunscreen use and sun behavior practices were more important determinants of status (McCarthy, 2008).

Collecting data on the individual factors contributing to sun exposure is extremely challenging, along with environmental influences, and the most effective methods have not yet been determined. However, Hall et al. (2010) found that daily sun exposure logs assessing time outside and clothing worn accurately captured sun exposure as compared to the objective measure of sun exposure, polysulphone (PS) dosimeter badges, used in their study. Hall et al. (2008) also validated sun exposure recall questionnaires against the daily sun logs and found that they accurately captured individual-level sun exposure thereby demonstrating the usefulness of simple tools. The present study employed this previously validated tool (Hall et al., 2008).

The strengths of the present study include the large number of assessment tools employed. Data were collected on dietary intake, sun exposure, skin reflectance, serum levels of multiple assays, blood pressure, anthropometrics, and body composition/bone density using DXA analysis. Participants were recruited continually over all four seasons of the year. This rolling recruitment allowed for a more comprehensive evaluation of seasonal sun exposure and corresponding serum 25(OH)D levels to capture fluctuations in sun intensity or time spent outdoors.

While the amount of information gathered from each participant was maximized, the overall power of the study was low due to the small sample size. In fact, it is likely that many more correlations would be significant with a larger study population. Other limitations include the fairly homogeneous group of participants. To ensure that future results are not impacted by an insufficient number of participants or the inability to test for interpersonal variability, future studies should include a larger, more heterogeneous sample. Additionally, it is important to consider the inherent limitations accompanied by memory-based questionnaires, including the recall bias often associated with the FFQ used to assess vitamin D intake and sun exposure recall.

Ideally, this study's data will strengthen the understanding of how to better predict and employ the most effective tools to improve vitamin D status. For instance, a simple questionnaire which addresses weekend sun exposure could be used. Although vitamin D is uniquely

synthesized from exposure to sunlight, there are no current public health recommendations or guidelines for sun exposure. In the quest to reduce the prevalence of worldwide deficiency, adequate sun exposure seems to surface as the most economical and reasonable of solutions, however the risk of skin cancer prevails in this journey to pinpoint appropriate prevention methods and therapeutic tools when confronting vitamin D deficiency. Thus, a greater argument can be made for tailoring dietary recommendations to be based upon individual needs versus grouped by age and gender, and that are ideally customized for those individuals who are at greater risk of deficiency.

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APPENDIX A: Screening Questionnaire

Name: _____ Where did you hear about the study? _____ Date of birth: _____ Age: _____ Gender: _____
 Street Address: _____ Apt # _____ City: _____ State: _____ Zip: _____
 Phone: _____ Email: _____ Height: _____ Weight: _____ BMI: (kg/m²) _____ Ethnicity: _____

QUESTION	YES	No	DECLINE	COMMENT
1. Are you willing to take either a vitamin D supplement or a placebo (ie. sugar pill) daily for 6 months?				Must be YES to participate.
2. Are you willing to have your ht and wt measured twice?				Must be YES to participate.
3. Are you willing to have your bone mineral density and body fat (ie. DEXA scan) measured twice in Santa Maria or San Luis Obispo?				Must be YES to participate.
4. Are you willing to have your skin pigmentation measured twice?				Must be YES to participate.
5. Are you willing to fill out exercise and diet recall questionnaires twice? Are you willing to keep a sun exposure log throughout the 6 months?				Must be YES to participate.
6. Are you willing to have your blood drawn three times to determine serum vitamin D, lipids, insulin, glucose and markers of inflammation? Have you ever had problems getting your blood drawn?				Must be YES to participate. Record answer.
7. Are you willing to take an oral glucose tolerance test three times (each time takes 2 hours)?				Must be YES to participate.
8. Are you willing to give a urine sample 5 times?				Must be YES to participate.
9. Have you ever used a tanning bed? If yes, how often? Have you used one in the past 2 months? Do you plan on using one in the next 6 months? Do you use bronzer (or Spray tans)? Are you willing not to use them?				Record answer. Must be NO for the past two months and during the study to participate. Must be NO to using bronzer during study.
10. Do you wear sunscreen every day? Where on your body?				Record answer. Must be NO to everywhere/everyday (Face is ok)
11. Do you have any short-term or long-term health problems? (ie. Diabetes (DM)) Do you have fat malabsorption problems or kidney or liver problems? Have you started menopause?				Please list: Must be NO to DM , malabsorption problems, menopause, kidney, and/or liver disease to participate.
12. Do you take any prescription medications? For how long?				Please list:
13. Do you regularly take over the counter medications (allergy, antihistamine, antacids, pain relievers, laxatives?) If so, how often?				Record answer. Must be NO to laxatives to participate.
14. Do you smoke or use any tobacco products? If no, have you quit smoking in the past year?				Must be NO to participate. Must have quit more than 1 yr ago.
15. Do you currently take any supplements that contain vitamin D? Multivitamin/mineral supplement? Calcium and vitamin D supplement? Vitamin D supplement? Amount? Cod liver oil or cod liver oil capsules? Amount?				Record answer. Must be NO to high dose vitamin D supplements (>1,000 IU/day) to participate.
16. Are you pregnant? Do you plan on becoming pregnant in the next 6 months?				Must be NO to participate. You must let us know if you become pregnant and if you do, we will ask that you not continue in the study.
17. Medical questions: When was your last medical exam? Do you see your doctor regularly? Do you feel comfortable talking to your doctor about symptoms you experience? Would you report to your doctor any unusual symptoms you may have related to the study?				

Completed by: _____ Date: _____ Blood draw appt set up? _____ DXA appt? _____
 _____ Qualified _____ Disqualified (DQ) Reason for DQ: _____

APPENDIX B:

Vitamin D Study Form

Study ID #: _____

Date: _____

☐ Skin Pigmentation Measurement:

Inner Arm	SCI (L*):	SCI (L*):	SCI (L*):
Hand	SCI (L*):	SCI (L*):	SCI (L*):
Forehead	SCI (L*):	SCI (L*):	SCI (L*):

☐ Ht: _____

☐ Wt: _____

☐ List Medical/Medication Changes: _____

Completed:

☐ DEXA

☐ Calcium/Vit D Screener

☐ Vit D-Specific FFQ

☐ Physical Activity Screener

☐ Sun Exposure Questionnaire

☐ Fasting Blood Draw/OGTT/Urine Sample Collected (Date: _____)

APPENDIX C:

Vitamin D Food Frequency Questionnaire

Study ID #: _____

Date: _____

Food / Beverage	Frequency/ # of servings in the past week (past 7 days)	Frequency/ # of servings in the past 4 weeks (past 28 days)	Typical serving size(s) each time	Comments
Supplements/multivitamins (Some calcium supps contain vit D)				Type: Brand: Amt. of vit D:
Milk				Type: Don't forget about milk in sauces/casseroles.
Milk beverages (latte, mocha, cappuccino, etc.)				
Soy milk				Brand:
Chocolate milk				
Homemade Pudding/Flan				Which one:
Ice cream				Brand/type:
Whipped cream /Coolwhip				Which one:
Yogurt				Brand:
Cheese (Consider cheese alone & in mixed dishes, such as enchiladas, pizza, casseroles, pasta, etc.)				Note type(s) of cheese:
Butter				
Margarine				
Eggs				Excluding egg whites. Don't forget about omelets, soufflés, frittatas and quiche.
Fish				Don't forget about sushi/sashimi pieces:
Salmon				
Mackerel				
Tuna				
Sardines				
Catfish				
Cod liver oil (NOT including omega 3 supplements)				Note any fish oil sup.
Other				
Mushrooms				Brand:
Liver				
Ready-to-eat cereals				Type/brand:
Ensure or slim fast				Which beverage:
Vit D fortified OJ				
Other vit D fortified food/beverage				

APPENDIX D:

Physical Activity Questionnaire

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

No vigorous physical activities: ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

No moderate physical activities: ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

No walking: ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day** Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day** Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

APPENDIX E:

Sun Exposure Questionnaire

ID#: _____

Date: _____

In your opinion, is your typical sun exposure “low” or “high” ?

☐ Low ☐ High

How would you characterize your skin type with regard to risk of sunburn?

- ☐ Always burn, never tan
☐ Burn slightly, then tan slightly
☐ Rarely burn, then tan moderately
☐ Never burn, tan darkly

During the Fall (Sept, Oct, Nov):

How much time do you typically spend outdoors *per day* on **weekdays**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

How much time do you typically spend outdoors *per day* on **weekends**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

Using the clothing key, what do you typically wear? (use numbers)

A ☐ B ☐ C ☐ D ☐ E ☐

Using the clothing key, where do you typically put sunscreen? (check box(es))

A ☐ B ☐ C ☐ D ☐ E ☐

SPF: _____

During the Winter (Dec, Jan, Feb):

How much time do you typically spend outdoors *per day* on **weekdays**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

How much time do you typically spend outdoors *per day* on **weekends**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

Using the clothing key, what do you typically wear? (use numbers)

A ☐ B ☐ C ☐ D ☐ E ☐

Using the clothing key, where do you typically put sunscreen? (check box(es))

A ☐ B ☐ C ☐ D ☐ E ☐

SPF: _____

During the Spring (March, April, May):

How much time do you typically spend outdoors *per day* on **weekdays**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

How much time do you typically spend outdoors *per day* on **weekends**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

Using the clothing key, what do you typically wear? (use numbers)

A ☐ B ☐ C ☐ D ☐ E ☐

Using the clothing key, where do you typically put sunscreen? (check box(es))

A ☐ B ☐ C ☐ D ☐ E ☐

SPF: _____

During the Summer (June, July, Aug):

How much time do you typically spend outdoors *per day* on **weekdays**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

How much time do you typically spend outdoors *per day* on **weekends**?

☐ <30 min ☐ 30-60 min ☐ 60-90 min ☐ >90 min

Using the clothing key, what do you typically wear? (use numbers)

A ☐ B ☐ C ☐ D ☐ E ☐

Using the clothing key, where do you typically put sunscreen? (check box(es))

A ☐ B ☐ C ☐ D ☐ E ☐

SPF: _____

KEY FOR SUN EXPOSURE LOG:

→ Pick a number from each area A-D that best represents what you're wearing (or where you've put on sunscreen for the exposed skin):

<p>A</p> <p>1 Nothing</p>	<p>2</p> <p>2 Beanie over forehead/ Backwards baseball cap/ Bandana/ Swim cap/ Helmet/ Hood</p>	<p>3</p> <p>3 Baseball cap/ Helmet with brim</p>	<p>4</p> <p>4 Large brimmed hat/ Cowboy hat</p>	<p>B</p> <p>1 Shirtless</p>	<p>2</p> <p>2 Bikini top/ Sportsbra</p>	<p>3</p> <p>3 Tank top/ Sleeveless top</p>	<p>4</p> <p>4 Tee shirt</p>	<p>5</p> <p>5 Quarter-length sleeved shirt</p>	<p>6</p> <p>6 Long-sleeved shirt/ Jacket/ Sweater</p>
<p>C</p> <p>1 Bikini bottom/ "Speedos"</p>	<p>2</p> <p>2 Shorts/ Short skirt</p>	<p>3</p> <p>3 Shorts or skirt near the knees/ Capris</p>	<p>4</p> <p>4 Pants/ Long skirt</p>	<p>D</p> <p>1 Bare feet</p>	<p>2</p> <p>2 Sandals</p>	<p>3</p> <p>3 Closed-toe shoes</p>	<p>E</p> <p>1 Gloves</p>	<p>2</p> <p>2 Scarf</p>	

APPENDIX F:

Sun Exposure Clothing Key