# Nutrition, Vitamin D and Refractive Error

Thesis

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By

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Abstract

**Purpose:** Several factors have been found to be candidate risk factors in myopic development and progression. Outdoor activity, in particular, has been found to be protective in the development of myopia. A possible component of outdoor activity could be vitamin D. We are investigating the effects of activity as well as dietary and circulating levels of vitamin D on myopes and non-myopes.

**Methods:** Thirty-two subjects provided information regarding diet and activity by means of surveys. A smaller number of subjects that met refractive error criteria (n=22) provided 200 μl of blood to analyze circulating vitamin D as well as a 2 ml sample of saliva for SNP analysis of the vitamin D receptor gene.

**Results**: Activity, both indoors and outdoors, were not significantly different for myopes versus non-myopes. Unadjusted levels of circulating vitamin D were not significant as well. Linear regression adjusted for four dietary variables (calcium, food folate, theobromine, and total sugar) and age showed myopes had 3.41 ng/ml less circulating blood vitamin D than non-myopes (p=0.005, R-squared=0.76). Odds ratios from SNP analysis of *VDR* gene were not significant in increasing the risk of being myopic

**Conclusions:** Outdoor and indoor activities were not significantly associated with circulating levels of vitamin D. Calcium and theobromine were positively associated while food folate and total sugar were negatively associated with blood vitamin D levels. Blood vitamin D levels were lower in myopes once adjusted for age, and dietary variables. Other intrinsic factors including single nucleotide polymorphisms in the vitamin D receptors were not significant for increasing the risk of being myopic.

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## Chapter 1: Literature Review

Myopia, or nearsightedness is one of several refractive conditions that cause optical defocus or blur. Research in refractive error has investigated the contributions of the many ocular components including corneal curvature, lens power, anterior chamber depth and axial length (Goss and Jackson, 1995; Grosvenor and Scott, 1991). Particularly, studies showed a difference in axial length, vitreal chamber depth and corneal curvature when comparing myopic subjects to emmetropic. Myopes generally have longer axial length, deeper vitreal chambers and steeper corneal curvature (Goss et al., 1995; Grosvenor et al., 1991). Emmetropia is when the eye focuses the light from distant objects on the retina. In contrast, myopia is an optical anomaly that causes parallel light to be refracted and focused in front of the retina rather than on the light-sensitive retinal tissue, creating symptoms of blur for distant objects. Other refractive conditions include hyperopia (light focused behind retina), astigmatism (light blurred due to meridional differences in corneal or lenticular curvatures) and presbyopia (inability to focus at near due to lenticular sclerosis and ciliary changes).

Although no treatment or medicine has been developed to alleviate the progression of myopia, there are other options to eliminate the optical blur

induced by nearsightedness. Corrective lenses, such as spectacles and contact lenses can change the vergence of light rays to focus distant objects onto the retina. The development of refractive surgeries makes this option a supplemental alternative for the correction of nearsightedness, farsightedness, and astigmatism to eliminate the necessity of spectacles or contact lenses.

The research regarding refractive conditions has been extensive. Myopia is responsible for a huge financial burden placed on the public. Vitale uses National Health and Nutrition Examination Survey (NHANES) data to show the direct cost of correcting distance visual impairment ranges from $3.9-7.2 billion per year (Vitale, Cotch, Sperduto and Ellwein, 2006).

The high cost of eye care has led to past and present research attempting to find cures or treatments to slow the progression of myopia. Saw (Saw, Shih-Yen, Koh and Tan, 2002b) reviewed the randomized clinical trials regarding interventions to slow myopia progression. Interventions discussed include the use of eye drops, such as anti-cholinergics (i.e.: tropicamide, cyclopentolate and atropine) and beta-adrenergic blockers (i.e.: timolol), in addition to the use of corrective lenses including different modalities of spectacle lenses (i.e.: single vision and multi-focal lenses) and contact lenses. Literature shows that everything except atropine use was statistically insignificant in slowing myopia progression. Although atropine had some effect on progression, the risks outweighed the benefits with atropine use due to the light sensitivity experienced (Saw, Gazzard, Au Eong and

Tan, 2002a). Saw's review shows that no conclusive data point an effective technique to retard the progression of myopia (Saw et al., 2002b).

The risk factors for myopia most frequently investigated have included near work (Bear, Richler and Burke, 1981; Jones, Sinnott, Mutti, Mitchell, Moeschberger and Zadnik, 2007; Saw, Shankar, Tan, Taylor, Tan, Stone and Wong, 2006; Young, Leary, Baldwin, West, Box, Harris and Johnson, 1969; Zylbermann, Landau and Berson, 1993), intelligence (Saw, Tan, Fung, Chia, Koh, Tan and Stone, 2004), socioeconomic status (Dirani, Chamberlain, Garoufalis, Chen, Guymer and Baird, 2006), outdoor activities and sports (Ashby, Ohlendorf and Schaeffel, 2009; Dirani, Tong, Gazzard, Zhang, Chia, Young, Rose, Mitchell and Saw, 2009; Norton, Siegwart and Amedo, 2006; Rose, Morgan, Ip, Kifley, Huynh, Smith and Mitchell, 2008a; Zhu, Winawer and Wallman, 2003) and of course genetics and heredity (Dirani et al., 2006; Hammond, Snieder, Gilbert and Spector, 2001; Lyhne, Sjolie, Kyvik and Green, 2001). Although the picture of myopic risk is still incomplete, the following sections discuss how each study has advanced the understanding of myopic risk factors regarding onset and progression.

Twin study reviews (Dirani et al., 2006; Hammond et al., 2001; Lyhne et al., 2001) discuss the history of twin studies researching myopia heritability. Twin studies are important in heredity research of myopia because monozygotic twins have identical genetic material whereas dizygotic twins, on average, share half of

the genetic material like other siblings because they are formed from two separate eggs. A stronger relationship for a trait between monozygotic twins than between dizygotic twins predicts a genetic component of the condition since environment is assumed to have the same degree of commonality between twin pairs (Dirani et al., 2006; Hammond et al., 2001; Lyhne et al., 2001). A correlation for a trait of more than 50% in monozygotic twins support that genetics play a major role in developing a condition (Hammond et al., 2001). Correlations between siblings that shows no significant differences between monozygotes and dizygotes support no genetic component to the trait.

Dirani et al. (Dirani et al., 2006) summarized the heritability of myopia from several twin studies. Although the data vary, the general consensus was that high myopia shows a high heritability. One of the strongest twin studies to date is the Hammond's Twin Eye Study (Hammond et al., 2001). This study is important in the literature because of its impressive study design. The large sample size reduces bias while allowing for data analysis involving multivariate statistics to evaluate dominant and additive effects as well as unique and common environmental influences on myopia.

British female twins (n=506) were divided by zygosity. Short tandem repeat fingerprinting confirmed the zygosity of 226 monozygotic twins and 280 dizygotic twins between the ages of 50 and 79 years. They were examined for spherical equivalent, total astigmatism and corneal astigmatism to find the

correlations between twins for each trait and therefore the hereditability. Multivariate analysis found heritability for the full range of spherical equivalent of 86% with 14% of variance attributed to environmental factors. Heritability for myopia and hyperopia were 90% and 89%, respectively (Hammond et al., 2001).

Another twin study (Lyhne et al., 2001) included 20 through 45 year-old same sex twins in order to look at the heritability of ocular refraction. This study involved 114 twin pairs from the Danish Twin Registry. It differed from Hammond et al. (Hammond et al., 2001) in that it looked at ocular refraction, lens thickness, corneal curvature, axial length, anterior chamber depth as well as length of education. Fifty-three monozygotic and 61 dizygotic twins showed heritability which was high for ocular refraction (0.91) and was found to be due to additive genetic effects, similar to findings from Hammond's Twin Eye Study (Hammond et al., 2001). Gene-environment interactions were examined in monozygotic twins to see if certain genotypes were expressed differently in certain environments than in others. A statistically significant qualitative interaction was found to support an environmental involvement in myopia development and progression. This qualitative interaction was inferred from a significant correlation between the differences between twins in refractive error as a function of the magnitude of the average refractive error of the twins. A potential gene-environment interaction may be the association of higher education achievement leading to higher levels of myopia (Lyhne et al., 2001). This hypothesis was not supported by the data,

however, as difference between twins in refractive error were not correlated with the differences in the level of education between twins.

If there were a gene-environmental interaction involved in myopia as described by Lyhne et al., there would be interesting clinical implications. Environmental precautionary measures could be taken by those at risk for developing myopia based on their genetic information (Lyhne et al., 2001). This supports the need to understand the underlying genetic information that leads to or puts people at risk for developing myopia. Mutti and Zadnik reviewed the literature involving the important genetic regions and mapping that have been studied in regards to refractive error. There are fourteen regions that have been given MYP designation by Human Genome Organization Gene Nomenclature Committee (http://www.hugo-international.org). Many MYP regions are responsible for high levels of myopia (MYP1, MYP2, MYP3, MYP4, MYP5, MYP11, MYP12, MYP13), some for low/moderate levels of myopia (MYP6, MYP14), and some to the full range of refractive error (MYP7, MYP8, MYP9, MYP10) (Mutti). The existence of this level of heterogeneity supports that myopia is a trait with multi-region inheritance. It could be due to a few genes found in a small sample of families or many genes that are more widely distributed that have weak influence resulting in variable signals.

Recent reports of increased prevalence cannot fully be explained by genetics and heredity (Lin, Shih, Hsiao and Chen, 2004; Saw, Katz, Schein, Chew and Chan,

1996). Numerous studies support how environmental exposures may be responsible for an increase in the prevalence of myopia (Ashby et al., 2009; Dirani et al., 2006; Dirani et al., 2009; Hammond et al., 2001; Lyhne et al., 2001; Rose et al., 2008a; Saw et al., 2006). An environmental perspective was taken to explain the increased prevalence of myopia in the classic Eskimo study (Young et al., 1969). Elder subjects in the population had little to no schooling. If the subjects were in school between the years of 1890 and 1940, the school was ungraded and had little to no near work involved. After 1940, the local population increased causing an increase in school enrollment. The educational system changed to a graded system in attempts to become more comparable to American school systems. Young found that when splitting data by this historical year, the amount of myopia (-0.25D or more myopic) was drastically different between the two respective groups. Of 197 Eskimo subjects, myopia was present in those 30 years and older 8.6% of the time and 58.6% in those 30 years and younger. Furthermore, when comparing myopia prevalence of Eskimos subjects to Americans and Europeans of similar ages the prevalence of myopia was higher for Eskimos 11-40 years of age and lower in those 41-77 years of age. Minor changes to families' diet and work routine were not thought to be significant in this shift of myopia prevalence (Young et al., 1969). This historic study showed that increased amounts of near work may in fact affect the prevalence of myopia. Many studies that followed have looked to see the impact of near work on other risk factors including familial environment.

A study from the 1980s (Bear et al., 1981) looked at 971 subjects from an isolated Newfoundland population to show the influences near work and education have on refraction. The parent-child and sibling-sibling relationship correlations were generally low; however, the regression correlations were even lower after adjusting for age, sex, education and near work versus only age and sex. These trends were more apparent in the offspring-parent pairings. The average reduction in resemblance was 35% when including near work and education into the adjustment. This finding suggests that environmental influences such as near work during education may have a larger influence on the refractive error in children than the genetic influence of their parents.

Further reports of increasing myopia with higher levels of near work are seen in Zylbermann et al. (Zylbermann et al., 1993). This study looked at 870 teenagers between the ages of 14-18 in a Jerusalem population with varying intensities of schooling techniques. The general school was co-ed and had similar teaching techniques to Western schools with six hour school days, 45 minute classes separated by 15 minute breaks, and homework typically not exceeding three hours per day. In contrast, Orthodox schools were separated by gender. The male population began three hour school days at four years of age that lengthened in time and near demand through the teenage years. By 14 years of age, these young boys attended school for 16 hours a day where they did extensive near work on material of varying fonts while rocking their torso back and forth. This motion required a constant change in accommodative demand while doing near work

activities. The girls, however, had six hour school days with about two to three hours of near work that included drawing and sewing-type tasks. The results showed that myopia prevalence was 81.3% for the Orthodox males and ranged from 27.4-36.2% in the other three categories. Orthodox females and non-Orthodox females and males were not statistically different. The non-Orthodox general school teenagers and the Orthodox females had a myopia prevalence similar to those previously reported, 28.4% for teenage boys and 35% for teenage boys (Angle and Wissmann, 1980). The prevalence in the Orthodox males far exceeded the prevalence and amount of the average population likely secondary to their extended periods of high demand for accommodation but also the constant change in accommodation from the rocking motion.

The increased prevalence reported secondary to near work and intense education environments lead to the question of how near work might cause myopia to develop or progress further. Animal studies have been used to test different environmental manipulations for their effect on refraction. Animals such as tree shrews, leghorn chicks and macaque monkeys have the ability to compensate for lenses worn for extended or brief periods of time and are used frequently in animal myopia studies (Ashby et al., 2009; Norton et al., 2006; Zhu et al., 2003). Minus lens wear simulates hyperopic defocus and near work since the lenses move the image behind the retina causing the eye to accommodate to focus the target. These minus lenses cause ocular elongation in animals. Plus lenses move the image in front of the eye and simulate myopic defocus. Zhu et al. (Zhu et al.,

2003) used leghorn chicks to determine if plus lenses could compensate for long periods of minus lens wear. Three experiments were conducted in the Zhu et al. study. The first looked at normal vision with interrupted periods of monocular lens wear. Chicks wore lenses for varying cycles each with a specific period of time. They found that repeated periods of +6D lens wear caused inhibition of the normal deepening of the vitreous chamber. This was seen more in the group that had six cycles of two minute lens wear over two cycles of thirty minute lens wear. In the second experiment, chicks wore negative lenses which were interrupted by brief periods of positive lens wear which significantly decreased the myopic compensation for the minus lens. The last experiment used binocular negative lenses and compared the effect of brief periods of plus lens wear in one eye over plano lens wear in the other. The positive lenses interfered with compensation to the minus lenses more than the plano lenses did. Therefore this study concluded that brief, repeated exposure to myopic defocus will outweigh the effects of sustained hyperopic defocus.

This study raises several clinical implications. The thought was that a child whose myopia was left under-corrected would have some residual myopic defocus and that this would slow the progression of myopia. Prescribing bifocals to school-aged children was also hypothesized to slow progression of myopia by minimizing defocus during accommodation or by providing myopic defocus. However, these hypotheses have generated controversial results from one study to another. In order to fully understand myopic defocus Zhu et al. argued that one

needs to be able to look at the amount of defocus at any given time during a child's near work activity to determine how much defocus is present and for how long (Zhu et al., 2003).

Additional animal studies have been used to look at the relationships between defocus and lens compensation. Norton et al. used tree shrews to look at different levels of defocus on the development of myopia. The study design involved tree shrews wearing -5D lens for 23 hours a day with 45 minutes of constant viewing distance with another lens. The -5D lens was classified as an inducing lens. The second lens interrupted the induction of myopia. The second lens worn during the 45 minutes provided defocus classified as minimal, myopic or hyperopic. Results showed that minus lenses caused axial elongation, however lenses that were less minus than the -5D lens showed less dramatic elongation. Plus lenses either diminished the myopiagenic effect of the -5D lens or they slowed the progression of elongation. Minimal defocus simulated by a plano lens gave the best results toward blocking the myopiagenic effects of the -5D lens. The study results suggest that children should take breaks from myopiagenic near activities in hopes that occasional exposures to clear distance vision will compete with the effects of near work. Progressive addition lenses to reduce defocus might also hold some promise for reducing progression of myopia. Similar to the protective effect of time outdoors reported by the Orinda Longitudinal Study of Myopia (Jones et al., 2007), this experiment suggests that distance clarity in the presence of near work may be a useful habit in slowing myopia progression.

Risk factors other than defocus have been studied. The Singapore Cohort Study of the Risk Factors for Myopia (SCORM) study in particular looked at several risk factors for developing myopia. This longitudinal cohort looked at 994 Chinese children in grades one through three who did not have -0.75D of myopia. Their parents were given a questionnaire that surveyed total monthly income, paternal and maternal educational levels and the diopter-hours children spent doing certain activities such as reading and spending time outdoors. Children were also administered a verbal intelligence questionnaire. Relative risks were found to be higher in seven year olds over the nine year olds, in females over males, in children that had any myopic parent over those without myopic parents, as well as children in the $2^{nd}$ and $3^{rd}$ tertiles for IQ over those in the $1^{st}$ (lowest) tertile. No association was found between the risk of myopia onset and the number of books read per week or total family income. There were also no interactions found between parental myopia and child IQ, reading and IQ, or age and parental myopia that influenced the risk of onset of myopia. Outdoor activity, numbers of hours reading per day, hours playing video games per day and night lighting were also not found to be risk factors for developing myopia. The strongest risk factors found in the study were parental myopia and children's IQ in the youngest Singaporean children. These risks were found across three definitions of the level of myopia needed for onset which support the validity of the associated risk factors (Saw et al., 2006).

The data from the Orinda Longitudinal Study of Myopia (OLSM) were used to look at activities that could predict myopia onset. OLSM collected longitudinal data from children in grades one through eight. This particular study used non-myopes in third grade who had complete data through up to eighth grade as well as an activity survey of several activities requiring different levels of accommodative demand (Jones et al., 2007). The study supported previous findings that stated that parental myopia was in fact a risk factor for developing myopia. Those with two myopic parents were twice as likely to develop myopia compared to those with one myopic parent and five times as likely to develop myopia when compared to those without any myopic parents. In the Orinda Study, the only activity or environmental variable that was significant for predicting onset of myopia was the amount of time spent playing sports and being outdoors. Higher levels of outdoor activity were in fact associated with lower risk of onset of myopia. This finding was contrary to SCORM which did not find a statistical relationship between time outdoors and the risk of onset (Saw et al., 2006). One hypothesis for the protective effect of time outdoors was that the distance clarity found to be a potent inhibitor of axial elongation in the tree shrew is also a significant stop signal inhibiting ocular growth in children.

Dirani et al. surveyed the 1249 children from the SCORM study between 11-20 years of age about outdoor activity, specifically looking at outdoor leisure versus sporting activities. Those with myopia (69.6%) spent significantly less total time outdoors total, as well as in outdoor leisure activities. Non-myopes spent more

time playing sports than myopes. Those spending more time outdoors were 0.90 times as likely to have myopia and those participating in more sports were 0.81 times as likely. While time in sports as well as outdoor leisure were significantly less in myopes, indoor sports were not. The odds ratio for sports indoors was not statistically significant at 0.75, 95% CI = 0.51-1.12, p = 0.16 (Dirani et al., 2009). Because this relationship was different from SCORM results for younger children (Saw et al., 2006), Dirani et al. speculated that this difference might be due to the use of a comprehensive outdoor activity questionnaire answered by the 11 to 20 year olds participating in the study as opposed to one question about outdoor activity answered by parents of the participants. Dirani et al. suggested that outdoor activity alone may be a bigger protective factor than sports alone. They suggest that recommending outdoor activity to prevent the onset or progression of myopia.

Rose et al (Rose et al., 2008a) looked at 1740 children with an average age of 6.7 years and 2453 teenagers with an average age of 12.7 years to study their near, midworking distance and outdoor activity with respect to the proportion of subjects with myopia. Questionnaires were administered regarding a variety of near work, indoor and outdoor activities including reading, picnics and walking. Less hyperopia was found in the group of year 7 students with an average proportion with myopia of 12.8% versus 0.7% in year 1. There was also a significant increase in the proportion with myopia and the amount of myopia in East Asians versus Caucasians. Data showed that merely being outdoors instead

of engaging in sports per se may be the protective factor to slowing myopia progression. Hypotheses about the source of the protective effect of time outdoors included low accommodative demand causing myopia inhibition or a possible substitution effect with outdoor activity increasing with a corresponding near work decrease. There was no evidence of a substitution of near work for time outdoors. This lack of a trade-off led to the hypothesis that light intensity may be the source of the protective factor. Increased light intensity causing pupillary constriction increasing the depth of field as well as increasing the level of dopamine, possibly inhibiting growth when released because of the extra light stimulation from being outdoors.

Ashby et al. set out to determine whether light levels may be a relevant factor in myopia development (Ashby et al., 2009). It was thought that higher levels of ambient illumination caused pupillary constriction, increasing depth of focus and decreasing image blur. Also, illumination causes a release of dopamine which is known to be an inhibitor of ocular growth. One day old white leghorn chickens were grown on a 12/12 hour of light and dark cycle where light phase intensity was 500 lux. There were two different study designs to look at the effects of light intensity. The first was to find the effects on ocular growth of removing translucent diffusers for fifteen minutes per day under different intensities of light. The second design was to expose the chickens to different levels of light for 6 hours per day to see the effects on axial length, refraction and corneal radius of curvature. They found that there was in fact a significant effect of both diffuser

treatment and light intensity on refractive development. There was a decrease in myopia development when the diffuser was removed under increased levels of intensity. High indoor light levels also resulted in less myopia but those under reduced illumination were not affected. This study determined that the protective effect from being outdoors may in fact be driven by light-intensity.

The current study is designed to address some of the questions raised by this review of literature. The research supporting a genetic and hereditary component to myopia is extensive (Ashby et al., 2009; Bear et al., 1981; Dirani et al., 2006; Dirani et al., 2009; Hammond et al., 2001; Jones et al., 2007; Lyhne et al., 2001; Rose et al., 2008a; Saw et al., 2006; Young et al., 1969; Zhu et al., 2003; Zylbermann et al., 1993). Additionally there are several classic risk factors such as near work that could lead to myopia onset or progression (Bear et al., 1981; Jones et al., 2007; Saw et al., 2006; Young et al., 1969; Zylbermann et al., 1993). However, the recent research looking at preventative factors suggests that time outdoors may be the more important environmental variable. What about outdoor activity is protective? The hypothesis for the current study is that myopia and time outdoors are related through the involvement of vitamin D. There has been much recent research supporting a large dietary insufficiency with vitamin D (Chapuy, Preziosi, Maamer, Arnaud, Galan, Hercberg and Meunier, 1997; Newhook, Sloka, Grant, Randell, Kovacs and Twells, 2009; Looker, Dawson-Hughes, Calvo, Gunter and Sahyoun, 2002; Gozdzik, Barta, Wu, Wagner, Cole, Vieth, Whiting and Parra, 2008; Mark, Gray-Donald, Delvin, O'Loughlin, Paradis, Levy and

Lambert, 2008). This can be seen all over the world but particularly in Asia due to a diet low in fortified foods (Zhai, Wang, Du, He, Wang, Ge and Popkin, 2007). In Asian countries, particularly Taiwan prevalence of myopia has been shown to have increased over a 20 year period (Lin et al., 2004).

The current study design will investigate several aspects of vitamin D metabolism. Activity surveys were important to determine what each subject spent his or her time doing and whether the activities were indoors or outdoors. Dietary surveys answered questions of nutrition and supplement intake. Blood samples measured the circulating levels of vitamin D and saliva samples allowed for genotyping and SNP analysis. Each component was important in answering the question, do vitamin D levels differ between persons with myopia compared to those without myopia.

Vitamin D or 25-hydroxyvitamin D (25(OH)D) is a fat-soluble metabolite responsible for calcium homeostasis (Vieth, 1990). There is an extensive biochemical reaction required for the formation of 25(OH)D (Webb and Holick, 1988). Blood levels of vitamin D are primarily affected by ultraviolet radiation and dietary supplementation. According to Webb and Holick (Webb et al., 1988), it is UVB in the electromagnetic range of 280-320 nm that is most relevant to vitamin D production. Also, the amount of vitamin D production is dependent on quantity and quality of UV radiation. Many factors affect cutaneous production of vitamin D, a few being increased melanin (Holick, MacLaughlin and Doppelt,

1981; Webb et al., 1988), aging, use of sunscreens, geographic location, season, and atmospheric conditions (Chapuy et al., 1997). Sun exposure had a greater effect when more body surface area was exposed rather than when considering just duration or length of time spent in the sunlight (Barger-Lux and Heaney, 2002). Data show serum 25-(OH)D levels decrease significantly from late summer to winter (Barger-Lux et al., 2002).

| | Deficiency | Insufficiency | Sufficiency | Toxicity |
|---|---|---|---|---|
| (Alpert and Shaikh, 2007) | <20 ng/ml | 20-30 ng/ml | >30 ng/ml | >200 ng/ml |
| (Diehl and Chiu, 2010) | < 30 ng/ml (<75 nmol/l) | | | |
| (Lips, Wiersinga, van Ginkel, Jongen, Netelenbos, Hackeng, Delmas and van der Vijgh, 1988) | ≤ 12 ng/ml (≤ 30 nmol/l) | | >12 ng/ml (>30 nmol/l) | |
| (Malabanan, Veronikis and Holick, 1998) | <20 ng/ml (50 nmol/l) | | >50 nmol/l | |
| http://ods.od.nih.gov/factsheets/vitamind.asp#h3 | <11ng/ml <27.5 nmol/l | <10-15 ng/ml <25-37.5 nmol/l | 15-30 ng/ml 37.5-75 nmol/l | |

**Table 1: Cited Definitions of Vitamin D Sufficiency, Insufficiency, Deficiency**

Diet has been shown to affect levels of vitamin D. Asian children have a prevalence of being deficient due to a diet low in fortified foods and high in fiber (Zhai et al., 2007). If adequate dietary intake and sun exposure cannot be

achieved, it is difficult to prevent insufficiency and deficiency. Insufficiency levels range from 25-75 nmol/l (10-30 ng/ml), which was found in 14% of a healthy urban French population (Chapuy et al., 1997). Deficiency is defined as having blood levels less than 27.5 to 50 nmol/l (<11-20 ng/ml) (Vieth, 1990). Vitamin D deficiency is due to poor nutrition, deprivation of sunlight, decline in synthesis of cutaneous vitamin D and could lead to osteomalacia and rickets (Sahota, 2000).

Edwards looked at nutritional effects on myopia (Edwards, 1996). Although not statistically significant, myopes generally consumed less vitamin D (31.5 ± 23.8µg compared to 50.9 ± 74.4µg) and calcium (515.7 ± 384.5mg compared to 570.0 ± 309.5mg) over a four-day period than non-myopes based on food records of 102 seven-year olds followed for three years to study risk factors for the incidence of myopia. This study concluded that many foods (protein, fat, B vitamins, phosphorus, iron, and cholesterol) were consumed less in myopic children than in non-myopic children, supporting a potential for nutritional involvement. Myopic children however were not malnourished since the data showed that their height, weight, and head circumferences were not statistically different from non-myopic children. One of the limitations of the study was that the data were not analyzed in a multivariate model to determine the effects of each nutrient after adjustment for the effects of the others.

It has been reported that vitamin D receptors are located on almost all tissues in the body (Alpert et al., 2007). Mutti et al. found a strong association with myopia in a candidate gene, *COL2A1*. This candidate genes is located near the vitamin D receptor genes on chromosome 12 (Mutti, Cooper, O'Brien, Jones, Marazita, Murray and Zadnik, 2007). Fulk et al. showed less myopia progression during summer months in both groups treated with either single vision correction and bifocal correction (Fulk, Cyert and Parker, 2002). Are these seasonal variations due to changes in vitamin D levels and sun exposure? As reported, myopic children spend less time outdoors (Dirani et al., 2009; Rose, Morgan, Smith, Burlutsky, Mitchell and Saw, 2008b). Do these myopic children also have less vitamin D circulating in their blood because of either inadequate dietary intake or less time spent outdoors? The hypothesis of this study is that myopic children will have a lower level of vitamin D in their blood, less dietary consumption of vitamin D, and/or genetic variations in the vitamin D receptor gene *VDR*.

# Chapter 2: Methods

## Subject Recruitment

The research followed the tenets of the Declaration of Helsinki. All subjects signed written consent documents after being informed of the purposes of the study including its risks and benefits. For any child under the age of 18 years, the child signed an assent form and the parent or guardian signed the consent form.

Initially, IRB approval was sought for children between the ages of 13 and 19 years. The onset of myopia is generally thought to occur between 8-12 years of age (Kleinstein, Jones, Hullett, Kwon, Lee, Friedman, Manny, Mutti, Yu and Zadnik, 2003). Based on this assumption, the subjects most likely to develop myopia would have developed myopia and non-myopes and would have stabilized into their respective refractive categories.

Following IRB approval, one e-mail was sent by the Worthington City Schools district office to parents of high school age children in the school district inviting them to participate in the study. Responses were minimal with only 14 subjects scheduling and completing their appointment. Sample size calculations required group sizes to contain 25 subjects for myopes and non-myopes to obtain an effect

of two times, meaning myopic children had circulating vitamin D half of those of non-myopic children. The initial response was inadequate to obtain the defined effect size; therefore IRB approval was sought for age expansion to 13-25 year olds. A subsequent email was sent to Optometry students to recruit subjects fitting into the broadened age criteria. A total of thirty-two subjects were examined between the ages of 13 and 25 years of age, with no regard to gender or ethnicity. Nineteen females and 13 males were seen with a mean age of 19.84 ± 4.62 years.

**Exclusion/Inclusion Criteria**

Brief ocular and medical history screened patients for inclusion for further examination. Patients with any significant history of ocular disease, previous strabismus, refractive surgical procedures or myopic therapies were excluded from examination. Myopic therapies include corneal reshaping or the use of atropine. The exclusion of any treatment of myopia such as LASIK or CRT was for several reasons. With the presence of refractive surgery or CRT, accurate measurement of the subject's nearsightedness would be impossible. The patient's refractive error could possibly be non-myopic in measurement but their activity level might be myopic in character. Therefore, keeping these subjects in data analysis would artificially skew or falsify the data compared to those that did not undergo myopia therapies. Medical history screened for diabetes (Jacobsen, Jensen, Lund-Andersen and Goldschmidt, 2008), Marfan's syndrome and Down's syndrome (Woodhouse, Pakeman, Cregg, Saunders, Parker, Fraser, Sastry and Lobo, 1997) due to the known myopic ocular manifestations.

Subjects were initially required to reside with their parents or guardians to control for drastic dietary or activity changes that might occur when leaving for the college or vocational world. Inclusion of subjects living at home would allow for the most accurate data on lifestyle and diet in accordance with the age at which most subjects developed their respective refractive error. The increased age range required the assumption that diet was somewhat similar at the older age to that when myopia developed.

**Examination**

Subjects were assigned a subject identification number which was used for anonymity of subject data. Each person was asked to complete a history, including date of birth, ethnicity, as well as a brief ocular and medical history. Subjects' acuities were measured using a high contrast Bailey-Lovie acuity chart at 6 meters. Best corrected visual acuities were required to be 6/7.5 or better in each eye. Two drops of tropicamide 1.0% were instilled into each eye separated by five minutes to obtain cycloplegia following a thirty minute period (Egashira, Kish, Twelker, Mutti, Zadnik and Adams, 1993). During cycloplegia, subjects answered questions from two surveys being read to them by the investigator. The first was a modified Sydney Myopia Study activity survey (Ip, Huynh, Robaei, Rose, Morgan, Smith, Kifley and Mitchell, 2007) (http://www.cvr.org.au/sms.htm.) and the other was the Block Kids Food

Frequency Questionnaire (FFQ) version 2004 for children ages 8-17 (http://www.nutritionquest.com).

Following survey completion and thirty minutes of cycloplegia, subject's refraction was measured on a Grand Seiko WR-5100K. Subjects were classified as myopic, unknown or non-myopic based on an average of ten readings from auto-refraction. Myopes had at least –0.75D of myopia in each principal meridian. Non-myopes had at least +0.25D or more plus power in each principal meridian. Subjects failing to fall into either the myope or non-myope categories were classified as unknown (n=10). Subjects with more than 1.00D of astigmatism were included as long as both meridians classified the subject into one of the two refractive groups. Only two subjects with more than 1.00D of cylinder were eligible for inclusion. Only myopes (n=14) and non-myopes (n=8) proceeded into the final testing which included measurement of circulating blood levels of vitamin D and SNP genotyping for *VDR* on chromosome 12q13.11.

**Activity Survey**

The activity survey was modified to better represent typical American activities and nomenclature. Activities in question included close work (homework, leisure reading, computer work) and sports (exercise and athletic participation) were recorded based on number of hours per day and were further categorized into whether the activity was performed indoors or outdoors. Sun exposure was also added to the survey to measure how much time was spent in the sun and if

protective measures were used that might affect amount of UV exposure, such as clothing, sun block or hats. The survey may be found in Appendix A.

**Block FFQ**

The Block Kids Food Frequency Questionnaire (FFQ) used a series of 77 food items to decide how often a particular food or group of foods was consumed in the past week and the portion size eaten each day. Food frequency questionnaires are one of several methods for dietary data collection including 24-hour recall, food records, and diet history (McPherson, 2000). In comparison, FFQs are practical and economical methods for collection of comprehensive dietary data (Subar, Thompson, Kipnis, Midthune, Hurwitz, McNutt, McIntosh and Rosenfeld, 2001). These capture usual dietary intake for a specific period of time. Subar et al. compared a new dietary health questionnaire (DHQ), Block FFQ, and Willett FFQ to four 24-hour recalls throughout the year. DHQ and Block compared similarly to each other whereas Willett had a lower correlation with recall. After adjustment for the amount of energy consumed, the three performed similarly with Willett having the lowest correlation with recall (Subar et al., 2001). Willett defended the importance of adjusting for energy in his commentary of Subar's study design. The review defined energy adjustment as the measurement of nutrient composition of diets which is important in many nutrient epidemiological studies (Willett, 1998). Subar responded that energy adjusted nutrients allow studies to examine the effects of nutrient substitution within a diet.

Measuring dietary intake in school-aged children is difficult and imprecise. McPherson et al reviewed literature regarding different techniques for collecting dietary data. Overall, food recall and records were the most accurate, particularly when supported by parental consensus on responses. However, these add a significant financial aspect to collecting data. FFQs did have lower correlations with recall but were considered appropriate for certain study designs such as epidemiological studies, monitoring, intervention, and cross-sectional study designs (McPherson, 2000). Of the extensive list of FFQ available, Block and Willett are the most widely used (Subar et al., 2001).

Furthermore, to satisfy the purpose of this study, where a relationship of absolute intakes and frequency of intake were more important, FFQ was preferred. This cross-sectional study looked at the dietary intake of different nutrients for each subject in order to analyze if myopes consumed more of one nutrient than non-myopes. Also, based on the research of the many FFQs, Block has been widely recognized and validated as a sufficient method of dietary measurement (Subar et al., 2001; McPherson, 2000). Therefore the current study used the Block FFQ which has been reported to correlate with the "gold standard" of 24 hour recall as reported in a group of 20-70 year olds (Subar et al., 2001). Block FFQ for children 8-17 was ideal for our initial sample size which initially was intended for 13-19 year olds. It was later applied to subjects of ages up to 24 years for consistency in data collection and analysis. This particular FFQ was short, yet comprehensive and added visual aids to describe portion size. The questionnaire

of choice took about 25 minutes to complete. It was developed from National Health and Nutrition Examination Survey (NHANES) 1999-2002 (http://www.cdc.gov/nchs/data/nhanes/nhanes_general_guidelines_june_04.pdf) dietary recall data. The database was developed from the USDA Nutrient Database for Dietary Studies version 1.0. The survey was sent to Nutrition Quest (Berkeley, California) for analysis of levels of nutrients consumed in each subject's diet.

**Blood Samples**

Each investigator was qualified to handle biohazards following completion of Office of Environmental Health and Safety Biosafety Level 2 Practices online training. Blood samples were administered using a sterile single-use 1.5 mm wide spring-loaded lancet (Sarstedt Inc.) following sterilization of the site using a isopropyl alcohol pad. Sharps were disposed in a sharps container and biohazards were disposed in appropriately marked biohazard containers. Blood was collected in a Sarstedt Microvette 200 capillary tube with heparin as the anti-coagulant. Each eligible subject gave approximately 200 microliters of blood which was stored at –87 degrees Celsius. Twenty-two samples of blood were collected and sent to The Ohio State University College of Pharmacy to be analyzed using assays to detect the blood level of vitamin D. The assays were developed by the OSU Comprehensive Cancer Center Pharmacoanalytical Shared Resource. Liquid Chromatography/Mass Spectroscopy was applied as the assay to measure nanomolar amounts of vitamin D.

Vogeser and Parhofer reviewed the techniques and uses of mass spectroscopy (MS) in endocrinology (Vogeser and Parhofer, 2007). Mass spectrometry is responsible for the analysis, characterization and quantification of chemical compounds. The first step is ion generation, using an electromagnetic field and increasing pressure and temperature the analyte separates from the eluent and become gaseous. The particular analyte responds to the use of cations and anions creating charge on the analyte which will later be used for the separation of ions. Ion selection is done through another set of vacuums which create trajectories of ions. Again, there are several types of analyzers but the general theory involves the principle that smaller and less charged ions travel faster due to their more stable trajectory whereas larger and more charged ions are analyzed last. Tandem mass spectrometry (MS/MS) can be very specific due to the fact that it runs mass spectrometry on the analyte twice (Vogeser et al., 2007).

According to van den Ouweland et al. (van den Ouweland, Beijers, Demacker and van Daal, 2010), serum 25-OH vitamin D concentrations can be measured by competitive binding assay, radioimmunoassay (RIA), high performance liquid chromatography and liquid chromatography tandem-mass spectrometry (LC-MS/MS). LC-MS was compared to RIA and an automated chemiluminescence-based immunoassay (ECLIA) in the measurement of serum 25-OH-vitamin D. This study found agreement between LC-MS/MS and RIA, however, ECLIA overestimated values particularly in the deficient levels. Van den Ouweland

concluded that LC-MS/MS provides rapid, accurate, sensitive and cost-effective alternatives and compares well to other effective methods of measuring vitamin D levels (van den Ouweland et al., 2010).

The protocol for analysis of blood samples collected was developed and performed as described by Yonghua Ling and Mitch Phelps of the Pharmacoanalytic Shared Resource, College of Pharmacy, The Ohio State University. Samples were prepared for and input through high performance liquid chromatography to fragment vitamin D to a more accessible form for mass spectrometry. The ionized analytes were analyzed by a triple quadrupole system for tandem mass spectrometry. One-hundred microliter samples of whole blood were analyzed with intermittent analysis to ensure accuracy (±15%).

**SNP Analysis**

Each subject gave approximately 2 ml of saliva. Oragene OG-250 DNA Discs were used to collect saliva samples (DNA Genotek Inc.). Storage was at room temperature until all samples were collected which were then submitted to Dr. Jeffrey C. Murray's laboratory at the University of Iowa for genotyping and SNP analysis. DNA processing was carried out using Qiagen's corresponding QiaAmp Kits (Qiagen, Inc.). Genotyping was done using TaqMan® SNP Genotyping Assays on the ABI Prism 7900HT from Applied Biosystems (Applied Biosystems, Foster City, CA, USA) with a protocol slightly modified from the manufacturer's instructions. The SNP selection was performed using HapMap and

Haploview (www.hapmap.org <http://www.hapmap.org> ). The SNPs were picked for high heterozygosity from the haplotype blocks. Genotyping was carried out using 384 well plates containing dried DNA samples and the scoring of the alleles was performed using the Applied Biosystems Sequence Detection Systems (SDS version 2.3).

**Statistical Analysis**

Data from activity surveys and FFQs were input into Excel spreadsheets. Equations used to calculate outdoor and indoor activities as well as total reading, sports and other in hours per week can be found in Appendix B and C. Statistical analysis was performed using PASW software (SPSS Inc, Chicago, version 17.0) to find mean and standard deviation for the entire sample size (n=32) as well as for refractive groups (myopes, non-myopes and unknown). Paired t tests functioned as an internal validation of indoor and outdoor measurements reported by subjects. These means were compared with one way ANOVA to find interactions between refractive groups. Bivariate correlations were measured for all 49 dietary variables found in Block FFQ with further correlative relationships regarding circulating levels of blood vitamin D. Significant dietary variables were input into linear regression to measure the amount of variance impacting levels of blood vitamin D. Comparisons of blood vitamin D levels were made for those affected (myopes versus non-myopes) while controlling for significant covariates in a general linear model. Dietary variables were input into a one way ANOVA to examine the effect of refractive groups, looking for differences between groups.

Final analysis involved binary logistic regression of the individual SNPs to analyze odds ratios associated with having myopia.

## Chapter 3: Results

Of the initial 14 children within the 13-19 age criteria, 10 of the 14 refractive errors qualified the subjects for the collection and analysis of blood and saliva samples. Five were myopic and the remaining five were non-myopic. Following IRB approval for expansion of subject age limitations, 18 more subjects were examined with nine additional myopes and three additional non-myopes eligible for full data analysis including blood and saliva. All 32 subjects completed refractive error data as well as activity and dietary surveys. However, 22 met all inclusion criteria and were eligible for further testing which included blood and saliva samples.

In table 2, descriptive statistics were run on the entire sample size (n=32). The subjects' mean age was 19.84 ± 4.62 years, range of 13.08 through 25.21. There were a total of 19 females (21.28 ± 4.38 years) and 13 males (17.74 ± 4.28 years) enrolled in the study. Mean, standard deviation and ranges are summarized for activities, dietary vitamin D and blood vitamin D.

| Variable | n | Mean ± SD | Minimum | Maximum |
|---|---|---|---|---|
| Total Outdoors Stated (hrs/wk) | 32 | 15.49 ± 6.82 | 3.00 | 30.00 |
| Total Outdoors Estimated (hrs/wk) | 32 | 13.16 ± 5.94 | 0.00 | 30.50 |
| Total Indoors Stated (hrs/wk) | 32 | 117.56 ± 11.92 | 92.33 | 145.75 |
| Total Indoors Estimated (hrs/wk) | 32 | 113.96 ± 17.73 | 75.50 | 146.03 |
| Total Reading (hrs/wk) | 32 | 38.30 ± 13.37 | 14.68 | 69.00 |
| Total Sports (hrs/wk) | 32 | 6.72 ± 4.96 | 0.00 | 19.00 |
| Total Other (hrs/wk) | 32 | 5.02 ± 5.38 | 0.00 | 17.00 |
| Total Dietary Vitamin D (IU/day) | 32 | 245.33 ± 214.31 | 26.09 | 782.42 |
| Blood Vitamin D (ng/ml) | 22 | 14.70 ± 4.29 | 6.62 | 23.57 |

**Table 2: Descriptive statistics for each activity performed and for vitamin D levels**

Table 3 compares means of time outdoors estimated versus stated and time indoors estimated versus stated through paired t test analysis. Outdoor activity levels are statistically different, $t=2.38$ ($p<0.05$) while indoor activity levels are not statistically different, $t=1.86$ ($p>0.05$)

| | t value (df: 31) | p value |
|---|---|---|
| Outdoor: Stated versus Estimated | 2.36 | 0.03 |
| Indoor Stated versus Estimated | 1.86 | 0.07 |

**Table 3: Paired t test of Outdoor and Indoor Activity comparing means of Stated versus Estimated**

Each subject was classified into one of three groups as defined in Methods: myopes, non-myopes or unknown. The myopic group had 14 total subjects (six females and eight males) with a mean age of 20.18 ± 5.33. Eight subjects (five females and three males) were classified as non-myopes with an average age of 18.68 ± 3.63 years. Unknown subjects were those who did not fit into categories of at least –0.75D of myopia or +0.25 of hyperopia. There were a total of 10 unknown refractive error subjects, eight females, two males with an average age of 20.30 ± 4.53 years. Table 4 presents the mean and standard deviation of outdoor activity, indoor activity as well as total reading, sports and other performed by each refractive group. Both outdoor and indoor activity have two separate means, classified as estimated and stated due to the nature of the questions asked in the activity survey (Ip et al., 2007). Appendices B and C display the equations used to calculate the hours per week for each activity analyzed. These equations can be used to find the relevant questions in the activity survey found in Appendix A (Ip et al., 2007).

| | Myopes<br>n = 14 | Unknown<br>n = 10 | Non-Myopes<br>n = 8 |
|---|---|---|---|
| Age | 20.18 ± 5.33 | 20.30 ± 4.53 | 18.68 ± 3.63 |
| Gender<br><br>Female<br><br>Male | <br><br>n = 6<br><br>n = 8 | <br><br>n=8<br><br>n=2 | <br><br>n = 5<br><br>n = 3 |
| Total Outdoors Stated (hrs/wk) | 15.59 ± 7.74 | 15.55 ± 5.59 | 15.25 ± 7.38 |
| Total Outdoors Estimated (hrs/wk) | 12.88 ± 7.78 | 13.24 ± 2.84 | 13.56 ± 5.77 |
| Total Indoors Stated (hrs/wk) | 118.0 ± 13.39 | 120.14 ± 7.92 | 113.49 ± 13.65 |
| Total Indoors Estimated (hrs/wk) | 112.00 ± 18.09 | 117.59 ± 15.84 | 112.87 ± 11.71 |
| Total Reading (hrs/wk) | 37.85 ± 14.01 | 41.14 ± 15.88 | 35.56 ± 9.08 |
| Total Sports (hrs/wk) | 5.82 ± 3.72 | 6.50 ± 4.51 | 8.56 ± 7.18 |
| Total Other (hrs/wk) | 4.75 ± 5.39 | 4.10 ± 6.07 | 6.63 ± 4.72 |
| Total Dietary Vitamin D (IU/day) | 261.4 ± 215.2 | 267.14 ± 251.77 | 190.0 ± 176.8 |
| Blood Vitamin D (ng/ml) | 13.95 ± 3.75 | n/a | 16.02 ± 5.11 |

**Table 4: Mean Values ± Standard Deviations of Activity and Vitamin D Levels for Each Group Categorized by Refractive Error**

Table 4 also lists mean total dietary vitamin D (IU/day) for myopes, unknown, and non-myopes were 261.4 ± 215.2, 267.14 ± 251.77, and 190.0 ± 176.8 respectively. Blood vitamin D levels (ng/ml) were collected for myopes and non-

myopes. Average values (ng/ml) were 13.95 ± 3.75 and 16.02 ± 5.11. Data for circulating blood levels of vitamin D were not collected for unknowns.

One-way analysis of variance (ANOVA) was conducted with refractive group as the between-group factor to determine whether significant differences in the main environmental variables, in vitamin D dietary intake, or in blood levels of vitamin D. Outdoor activity, indoor activity, total reading, sports and other, total vitamin D and circulating vitamin D were not statistically significantly different between refractive error groups as seen in table 5 ($p>0.05$).

| **Variable** | **F (df = 2, 29)** | **p-value** |
|---|---|---|
| Total Outdoors Stated (hrs/wk) | 0.006 | 0.99 |
| Total Outdoors Estimated (hrs/wk) | 0.032 | 0.97 |
| Total Indoors Stated (hrs/wk) | 0.70 | 0.51 |
| Total Indoors Estimated (hrs/wk) | 0.38 | 0.69 |
| Total Reading (hrs/wk) | 0.38 | 0.68 |
| Total Sports (hrs/wk) | 0.78 | 0.47 |
| Total Other (hrs/wk) | 0.50 | 0.61 |
| Total Dietary Vitamin D (IU/day) | 0.34 | 0.71 |
| Blood Vitamin D (ng/ml) | 1.19 | 0.29 |

**Table 5: One Way Analysis of Variance for Activities, Dietary intake of Vitamin D and Circulating Blood Vitamin D**

Bivariate correlations were analyzed on 49 dietary variables returned from the Nutrition Quest analysis. Table 6 lists several pages of correlation matrices with significant relationships denoted. A legend listing full names for the abbreviations with respective units can be found in Appendix B.

| | DtKcal | DtProt | DtTFat | DtCarb | DtCalc | DtPhos | DtIron | DtSodi | DtPota | DtThia |
|---|---|---|---|---|---|---|---|---|---|---|
| DtKcal | 1 | | | | | | | | | |
| DtProt | 0.79+ | 1 | | | | | | | | |
| DtTFat | 0.85+ | 0.71+ | 1 | | | | | | | |
| DtCarb | 0.91+ | 0.56+ | 0.59+ | 1 | | | | | | |
| DtCalc | 0.76+ | 0.68+ | 0.50+ | 0.76+ | 1 | | | | | |
| DtPhos | 0.85+ | 0.92+ | 0.69+ | 0.71+ | 0.84+ | 1 | | | | |
| DtIron | 0.64+ | 0.66+ | 0.40* | 0.65+ | 0.74+ | 0.75+ | 1 | | | |
| DtSodi | 0.84+ | 0.84+ | 0.84+ | 0.62+ | 0.58+ | 0.82+ | 0.62+ | 1 | | |
| DtPota | 0.75+ | 0.81+ | 0.62+ | 0.64+ | 0.68+ | 0.89+ | 0.73+ | 0.85+ | 1 | |
| DtThia | 0.76+ | 0.67+ | 0.50+ | 0.76+ | 0.83+ | 0.79+ | 0.94+ | 0.67+ | 0.75+ | 1 |
| DtRibo | 0.78+ | 0.77+ | 0.49+ | 0.77+ | 0.94+ | 0.89+ | 0.85+ | 0.64+ | 0.75+ | 0.90+ |
| DtNiac | 0.76+ | 0.80+ | 0.55+ | 0.69+ | 0.80+ | 0.82+ | 0.91+ | 0.70+ | 0.71+ | 0.93+ |
| DtVitC | 0.29 | 0.20 | 0.35* | 0.22 | 0.17 | 0.24 | 0.29 | 0.51+ | 0.55+ | 0.33 |
| DtSFat | 0.85+ | 0.69+ | 0.89+ | 0.66+ | 0.63+ | 0.69+ | 0.39* | 0.71+ | 0.53+ | 0.50+ |
| DtMFat | 0.86+ | 0.72+ | 0.98+ | 0.60+ | 0.50+ | 0.67+ | 0.40* | 0.85+ | 0.60+ | 0.51+ |
| DtPFat | 0.51+ | 0.42* | 0.78+ | 0.26 | 0.14 | 0.43* | 0.21 | 0.64+ | 0.53+ | 0.28 |
| DtChol | 0.46+ | 0.69+ | 0.56+ | 0.189 | 0.21 | 0.50+ | 0.22 | 0.58+ | 0.38* | 0.23 |
| DtFibe | 0.45+ | 0.47+ | 0.42* | 0.38* | 0.26 | 0.57+ | 0.58+ | 0.64+ | 0.78+ | 0.50+ |
| DtFolFD | 0.62+ | 0.55+ | 0.38* | 0.65+ | 0.64+ | 0.66+ | 0.90+ | 0.63+ | 0.74+ | 0.90+ |
| DtZinc | 0.74+ | 0.80+ | 0.55+ | 0.65+ | 0.82+ | 0.83+ | 0.87+ | 0.69+ | 0.73+ | 0.89+ |
| DtAnZin | 0.65+ | 0.92+ | 0.57+ | 0.42* | 0.56+ | 0.80+ | 0.54+ | 0.72+ | 0.69+ | 0.53+ |
| DtVitB6 | 0.66+ | 0.66+ | 0.43* | 0.66+ | 0.72+ | 0.75+ | 0.91+ | 0.64+ | 0.77+ | 0.91+ |
| DtMagn | 0.74+ | 0.78+ | 0.62+ | 0.63+ | 0.64+ | 0.88+ | 0.76+ | 0.76+ | 0.92+ | 0.74+ |
| DtAcaro | -0.07 | 0.09 | 0.10 | -0.19 | -0.16 | 0.09 | 0.12 | 0.21 | 0.26 | 0.06 |
| DtBcaro | -0.01 | 0.08 | 0.13 | -0.11 | -0.11 | 0.12 | 0.19 | 0.30 | 0.38* | 0.09 |

continued

**Table 6: Bivariate Correlations Matrix of Dietary Variables from Block Dietary FFQ (+ p<0.01; * p<0.05)**

Table 6 continued

| | DtKcal | DtProt | DtTFat | DtCarb | DtCalc | DtPhos | DtIron | DtSodi | DtPota | DtThia |
|---|---|---|---|---|---|---|---|---|---|---|
| DtCrypt | 0.11 | 0.17 | 0.13 | 0.06 | 0.05 | 0.18 | 0.19 | 0.38* | 0.46+ | 0.23 |
| DtLutein | 0.02 | 0.10 | 0.10 | -0.04 | -0.03 | 0.14 | 0.23 | 0.29 | 0.37* | 0.14 |
| DtLycope | 0.17 | 0.28 | 0.18 | 0.09 | 0.17 | 0.34 | 0.16 | 0.39* | 0.41* | 0.18 |
| DtRetinol | 0.67+ | 0.74+ | 0.40* | 0.64+ | 0.79+ | 0.83+ | 0.61+ | 0.54+ | 0.70+ | 0.66+ |
| DtCarote | -0.02 | 0.10 | 0.13 | -0.12 | -0.11 | 0.13 | 0.19 | 0.31 | 0.39* | 0.10 |
| DtVitAR | 0.47+ | 0.60+ | 0.40* | 0.36* | 0.46+ | 0.69+ | 0.59+ | 0.64+ | 0.82+ | 0.55+ |
| DtVitEAt | 0.39* | 0.28 | 0.54+ | 0.26 | 0.34 | 0.39* | 0.48+ | 0.46+ | 0.48+ | 0.53+ |
| DtVit B12 | 0.62+ | 0.76+ | 0.33 | 0.59+ | 0.79+ | 0.81+ | 0.80+ | 0.56+ | 0.69+ | 0.79+ |
| DtCopper | 0.70+ | 0.67+ | 0.71+ | 0.55+ | 0.39* | 0.72+ | 0.57+ | 0.76+ | 0.81+ | 0.60+ |
| DtSelnium | 0.75+ | 0.95+ | 0.73+ | 0.51+ | 0.54+ | 0.87+ | 0.60+ | 0.86+ | 0.81+ | 0.62+ |
| DtFolfort | 0.46+ | 0.32 | 0.12 | 0.60+ | 0.69+ | 0.43* | 0.75+ | 0.26 | 0.34 | 0.81+ |
| DtFolFood | 0.37* | 0.44* | 0.43 | .025 | 0.14 | 0.48+ | 0.43* | 0.62+ | 0.71+ | 0.38* |
| DtFolDFE | 0.60+ | 0.50+ | 0.31 | .068+ | 0.71+ | 0.62+ | 0.90+ | 0.53+ | .64+ | 0.93+ |
| DtVitK | 0.01 | 0.06 | 0.11 | -0.05 | -0.08 | 0.10 | 0.19 | 0.28 | 0.34 | 0.09 |
| DtTheoBr | 0.19 | 0.08 | 0.17 | 0.19 | 0.05 | 0.07 | 0.01 | -0.00 | 0.01 | 0.13 |
| DtTotSug | 0.80+ | 0.44* | 0.46+ | 0.93+ | 0.73+ | 0.58+ | 0.49+ | 0.43* | 0.45+ | 0.64+ |
| DtOme3 | 0.41* | 0.35 | 0.64+ | 0.20 | 0.07 | 0.37* | 0.15 | 0.61+ | 0.56+ | 0.19 |
| DtOme6 | 0.50+ | 0.42* | 0.77+ | 0.25 | 0.13 | 0.42* | 0.21 | 0.62+ | 0.52+ | 0.28 |
| DtVit D | 0.53+ | 0.66+ | 0.20 | 0.56+ | 0.77+ | 0.75+ | 0.63+ | 0.38* | 0.60+ | 0.65+ |
| DtTranFat | 0.59+ | 0.31 | 0.50+ | 0.59+ | 0.49+ | 0.36* | 0.40* | 0.31 | 0.20 | 0.48+ |
| GrpSoldTo | 0.48+ | 0.57+ | 0.56+ | 0.29 | 0.18 | 0.56+ | 0.38* | 0.76+ | 0.79+ | 0.36* |
| TotalVitD | 0.36* | 0.63+ | 0.17 | 0.30 | 0.51+ | 0.63+ | 0.48+ | 0.39* | 0.56+ | 0.47+ |
| BloodVitD | -0.39 | -0.13 | -0.09 | -0.59+ | -0.34 | -0.30 | -0.42 | -0.28 | -0.34 | -0.39 |

continued

Table 6 continued

| | DtRibo | DtNiac | DtVitC | DtSFat | DtMFat | DtPFat | DtChol | DtFibe | DtFolFD | DtZinc |
|---|---|---|---|---|---|---|---|---|---|---|
| DtRibo | 1 | | | | | | | | | |
| DtNiac | 0.91+ | 1 | | | | | | | | |
| DtVitC | 0.11 | 0.22 | 1 | | | | | | | |
| DtSFat | 0.57+ | 0.55+ | 0.14 | 1 | | | | | | |
| DtMFat | 0.49+ | 0.58+ | 0.3 | 0.85+ | 1 | | | | | |
| DtPFat | 0.16 | 0.27 | 0.52+ | 0.45* | 0.74+ | 1 | | | | |
| DtChol | 0.37* | 0.44* | -0.00 | 0.42* | 0.58+ | 0.44* | 1 | | | |
| DtFibe | 0.38* | 0.42* | 0.47+ | 0.22 | 0.38* | 0.59+ | 0.21 | 1 | | |
| DtFolFD | 0.76+ | 0.83+ | 0.44* | 0.30 | 0.38* | 0.33 | 0.19 | 0.67+ | 1 | |
| DtZinc | 0.90+ | 0.94+ | 0.18 | 0.58+ | 0.56+ | 0.25 | 0.44 | 0.43* | 0.76+ | 1 |
| DtAnZin | 0.66+ | 0.67+ | 0.10 | 0.61+ | 0.57+ | 0.25 | 0.67+ | 0.37* | 0.39* | 0.78+ |
| DtVitB6 | 0.86+ | 0.91+ | 0.32 | 0.40* | 0.42* | 0.27 | 0.32 | 0.60+ | 0.91+ | 0.87+ |
| DtMagn | 0.72+ | 0.70+ | 0.39* | 0.53+ | 0.57+ | 0.56+ | 0.33 | 0.84+ | 0.77+ | 0.68+ |
| DtAcaro | -0.09 | 0.01 | 0.37* | -0.07 | 0.05 | 0.35 | 0.08 | 0.41* | 0.16 | 0.09 |
| DtBcaro | -0.10 | 0.00 | 0.64+ | -0.08 | 0.08 | 0.42* | -0.08 | 0.59+ | 0.33 | -0.01 |
| DtCrypt | 0.02 | 0.06 | 0.80+ | 0.01 | 0.11 | 0.26 | -0.10 | 0.35* | 0.29 | 0.04 |
| DtLutein | -0.04 | 0.05 | 0.61+ | -0.07 | 0.06 | 0.33 | -0.11 | 0.56+ | 0.38* | -0.01 |
| DtLycope | 0.17 | 0.13 | 0.19 | 0.16 | 0.20 | 0.13 | -0.08 | 0.33 | 0.17 | 0.19 |
| DtRetinol | 0.86+ | 0.68+ | 0.04 | 0.55+ | 0.36* | 0.10 | 0.34 | 0.32 | 0.55+ | 0.71+ |
| DtCarote | -0.10 | 0.01 | 0.65+ | -0.07 | 0.08 | 0.42 | -0.06 | 0.59+ | 0.32 | 0.01 |
| DtVitAR | 0.53+ | 0.49+ | 0.55+ | 0.33 | 0.33 | 0.43* | 0.20 | 0.73+ | 0.66+ | 0.50+ |
| DtVitEAt | 0.30 | 0.45+ | 0.54+ | 0.29 | 0.51+ | 0.73+ | 0.09 | 0.55+ | 0.57+ | 0.45+ |
| DtVit B12 | 0.90+ | 0.85+ | 0.04 | 0.41* | 0.35 | 0.05 | 0.42* | 0.37* | 0.67+ | 0.93+ |
| DtCopper | 0.48+ | 0.55+ | 0.48+ | 0.54+ | 0.65+ | 0.75+ | 0.36* | 0.83+ | 0.66+ | 0.56+ |

continued

Table 6 continued

| | DtRibo | DtNiac | DtVitC | DtSFat | DtI | DtPFat | DtChol | DtFibe | DtFolFD | DtZinc |
|---|---|---|---|---|---|---|---|---|---|---|
| DtSelnium | 0.68+ | 0.73+ | 0.27 | 0.60+ | 0.73+ | 0.59+ | 0.78+ | 0.58+ | 0.57+ | 0.73+ |
| DtFolfort | 0.76+ | 0.77+ | -0.00 | 0.21 | 0.16 | -0.12 | 0.03 | 0.13 | 0.74+ | 0.72+ |
| DtFolFood | 0.23 | 0.31 | 0.66+ | 0.20 | 0.37* | 0.64+ | 0.25 | 0.85+ | 0.60+ | 0.26 |
| DtFolDFE | 0.81+ | 0.87+ | 0.30 | 0.29 | 0.32 | 0.18 | 0.14 | 0.51+ | 0.97+ | 0.79+ |
| DtVitK | -0.09 | 0.01 | 0.61+ | -0.07 | 0.07 | 0.37* | -0.11 | 0.56+ | 0.35* | -0.06* |
| DtTheoBr | 0.09 | 0.08 | -0.21 | 0.34 | 0.12 | -0.09 | -0.07 | -0.08 | 0.01 | 0.12 |
| DtTotSug | 0.69+ | 0.57+ | 0.10 | 0.61+ | 0.47+ | 0.06 | 0.11 | 0.10 | 0.47+ | 0.57+ |
| DtOme3 | 0.09 | 0.15 | 0.65+ | 0.35 | 0.58+ | 0.89+ | 0.43* | 0.61+ | 0.28 | 0.18 |
| DtOme6 | 0.15 | 0.27 | 0.51+ | 0.44* | 0.73+ | 1.00+ | 0.43* | 0.58+ | 0.33 | 0.24 |
| DtVit D | 0.87+ | 0.67+ | -0.18 | 0.33 | 0.21 | -0.06 | 0.31 | 0.25 | 0.51+ | 0.69+ |
| DtTranFat | 0.44* | 0.51+ | -0.02 | 0.49+ | 0.57+ | 0.22 | 0.15 | 0.02 | 0.27 | 0.47+ |
| GrpSoldTo | 0.29 | 0.35 | 0.60+ | 0.35* | 0.52+ | 0.68+ | 0.42* | 0.85+ | 0.49+ | 0.40* |
| TotalVitD | 0.63+ | 0.55+ | 0.02 | 0.19 | 0.18 | 0.03 | 0.39* | 0.29 | 0.36* | 0.63+ |
| BloodVitD | -0.36 | -0.30 | -0.24 | -0.14 | -0.12 | -0.01 | 0.08 | -0.33 | -0.47* | -0.28 |

| | DtAnZin | DtVitB6 | DtMagn | DtAcaro | DtBcaro | DtCrypt | DtLutein | DtLycope | DtRetinol | DtCarote |
|---|---|---|---|---|---|---|---|---|---|---|
| DtAnZin | 1 | | | | | | | | | |
| DtVitB6 | 0.56+ | 1 | | | | | | | | |
| DtMagn | 0.61+ | 0.75+ | 1 | | | | | | | |
| DtAcaro | 0.16 | 0.07 | 0.22 | 1 | | | | | | |
| DtBcaro | 0.03 | 0.12 | 0.42* | 0.70+ | 1 | | | | | |

continued

Table 6 continued

| | DtAnZin | DtVitB6 | DtMagn | DtAcaro | DtBcaro | DtCrypt | DtLutein | DtLycope | DtRetinol | DtCarote |
|---|---|---|---|---|---|---|---|---|---|---|
| DtCrypt | 0.12 | 0.15 | 0.32 | 0.38* | 0.63+ | 1 | | | | |
| DtLutein | -0.02 | 0.16 | 0.45+ | 0.41* | 0.93+ | 0.62+ | 1 | | | |
| DtLycope | 0.31 | 0.09 | 0.32 | 0.32 | 0.28 | 0.35* | 0.19 | 1 | | |
| DtRetinol | 0.70+ | 0.67+ | 0.66+ | -0.09 | -0.17 | 0.04 | -0.15 | 0.27 | 1 | |
| DtCarote | 0.05 | 0.12 | 0.41* | 0.77 | 0.99+ | 0.65+ | 0.89+ | 0.31 | -0.16 | 1 |
| DtVitAR | 0.53+ | 0.58+ | 0.82+ | 0.55+ | 0.70+ | 0.55+ | 0.63+ | 0.43* | 0.58+ | 0.71+ |
| DtVitEAt | 0.10 | 0.46+ | 0.54+ | 0.42* | 0.51+ | 0.26 | 0.44* | 0.14 | 0.02 | 0.51+ |
| DtVit B12 | 0.79+ | 0.82+ | 0.62+ | 0.03 | -0.12 | -0.02 | -0.13 | 0.23 | 0.81+ | -0.10 |
| DtCopper | 0.55+ | 0.57+ | 0.89+ | 0.37* | 0.49+ | 0.34 | 0.44* | 0.34 | 0.44* | 0.49+ |
| DtSelnium | 0.86+ | 0.63+ | 0.79+ | 0.18 | 0.17 | 0.21 | 0.15 | 0.29 | 0.66+ | 0.18 |
| DtFolfort | 0.24 | 0.76+ | 0.31 | -0.19 | -0.27 | -0.15 | -0.21 | -0.00 | 0.54+ | -0.27 |
| DtFolFood | 0.29 | 0.44* | 0.77+ | 0.46+ | 0.81+ | 0.61+ | 0.82+ | 0.28 | 0.18 | 0.80+ |
| DtFolDFE | 0.36* | 0.91+ | 0.64+ | 0.03 | 0.12 | 0.14 | 0.17 | 0.12 | 0.59+ | 0.11 |
| DtVitK | -0.07 | 0.12 | 0.43* | 0.42* | 0.94+ | 0.59+ | 0.99+ | 0.17 | -0.19 | 0.90+ |
| DtTheoBr | 0.13 | 0.03 | 0.04 | 0.09 | -0.10 | -0.12 | -0.15 | 0.12 | 0.08 | -0.08 |
| DtTotSug | 0.37* | 0.50+ | 0.42* | -0.27 | -0.22 | -0.00 | -0.15 | -0.01 | 0.58+ | -0.23 |
| DtOme3 | 0.27 | 0.27 | 0.49+ | 0.38* | 0.44* | 0.38* | 0.34 | 0.11 | 0.11 | 0.46+ |
| DtOme6 | 0.24 | 0.27 | 0.56+ | 0.35 | 0.42* | 0.25 | 0.34 | 0.11 | 0.09 | 0.42* |
| DtVit D | 0.62+ | 0.63+ | 0.58+ | -0.18 | -0.25 | -0.13 | -0.20 | 0.18 | 0.86+ | -0.25 |
| DtTranFat | 0.19 | 0.29 | 0.21 | -0.14 | -0.24 | -0.32 | -0.23 | -0.02 | 0.11 | -0.24 |
| GrpSoldTo | 0.54+ | 0.47+ | 0.71+ | 0.51+ | 0.53+ | 0.47+ | 0.42* | 0.44* | 0.37* | 0.56+ |
| TotalVitD | 0.71+ | 0.54+ | 0.48+ | 0.06 | -0.01 | 0.04 | -0.01 | 0.11 | 0.61+ | 0.01 |
| BloodVitD | -0.09 | -0.45* | -0.33 | 0.14 | -0.08 | -0.15 | -0.14 | -0.08 | -0.29 | -0.06 |

continued

Table 6 continued

| | DtVitAR | DtVitEAt | DtVit B12 | DtCopper | DtSelnium | DtFolfort | DtFolFood | DtFolDFE | DtVitK | DtTheoBr |
|---|---|---|---|---|---|---|---|---|---|---|
| DtVitAR | 1 | | | | | | | | | |
| DtVitEAt | 0.44* | 1 | | | | | | | | |
| DtVit B12 | 0.49+ | 0.20 | 1 | | | | | | | |
| DtCopper | 0.73+ | 0.64+ | 0.43* | 1 | | | | | | |
| DtSelnium | 0.62+ | 0.36* | 0.68+ | 0.76+ | 1 | | | | | |
| DtFolfort | 0.15 | 0.24 | 0.71+ | 0.15 | 0.24 | 1 | | | | |
| DtFolFood | 0.80+ | 0.55+ | 0.15 | 0.80+ | 0.56+ | -0.10 | 1 | | | |
| DtFolDFE | 0.51+ | 0.48+ | 0.73+ | 0.50+ | 0.48+ | 0.89+ | 0.37* | 1 | | |
| DtVitK | 0.62+ | 0.45* | -0.19 | 0.44* | 0.13 | -0.25 | 0.82+ | 0.14 | 1 | |
| DtTheoBr | -0.01 | -0.02 | 0.03 | 0.18 | -.01 | 0.06 | -0.06 | 0.03 | -0.14 | 1 |
| DtTotSug | 0.22 | 0.10 | 0.53+ | 0.33 | 0.34 | 0.56+ | 0.03 | 0.54+ | -0.17 | 0.26 |
| DtOme3 | 0.48+ | 0.55+ | 0.07 | 0.66+ | 0.53+ | -0.21 | 0.66+ | 0.11 | 0.38* | -0.16 |
| DtOme6 | 0.43* | 0.74+ | 0.04 | 0.74+ | 0.58+ | -0.12 | 0.63+ | 0.18 | 0.37* | -0.09 |
| DtVit D | 0.41* | -0.03 | 0.84+ | 0.29 | 0.56+ | 0.60+ | 0.06 | 0.58+ | -0.25 | 0.08 |
| DtTranFat | -0.12 | 0.38* | 0.31 | 0.24 | 0.25 | 0.42* | -0.11 | 0.34 | -0.22 | 0.25 |
| GrpSoldTo | 0.73+ | 0.45+ | 0.34 | 0.81+ | 0.69+ | -0.05 | 0.79+ | 0.32 | 0.43* | -0.07 |
| TotalVitD | 0.42* | 0.04 | 0.75+ | 0.34 | 0.58+ | 0.29 | 0.18 | 0.36* | -0.07 | 0.06 |
| BloodVitD | -0.24 | -0.14 | -0.29 | -0.21 | -0.11 | -0.33 | -0.22 | -0.45* | -0.13 | 0.23 |

continued

Table 6 continued

| | DtTotSug | DtOme3 | DtOme6 | DtVit D | DtTranFat | GrpSoldTo | TotalVitD | BloodVitD |
|---|---|---|---|---|---|---|---|---|
| DtTotSug | 1 | | | | | | | |
| DtOme3 | 0.03 | 1 | | | | | | |
| DtOme6 | 0.05 | 0.87+ | 1 | | | | | |
| DtVit D | 0.55+ | -0.09 | -0.07 | 1 | | | | |
| DtTranFat | 0.54+ | 0.02 | 0.22 | 0.20 | 1 | | | |
| GrpSoldTo | 0.04 | 0.79+ | 0.66+ | 0.14 | -0.06 | 1 | | |
| TotalVitD | 0.27 | 0.08 | 0.03 | 0.70+ | 0.12 | 0.29 | 1 | |
| BloodVitD | -0.54+ | -0.07 | -0.00 | -0.25 | -0.07 | -0.18 | -0.08 | 1 |

Table 7 summarizes the significant supplement correlations from the bivariate analysis to total vitamin D from dietary survey and circulating blood vitamin D levels. Dietary variables significantly correlated with blood vitamin D include dietary carbohydrates, total folate, vitamin B6, folate/folic acid, and total sugar. These five correlations can be seen in Figures 1-5 where the relationship is shown as a scatterplot between each significant dietary variable and circulating vitamin D blood levels.

| | Total Vitamin D | Blood Vitamin D | | Total Vitamin D | Blood Vitamin D |
|---|---|---|---|---|---|
| **Dt Kcal** | 0.36* | | **Dt Bcaro** | | |
| **Dt Prot** | 0.63+ | | **Dt Crypt** | | |
| **Dt TFat** | | | **Dt Lutein** | | |
| **Dt Carb** | | -0.59+ | **DtLycope** | | |
| **Dt Calc** | 0.51+ | | **DtRetinol** | 0.61+ | |
| **Dt Phos** | 0.63+ | | **Dt Carote** | | |
| **Dt Iron** | 0.48+ | | **DtVitAR** | 0.42* | |
| **Dt Sodi** | 0.39* | | **DtVitEAt** | | |
| **Dt Pota** | 0.56+ | | **Dt Vit B12** | 0.75+ | |
| **Dt Thia** | 0.47+ | | **Dt Copper** | | |
| **Dt Ribo** | 0.63+ | | **Dt Selnium** | 0.58+ | |
| **Dt Niac** | 0.55+ | | **Dt Folfort** | | |
| **Dt VitC** | | | **Dt FolFood** | | |
| **Dt SFat** | | | **Dt FolDFE** | 0.36* | -0.45* |
| **Dt MFat** | | | **Dt VitK** | | |
| **Dt PFat** | | | **Dt TheoBr** | | |
| **Dt Chol** | 0.39* | | **Dt TotSug** | | -0.54+ |
| **Dt Fibe** | | | **Dt Ome3** | | |
| **Dt FolFD** | 0.36* | -0.47* | **Dt Ome6** | | |
| **Dt Zinc** | 0.63+ | | **Dt Vit D** | 0.70+ | |
| **Dt AnZin** | 0.71+ | | **Dt TranFat** | | |
| **Dt Vit B6** | 0.54+ | -0.45* | **GrpSolidTo** | | |
| **Dt Magn** | 0.48+ | | **TotalVitD** | | |
| **Dt Acaro** | | | **Blood Vit D** | | |

**Table 7: Significant Values Correlating with Total Vitamin D and Circulating Vitamin D (+ p<0.01; * p<0.05)**

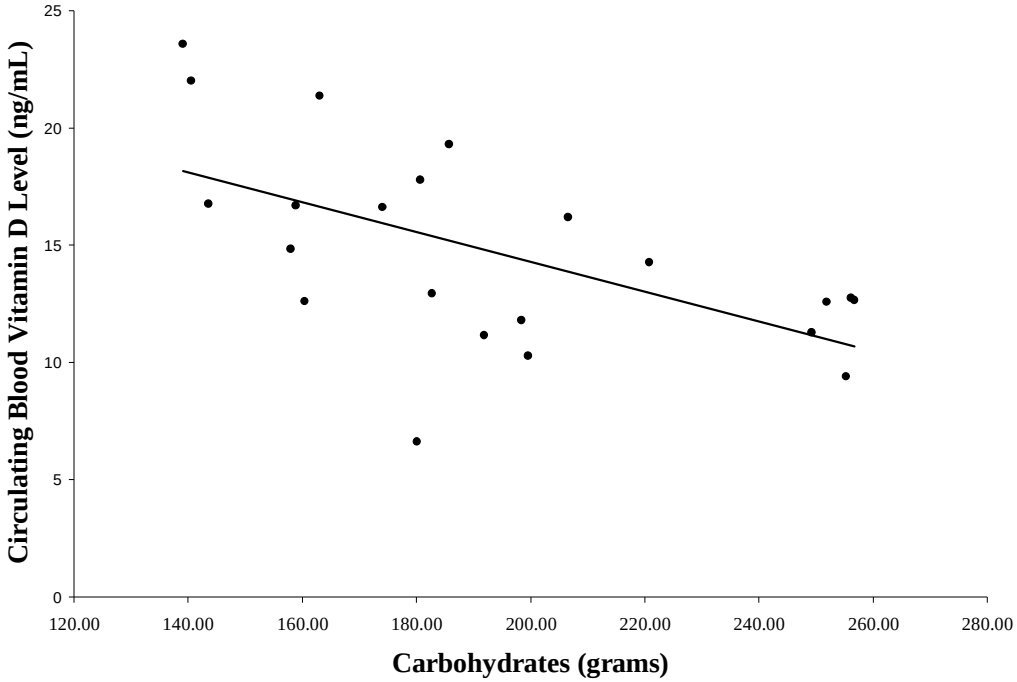


**Figure 1: Circulating Blood Vitamin D versus Carbohydrates ($p<0.01$)**

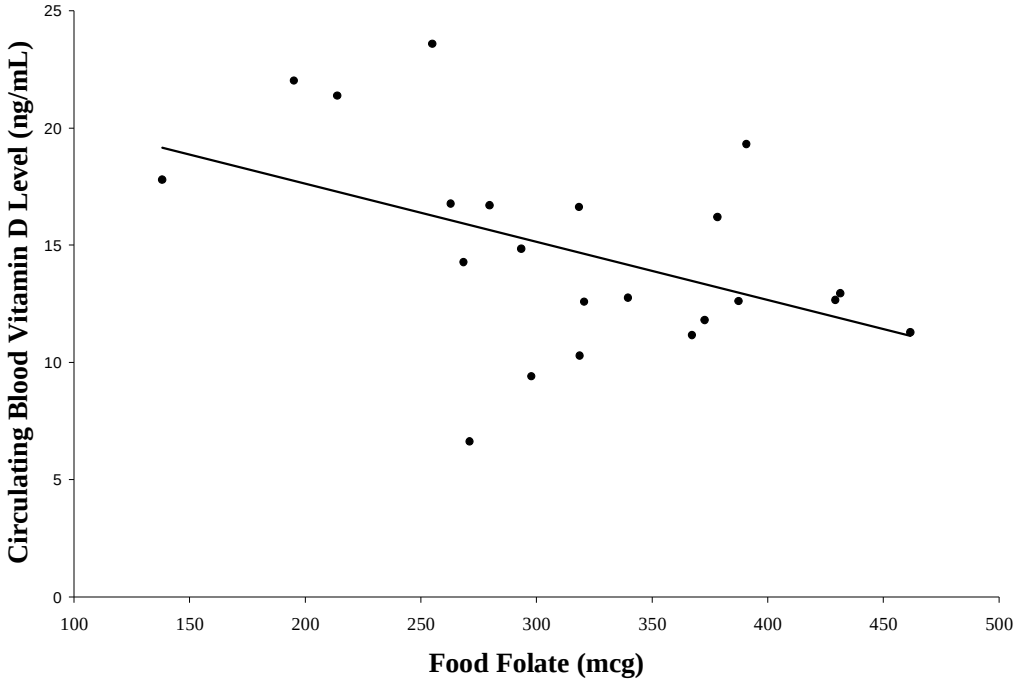


**Figure 2: Circulating Blood Vitamin D versus Food Folate ($p<0.05$)**

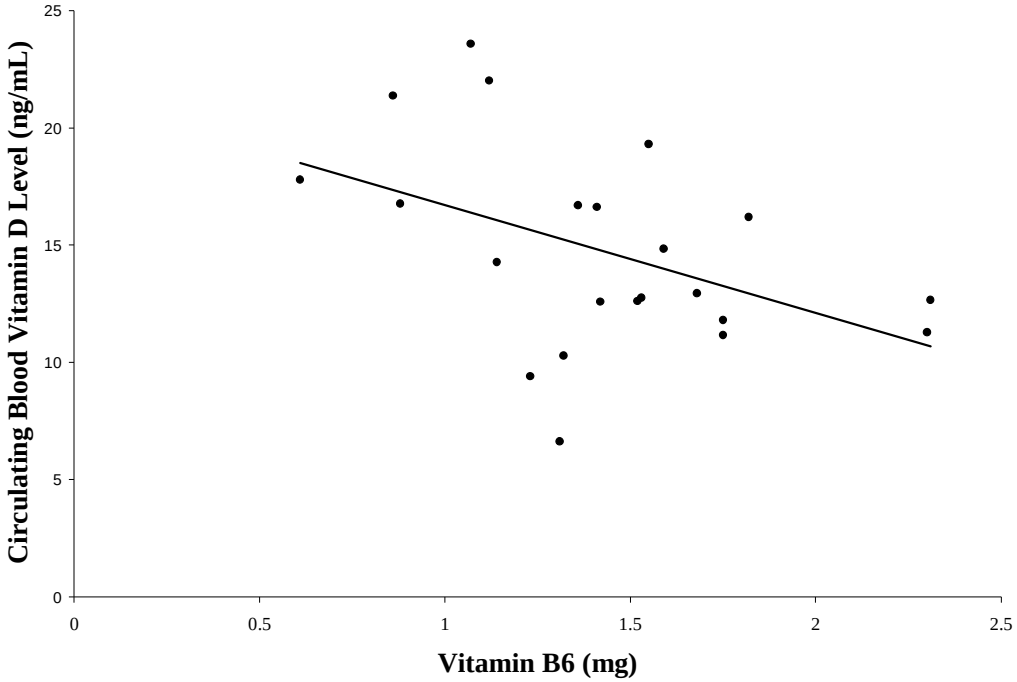


**Figure 3: Circulating Blood Vitamin D versus Dietary Vitamin B6 ($p<0.05$)**

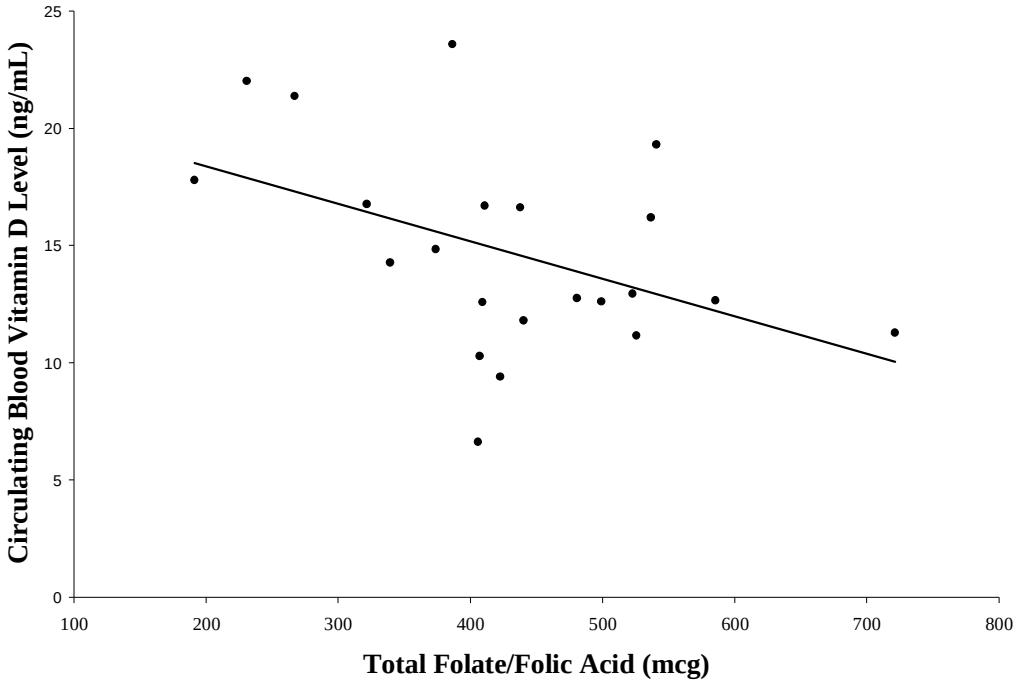


**Figure 4: Circulating Blood Vitamin D Levels versus Total Folate/Folic Acid ($p<0.05$)**

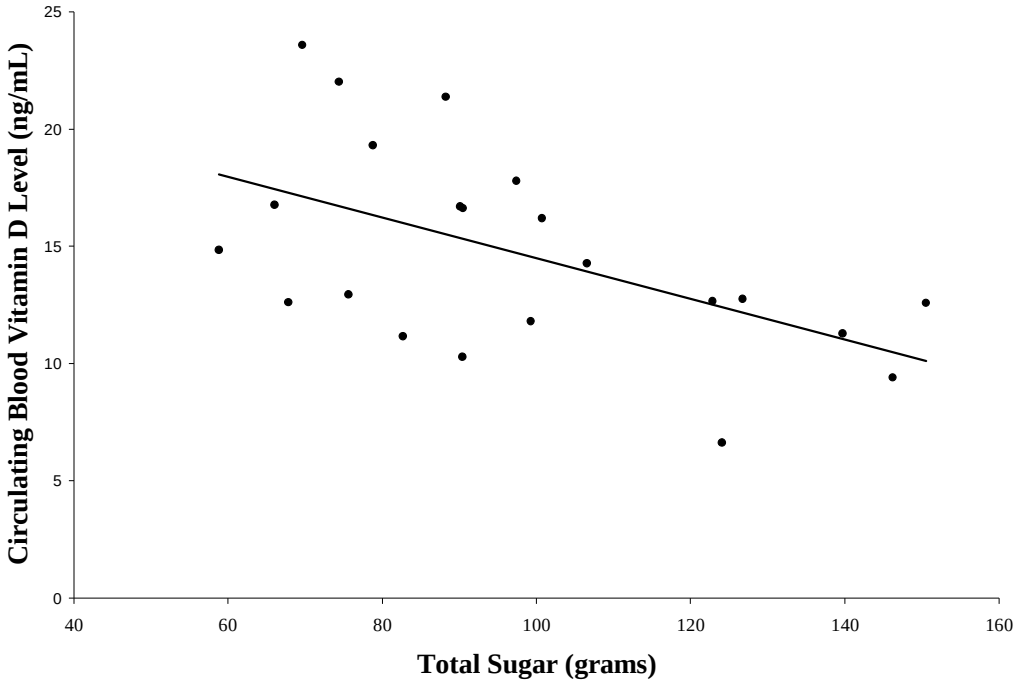


**Figure 5: Circulating Blood Vitamin D versus Total Sugar (p<0.01)**

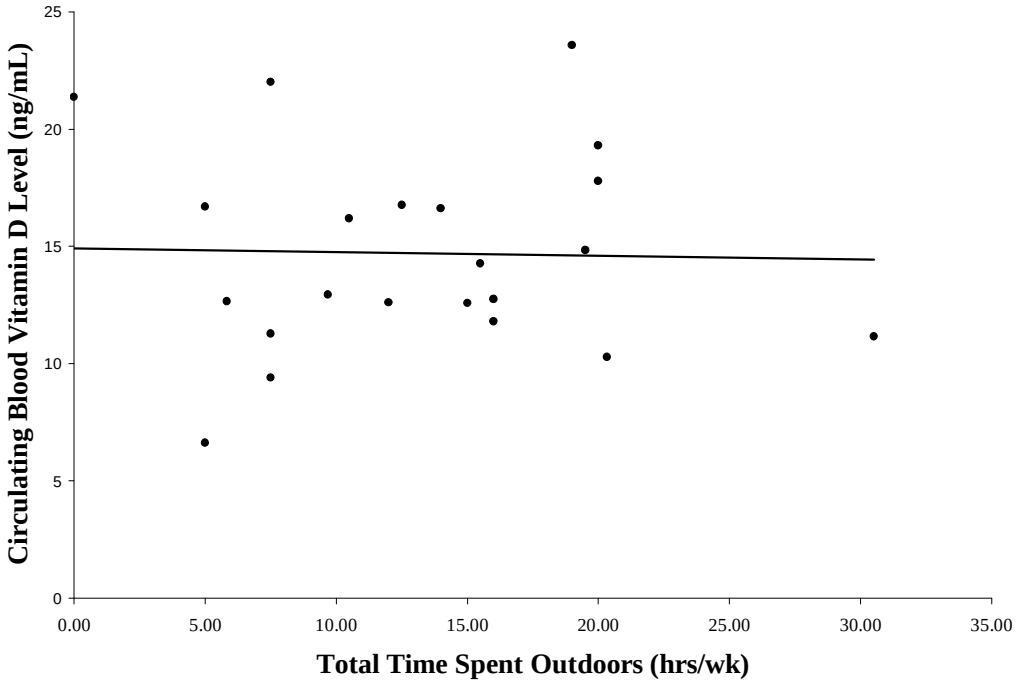


**Figure 6: Total Time Spent Outdoors (estimated) (r=–0.206, p=0.91)**

Bivariate correlations were performed to look for associations between time spent outdoors and indoors and circulating blood vitamin D levels. Figures 6 and 7 show estimated time spent outdoors and indoors (r=–0.206, p=0.91; r=–0.044, p=0.85 respectively). When the variables were the stated levels of each activity, the correlations and p values were r=0.000, p=1.000 for time outdoors and r=-0.155, p=0.49 for time indoors.

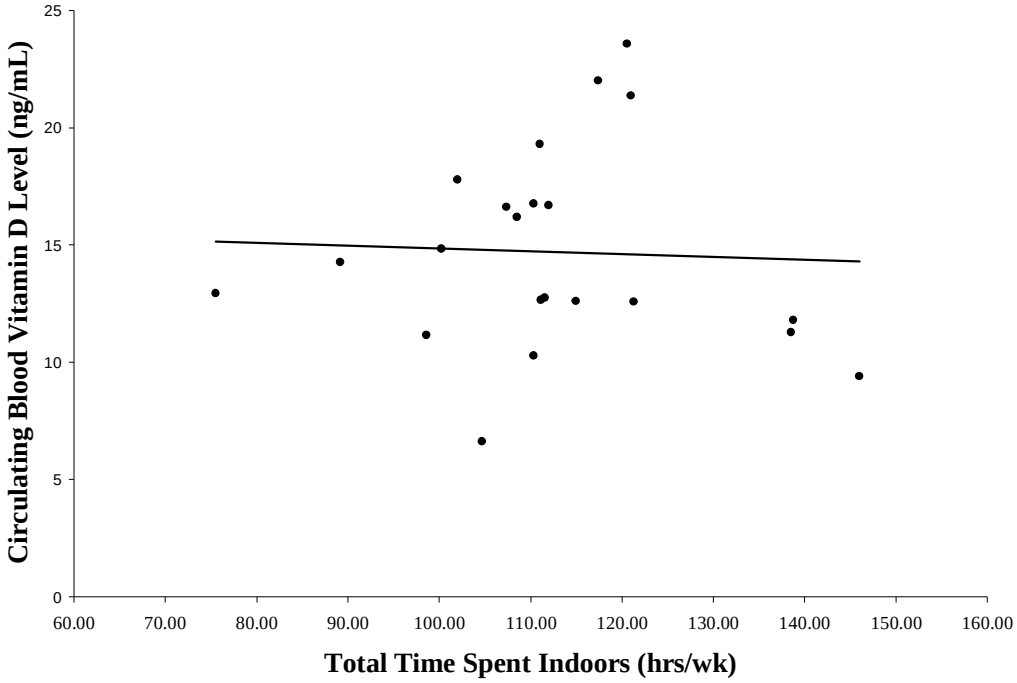


**Figure 7: Total Time Spent Indoors (estimated) (r=–0.044, p=0.85)**

Table 8 repeats and summarizes the bivariate correlations and significance level for the five dietary variables significantly correlated with circulating blood vitamin D levels.

| | DtCarb | DtFolFD | DtVitB6 | DtFolDFE | DtTotSug | BloodVitD |
|---|---|---|---|---|---|---|
| DtCarb | 1 | | | | | |
| DtFolFD | 0.65+ | 1 | | | | |
| DtVitB6 | 0.66+ | 0.91+ | 1 | | | |
| DtFolDFE | 0.68+ | 0.97+ | 0.91+ | 1 | | |
| DtTotSug | 0.93+ | 0.47+ | 0.50+ | 0.54+ | 1 | |
| BloodVitD | -0.59+ | -0.47* | -0.45* | -0.45* | -0.54+ | 1 |

**Table 8: Summarized Correlations Matrix including Significant Dietary Variables**

Table 9 shows the results from a backwards linear regression between blood levels of vitamin D and the six significant dietary covariates in table 8. Each model eliminates the least significant variable, first dietary vitamin B6 followed by dietary carbohydrates, dietary folate/folic acid. The regression leaves two significant variables: total food folate (p=0.048) and total sugar (p=0.016).

| | Variable | Unstandardized Coefficient | Std Error | p value | Adjusted R Squared |
|---|---|---|---|---|---|
| 1 | DtVitB6 | 0.198 | 4.766 | 0.97 | 0.29 |
| | DtCarb | 0.016 | 0.055 | 0.78 | |
| | DtFolDFE | 0.022 | 0.025 | 0.39 | |
| | DtFolFD | -0.054 | 0.040 | 0.19 | |
| | DtTotSug | -0.105 | 0.076 | 0.18 | |
| | *Removing VitB6 for Model 2 | | | | |
| 2 | DtCarb | 0.016 | 0.053 | 0.77 | 0.33 |
| | DtFolDFE | 0.022 | 0.023 | 0.35 | |
| | DtTotSug | -0.105 | 0.074 | 0.17 | |
| | DtFolFD | -0.053 | 0.037 | 0.16 | |
| | *Removing DtCarb for Model 3 | | | | |
| 3 | DtFolDFE | 0.020 | 0.022 | 0.36 | 0.37 |
| | DtFolFD | -0.048 | 0.031 | 0.14 | |
| | DtTotSug | -0.086 | 0.030 | 0.012 | |
| | *Removing DtFolDFE for Model 4 | | | | |
| 4 | DtFolFD | -0.020 | 0.009 | 0.048 | 0.37 |
| | DtTotSug | -0.075 | 0.028 | 0.016 | |

**Table 9: Backwards Linear Regression of Circulating Blood Vitamin D as a Function of Significant Dietary Variables**

Adding each dietary variable back into the model in table 9 that includes total folate and total sugar yielded two additional significant relationships. The linear regression coefficients can be seen in table 10 where calcium and theobromine have been added, with an adjusted R squared of 0.60.

| | Variable | Unstandardized Coefficient | Std Error | p value | Adjusted R Squared |
|---|---|---|---|---|---|
| 1 | DtFolFD | -0.037 | 0.011 | 0.003 | 0.60 |
| | DtTotSug | -0.146 | 0.031 | 0.000 | |
| | DtTheo | 0.064 | 0.026 | 0.024 | |
| | DtCalc | 0.011 | 0.004 | 0.014 | |

**Table 10: Linear Regression of Circulating Blood Vitamin D as a Function of Total Dietary Folate, Total Sugar, Theobromine and Calcium**

Affected status was then added to the model in table 10. Myopia was significant when adjusted for food folate, total sugar, theobromine and calcium. The adjusted R squared increased to 0.68 (table 11). The adjusted mean difference between myopes and non-myopes was a lower blood level of vitamin D in myopes by 2.60 ng.ml.

| Variable | Unstandardized Coefficient | Std Error | p value | Adjusted R Squared |
|---|---|---|---|---|
| DtFolFD | -0.035 | 0.010 | 0.002 | 0.68 |
| DtTotSug | -0.149 | 0.028 | 0.000 | |
| DtTheo | 0.079 | 0.024 | 0.005 | |
| DtCalc | 0.012 | 0.004 | 0.007 | |
| Affected | 2.60 | 1.12 | 0.033 | |

**Table 11: Linear Regression of Circulating Blood Vitamin D as a Function of Food Folate, Total Sugar, Theobromine, Calcium and Affected Group**

| Variable | Unstandardized Coefficient | Std Error | p value | Adjusted R Squared |
|---|---|---|---|---|
| DtFolFD | -0.035 | 0.008 | 0.001 | |
| DtTotSug | -0.12 | 0.028 | 0.001 | |
| DtTheo | 0.10 | 0.023 | 0.000 | 0.76 |
| DtCalc | 0.010 | 0.003 | 0.006 | |
| Age | 0.32 | 0.13 | 0.026 | |
| Affected | 3.41 | 1.03 | 0.005 | |

**Table 12: Linear Regression of Circulating Blood Vitamin D as a Function of Food Folate, Total Sugar, Theobromine, Calcium, Affected Group and Age**

Other covariates such as age and gender were then tested in the model. Age was significant once adjusted for covariates: food folate, total sugar, theobromine, calcium, and myopia affected status. The adjusted R squared increased to 0.76 (table 12). The adjusted mean difference between myopes and non-myopes was a lower blood level of vitamin D in myopes by 3.41 ng.ml. Food folate and total sugar were associated with lower blood levels of vitamin D and that theobromine, calcium, and older age were associated with higher blood levels of vitamin D. Gender was not a significant term in the model ($F=0.25$, $df=1, 14$, $p=0.63$).

| Variable | F (df=1, 20) | Sig | Variable | F (df=1, 20) | Sig |
|---|---|---|---|---|---|
| DtKcal | 2.71 | 0.12 | DtCrypt | 1.72 | 0.20 |
| DtProt | 2.22 | 0.15 | DtLutein | 1.72 | 0.20 |
| DtTFat | 3.84 | 0.06 | DtLycope | 0.34 | 0.57 |
| DtCarb | 0.96 | 0.34 | DtRetinol | 0.43 | 0.52 |
| DtCalc | 0.10 | 0.76 | DtCarote | 1.64 | 0.22 |
| DtPhos | 3.41 | 0.08 | DtVitAR | 3.22 | 0.09 |
| DtIron | 0.13 | 0.72 | DtVitEAt | 0.49 | 0.49 |
| DtSodi | 3.95 | 0.06 | DtVit B12 | 0.00 | 1.00 |
| DtPota | 4.10 | 0.06 | DtCopper | 8.45 | 0.01+ |
| DtThia | 0.00 | 0.95 | DtSelnium | 2.08 | 0.16 |
| DtRibo | 0.15 | 0.71 | DtFolfort | 2.34 | 0.14 |
| DtNiac | 0.00 | 1.00 | DtFolFood | 5.97 | 0.02* |
| DtVitC | 0.16 | 0.70 | DtFolDFE | 0.14 | 0.72 |
| DtSFat | 3.65 | 0.07 | DtVitK | 2.11 | 0.16 |
| DtMFat | 2.21 | 0.15 | DtTheoBr | 1.37 | 0.26 |
| DtPFat | 3.32 | 0.08 | DtTotSug | 0.05 | 0.83 |
| DtChol | 0.93 | 0.35 | DtOme3 | 3.95 | 0.06 |
| DtFibe | 8.26 | 0.01+ | DtOme6 | 3.16 | 0.09 |
| DtFolFD | 0.12 | 0.73 | DtVit D | 0.30 | 0.59 |
| DtZinc | 0.03 | 0.87 | DtTranFat | 0.05 | 0.83 |
| DtAnZin | 1.08 | 0.31 | GrpSoldTo | 5.02 | 0.04* |
| DtVitB6 | 0.28 | 0.60 | TotalVitD | 0.63 | 0.44 |
| DtMagn | 6.90 | 0.02* | BloodVitD | 1.19 | 0.29 |
| DtAcaro | 0.62 | 0.44 | | | |
| DtBcaro | 1.91 | 0.18 | | | |

**Table 13: One Way ANOVA Comparing Dietary Intake in Myopes versus Non-Myopes (+ $p \leq 0.01$; *$p \leq 0.05$)**

Table 13 compares means of dietary variables in myopes and non-myopes. Significant differences between groups include fiber, magnesium, copper, and food folate.

Adding magnesium (F=0.40, df=1, 14, p=0.54), fiber (F=0.78, df=1, 14, p=0.39) or copper (F=2.84, df=1, 14, p=0.11) to the linear regression did not produce any significant relationships or improvements to the model.

The genetics analysis was conducted using logistic regression. None of the SNPs within *VDR* was significantly associated with being myopic at this sample size.

| SNP (alleles) | Odd Ratio | 95% CI | p value |
|---|---|---|---|
| rs7975232 (C:A) | 2.29 | 0.67-7.81 | 0.19 |
| rs2239182 (G:A) | 0.35 | 0.062-1.97 | 0.23 |
| rs2189480 (C:A) | 0.50 | 0.14-1.73 | 0.27 |
| rs3819545 (C:T) | 2.14 | 0.51-9.04 | 0.30 |
| rs3782905 (C:G) | 0.46 | 0.11-1.94 | 0.29 |
| rs10735810 (A:G) | 1.67 | 0.28-9.82 | 0.57 |
| rs2853559 (C:T) | 0.51 | 0.11-2.35 | 0.39 |
| rs4516035 (C:T) | 3.33 | 0.50-22.1 | 0.21 |
| rs10877013 (C:T) | 1.44 | 0.32-6.40 | 0.63 |

**Table 14: Binary Logistic Regression of SNP genotype categories. The odds ratios represent the increase in the odds of being myopic associated with each copy of the first allele compared to the second.**

## Chapter 4: Discussion

The recorded increase in myopic prevalence is likely to have an environmental background. Near work has become a controversial topic regarding development and progression of myopia. Some research supports an effect of near work on the prevalence of myopia (Bear et al., 1981; Young et al., 1969; Zylbermann et al., 1993). In contrast, no statistical effects of near work were found between refractive groups in other articles (Jones et al., 2007). Myopes and non-myopes spent a similar amount of time reading (37.85 ± 14.01, 35.56 ± 9.08 hours per week respectively; $p>0.05$). Similarly, Jones et al. found no effect of near work on the risk of 3$^{rd}$ graders becoming myopic by the 8$^{th}$ grade (Jones et al., 2007). Third graders with two myopic parents and outdoor activity in the lowest quartile were at greatest risk for developing myopia by the eighth grade. This finding suggests that outdoor activity may be a more important environmental variable than near work.

Outdoor activity was in fact found to be protective for developing myopia (Dirani et al., 2009). A previous report from Dirani et al. (Dirani et al., 2006) did not find this relationship, however, this is likely due to the format in which the survey addressed outdoor activity. Similarly, it is possible that the small sample size of

this study may not have had sufficient statistical power for finding significance. A larger sample size might have yielded significance between the two populations due to the substantial size of this protective role (Dirani et al., 2009; Jones et al., 2007; Rose et al., 2008a). On the other hand, the protective effects of time outdoors might not apply to Asian children. The recent discovery of the protective impact of outdoor activity (Rose et al., 2008a) has changed thinking away from near work to high intensity light involvement (Ashby et al., 2009) and myopic defocus (Zhu et al., 2003) as a possible player in this protective role.

The protective effect of time outdoors and additional research showing the slowing of myopic progression in the summer months (Fulk et al., 2002) suggest a role for vitamin D involvement. The hypothesis being studied is that higher vitamin D levels are seen in non-myopes due to their increased levels of outdoor activity that has been seen in past research (Dirani et al., 2009; Rose et al., 2008a). Myopes did have lower blood levels of vitamin D compared to non-myopes by 3.41 ng/ml when adjusted for age and dietary variables that affect blood vitamin D levels (table 12). Dietary sugar and folate were negatively associated with vitamin D, while theobromine and calcium were positively associated with vitamin D levels. Further analysis including one way ANOVA compared the means of each refractive group (myope, non-myope and unknown). As seen in tables 4 and 5, the means of each variable analyzed were not significantly different between the three refractive groups. Based on the reported hours per week spent engaging in each activity of interest there was no difference

between myopes and non-myopes for outdoor activity, indoor activity, reading, sports or other. Post-hoc testing was not necessary due to the absence of any significant relationships. Outdoor activity was also not a significant factor in the multiple regression with vitamin D. Therefore it seems that the differences in blood vitamin D level in this sample were more intrinsic differences rather than differences due to diet or time outdoors. The lack of significance in the genetic analysis did not suggest that the source of any intrinsic difference was due to variation in the tested SNPs in *VDR*.

**Activity Validation**

One reason that time outdoors was not significant might have been the small sample size in the current study. Another reason might be that the activity survey is not very accurate. However the activity survey has been used successfully for several measurements of time spent outdoors (Ip et al., 2007; Ip, Rose, Morgan, Burlutsky and Mitchell, 2008; Ojaimi, Rose, Smith, Morgan, Martin and Mitchell, 2005). The Sydney Myopia Group has attempted to produce a survey that breaks down the activities done and their respective location (indoors versus outdoors). The survey asks questions similarly for weekday and non-weekday, however they were not exactly the same.

The use of this survey allowed for well-known method of measurement rather than the need for development of a new activity survey. It was a useful starting point, but through analysis, some limitations have been discovered. The

comparison of stated time indoors or outdoors versus summing time spent on activities indoors or outdoors was used as an internal validation. For outdoor activities, stated versus estimated hours were significantly different (table 3, p=0.03). Indoor stated versus estimated hours were not significantly different (table 3, p=0.07). However, subjects were only asked to state the time indoors for non-weekdays, not for weekdays. This makes things difficult to infer for indoor activity validation.

Outdoor and indoor activities were calculated as shown in Appendix B and C. The location of activities (i.e.: indoors and outdoors) were asked in two separate ways. The first approach asked the subject to state the hours spent outdoors and indoors on a typical day. Outdoor activity asked the hours per day on a typical weekday and non-weekday. In regards to indoor activity, subjects were only asked how much time per day they spent indoors on a non-weekday. Subjects were also asked to estimate the length of time they spent doing predetermined activities while outdoor and indoors. These were summed to estimate time spent indoors and outdoors. Activities in question included reading, computer work, sports, handwriting, and school work. The activities were classified into one of three categories, reading, sports or other. The categories were then summed into time spent participating in activities either indoor or outdoor.

For a cross-sectional study design, this was probably not ideal. This research was conducted from early January through late September. Subjects found it difficult

to answer the questions about specific activities when thinking about the entire year due to the variation in activity conducted throughout the year. It was recommended to answer on a typical day.

**Vitamin D and Diet**

Vieth reports that 200 IU/day may prevent osteomalacia in the absence of sunlight but levels near 800-1000 IU per day may be more beneficial particularly in the elderly populations (Vieth, 1999). Block FFQ measured a mean total dietary intake of vitamin D of 245.33 ± 214.31 IU/day, with a range of 26.09 to 782.42 IU/day for the entire sample, with myopes and non-myopes usual dietary consumption of 261.40 ± 215.20 and 190.00 ± 176.80 IU/day respectively. The unknown group had the highest level of vitamin D intake, 267.14 ± 251.77 IU/day, which was not statistically different from myopes and non-myopes. These levels are higher than the 136 IU/day reported for French adults (Chapuy et al., 1997).

Circulating vitamin D levels were on average 14.70 ± 4.29 ng/ml (6.62-23.57 ng/ml). Insufficiency definitions have varied (table 1) (Alpert et al., 2007; Diehl et al., 2010; Lips et al., 1988; Malabanan et al., 1998). Myopes had blood levels of 13.95 ± 3.75 ng/ml while non-myopes had blood levels of 16.02 ± 5.11ng/ml. As table 5 demonstrates, these values are not significantly different, however, non-myopes overall had higher circulating levels once dietary covariates were adjusted for in the multiple regression (table 12).

The amount of blood vitamin D measured for a subject did not show any relationship with time spent outdoors. No positive or negative relationships were seen in Figures 6 and 7. Correlations were not significant and blood levels were not related to time spent either outdoors or indoors.

Block FFQ gave extensive data regarding subjects' typical dietary habits. Bivariate correlations compare two variables to measure the significance of the relationship between two dietary variables. The significant variables are listed in Table 7. Dietary variables of interest were those with significant correlations with circulating levels of blood vitamin D. The five significant correlations included total sugar, food folate, folate/folic acid, vitamin B6 and carbohydrates. Figures 1-5 plot the corresponding relationships. Interestingly, the greater the amount of each nutrient consumed in a subject's diet the lower the circulating vitamin D. Dietary food folate and total sugar were significant in the multiple regression with circulating blood vitamin D.

Zamboni et al followed 16 obese children with diets high in carbohydrates and calories. They observed a negative relationship between high carbohydrates and calcium levels. Furthermore, these children were found to have corresponding low vitamin D levels. Similarly, our data supports this positive correlation between calcium and vitamin D and the negative correlation between sugar and vitamin D (Zamboni, Soffiati, Giavarina and Tato, 1988).

Folate is a water-soluble vitamin responsible for RNA and DNA synthesis (Hatzis, Bertsias, Linardakis, Scott and Kafatos, 2006) as well as production of neurotransmitters and amino acids. Insufficiency can lead to vascular disease, anemia and increased risk for cancer (Tapiero, Tew, Gate and Machover, 2001). Sources of dietary food folate can be found in breads and cereals, fruits and vegetables (Suitor and Bailey, 2000). Theobromine on the other hand, is most often found in snacks, such as Coca-Cola, chocolate and cocoa beverages (Eteng, Eyong, Akpanyung, Agiang and Aremu, 1997). Many children obtain theobromine through chocolate covered snacks (Ahuja, 2001). Somewhat like caffeine, the major effect of theobromine in the diet is as a central nervous system stimulant (Eteng et al., 1997).

**SNP Analysis**

SNPs entered into a logistic regression in backwards fashion had no significant associations with myopia. Odds ratios were not significant for any SNP entered as individual variables either. SNP rs4516035 had the highest odds ratio associated with myopia at 3.33 (95% CI = 0.50-22.14). Odds ratio quantify the increase in odds of having a disease when comparing exposure versus no exposure to risk. However the sample size is very small to expect any significant associations in a SNP analysis. Only 22 saliva samples were submitted for SNP analysis. Genome-wide SNP studies often use hundreds to thousands of subjects.

**Limitations**

There are many limitations to this study. The first is the small sample size. The low response rate from Worthington School Districts required an expansion to easily accessible subjects, which included Ohio State College of Optometry students. Although not ideal for data collection the Optometry students made up 56% of the subject data. Initially college students were not intended for the study in hopes to prevent any bias in data due to any changes in diet and activity when moving to the collegiate lifestyle which likely would not reflect the lifestyle the subject had while becoming myopic. However, in attempts to achieve a larger sample size to achieve the greater power desired, a more accessible sample was necessary. Remarkably, there was a statistically significant lower blood level of vitamin D in myopes compared to non-myopes by 3.41 ng/ml (8.52 nmol). Despite the small sample size, the final model accounted for a large amount of the variance in blood vitamin D at 76%.

Over the several months of research, seasons changed along with their activities and sun exposure. This may have lead to artificially high or low measurements from one subject to another. It may be best to ask questions regarding seasonal activities or collect data on several days throughout the year to have more comprehensive data of each subjects' activities throughout the year.

**Further Directions**

The goal of this pilot cross-sectional study was to collect preliminary data on vitamin D. The idea was to see if there were any significant relationship that could continue to unveil information about the development and progression of myopia. To further develop information on vitamin D, it will be important to get a larger sample size first and foremost. An activity survey needs to be developed and validated to best accommodate the activities of interest. The study conducted may have better benefitted from a list of activities that would be asked about indoor and outdoor time and the subject could state if they engaged in the activity, the length of time, and whether it was indoors or outdoors. The Block FFQ seemed to work well for the design of this study; it was short yet covered the foods that our subjects ate. The original question about whether the effect of time outdoors is due to cutaneous production of vitamin D still remains unanswered. The significantly lower value in blood levels of vitamin D among myopes suggests that this hypothesis is still a reasonable one.

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## Appendix A: Activity Survey

Child ID ☐☐☐

**CONTACT DETAILS**

Child Name: _____

Home phone: _____

Mobile phone: _____

Email: _____

**QUESTIONS ABOUT HOW YOU SPEND A TYPICAL SCHOOL/WORK DAY**

1) **SCHOOL/WORK:** How many days per week do you attend school or work ☐
*(days)*

2) **SLEEP:** When do you usually go to sleep at night? ☐☐.☐☐
*(hour)*. *(minute)*

3) **SLEEP:** What time do you usually wake up in the morning? ☐☐.☐☐
*(hour)*. *(minute)*

4) **BEFORE YOU LEAVE FOR SCHOOL:** After you wake up in the morning and before you leave for school/work, do you spend any time outside?
☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

5) **TRAVEL:** What time do you leave home to go to school/work? ☐☐.☐☐
*(hour)*. *(minute)*

6) **TRAVEL:** How do you travel to school/work and for how long?
☐ Bus, train or tram ☐☐
☐ Car ☐☐
☐ Walking, bicycle or motorbike ☐☐
*(minutes)*

7) **TRAVEL:** What time do you arrive at school/work? ☐☐.☐☐
*(hour)*. *(minute)*

8) **SCHOOL/WORK:** After you arrive at school/work, do you spend any time outside before school starts?
   - ☐ Not at all
   - ☐ less than an hour
   - More than one hour (please specify) ☐☐ *(hours)*

9) **SCHOOL/WORK:** What time do you usually start school/work ☐☐.☐☐ *(hour)***.** *(minute)*

10) **SCHOOL/WORK:** In the middle of your school/work day, do you spend any time outside?
   - ☐ Not at all
   - ☐ less than an hour
   - More than one hour (please specify) ☐☐ *(hours)*

11) **SCHOOL/WORK:** What time do you usually finish school/work ☐☐.☐☐ *(hour)***.** *(minute)*

12) **SCHOOL/WORK:** After school/work finishes, do you spend any time outside before leaving to go home?
   - ☐ Not at all
   - ☐ less than an hour
   - More than one hour (please specify) ☐☐ *(hours)*

13) **TRAVEL:** What time do you leave school/work to go to home? ☐☐.☐☐ *(hour)***.** *(minute)*

14) **TRAVEL:** Do you travel to home the same way as you traveled in the morning?
   - ☐ Yes
   - ☐ No, *if so how do you travel?*
     - ☐ Bus, train or tram ☐☐
     - ☐ Car ☐☐
     - ☐ Walking, bicycle or motorbike ☐☐

     *(minutes)*

15) **TRAVEL:** What time do you arrive at home? ☐☐.☐☐ *(hour)***.** *(minute)*

16) **AFTER YOU ARRIVE HOME:** After you arrive home and before nighttime do you spend any time outside?
   - ☐ Not at all
   - ☐ less than an hour
   - More than one hour (please specify) ☐☐ *(hours)*

*We would now like to ask you about how you spend your time when you are not in school or asleep. We need to know how long you are indoors or outdoors and what kinds of activities you do. We will start with indoor activities. Remember **do not** include school/work or sleep time. Indoor time can include bus, car or train travel, but not walking, riding a bicycle or motorbike.*

17) On a typical school/work day, **WHILE YOU ARE INDOORS**, for how long (per day) do you do the following activities:

17a)Read printed material for pleasure, for example reading a magazine or novel?
☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

17b)Read printed material or do handwriting for study/work?
☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

17c)Use computers for study/work/pleasure?
☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

17d)Watch television/go to the movies?
☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

17e)Play sports or exercise indoors?
☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

17f)Are there any other indoor activities that you would do for **more than 1 hour** in a typical day? ☐ Not at all
☐ Yes 1. Please specify the activity _____ ☐☐
*(hours)*

2. Please specify the activity _____ ☐☐
*(hours)*

3. Please specify the activity _____ ☐☐
*(hours)*

18) **OUTDOORS:** How many hours do you spend outdoors in a day ☐☐ ***DO NOT*** *include school/work/sleep* *(hours)*

18a) While you are outdoors, do you do any close work activities such as; reading for pleasure or study, use computers or watch television?

☐ No *(if answered no, proceed to question 18b)*

☐ Yes Please specify the activity _____ ☐☐ *(hours)*

☐ Another? Please specify the activity _____ ☐☐ *(hours)*

18b)Play sports or exercise outdoors?

☐ Not at all

☐ less than an hour

More than one hour (please specify) ☐☐ *(hours)*

18c)Are there any other outdoor activities that you would do for **more than 1 hour** in a typical day, for example walking, gardening or shopping outside?

☐ Not at all

☐ Yes Please specify the activity _____ ☐☐ *(hours)*

☐ Another? Please specify the activity _____ ☐☐ *(hours)*

**QUESTIONS ABOUT HOW YOU SPEND A TYPICAL NON-SCHOOL/WORK DAY**

19) **SCHOOL/WORK:** Do you attend any academic tuition classes, for example mathematics or language or music classes on a typical non-school/work day?

☐ Not at all

☐ less than an hour

More than one hour (please specify) ☐☐ *(hours)*

20) **SLEEP:** When do you usually go to sleep at night? ☐☐**.**☐☐ *(hour)***.** *(minute)*

21) **SLEEP:** What time do you usually wake up in the morning? ☐☐**.**☐☐ *(hour)***.** *(minute)*

22) **INDOORS:** How many hours do you spend indoors in a day ☐☐ ***DO NOT*** *include sleep or academic tuition classes* *(hours)*

23) On a typical non- school/work day, **WHILE YOU ARE INDOORS**, for how long (per day) do you do the following activities:

23a)Read printed material for pleasure, for example a magazine or novel?

☐ Not at all

☐ less than an hour

More than one hour (please specify) ☐☐ *(hours)*

23b)Read printed material or do handwriting for study/work?

☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

23c)Use computers for study/work/pleasure?

☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

23d)Watch television/go to the movies?

☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

23e)Play sports or exercise indoors?

☐ Not at all
☐ less than an hour
More than one hour (please specify) ☐☐
*(hours)*

23f)Are there any other indoor activities that you would do for **more than 1 hour** in a typical day?

☐ Not at all
☐ Yes 1. Please specify the activity _____ ☐☐
*(hours)*

2. Please specify the activity _____ ☐☐
*(hours)*

3. Please specify the activity _____ ☐☐
*(hours)*

24) **OUTDOORS:** How many hours do you spend outdoors in a day ☐☐ ***DO NOT*** *include school/work/sleep* *(hours)*

25) On a typical non-school/work day, **WHILE YOU ARE OUTDOORS**, for how long (per day) do you do the following activities:

25a)While you are outdoors, do you do any close work activities such as; reading for pleasure or study, use computers or watch television?

☐ No *(if answered no, proceed to question 18b)*
☐ Yes Please specify the activity _____ ☐☐
*(hours)*

☐ Another? Please specify the activity _____ ☐☐
*(hours)*

25b)Play sports or exercise outdoors?

☐ Not at all
☐ less than an hour

More than one hour (please specify) ☐☐
*(hours)*

25c)Are there any other outdoor activities that you would do for **more than 1 hour** in a typical day, for example walking, gardening, shopping outside, lying by a pool, barbecue, or picnic?

☐ Not at all
☐ Yes Please specify the activity _____ ☐☐
*(hours)*

☐ Another? Please specify the activity _____ ☐☐
*(hours)*

Comments: _____

_____

## QUESTIONS ABOUT SUN AND YOUR HEALTH

*The next few questions are about occasions last summer when you were outside in the sun for at least 15 minutes. Please think about actions you usually took for sun protection on these occasions.*

26. Thinking back to last summer, how often did you go out in the sun for more than 15 minutes between 11 am and 3 pm?
□ Always
□ Often
□ Sometimes
□ Rarely or never
□ Never in the sun for more than 15 minutes
□ Don't know

27. Still thinking of last summer, how often did you get sun burnt, so your skin was still sore or tender the next day?
□ Not at all
□ Once
□ Twice
□ 3 or 4 times
□ 5 or more times
□ Don't know

28. Still thinking of last summer, when you were out in the sun for more than 15 minutes, how often did you wear a broad brimmed hat or cap.
□ Always
□ Often
□ Sometimes
□ Rarely or never
□ Don't know

29. Still thinking about last summer, how often did you apply broad-spectrum sunscreen with an SPF of 15 or more?
□ Always
□ Often

□ Sometimes
□ Rarely or never
□ Don't know

30. Still thinking about last summer, how often did you dress in clothing to protect yourself from the sun?
□ Always
□ Often
□ Sometimes
□ Rarely or never
□ Don't know

31. In the past 48 hours, how often did you go out in the sun for 15 minutes or more between 11 am or 3 pm?
□ Always
□ Often
□ Sometimes
□ Rarely or never
□ Never in the sun for more than 15 minutes
□ Don't know

32. Still thinking about the past 48 hours, how often did you get sun burnt, so your skin was still sore or tender the next day?
□ Not at all
□ Once
□ Twice
□ 3 or 4 times
□ 5 or more times
□ Don't know

33. Still thinking about the past 48 hours, when you were out in the sun for more than 15 minutes, how often did you wear a broad brimmed hat or cap.
□ Always
□ Often
□ Sometimes
□ Rarely or never
□ Don't know

34. Still thinking about the past 48 hours, how often did you apply broad-spectrum sunscreen with an SPF of 15 or more?
□ Always
□ Often
□ Sometimes
□ Rarely or never
□ Don't know

35. Still thinking about the past 48 hours, how often did you dress in clothing to protect yourself from the sun?
□ Always
□ Often
□ Sometimes
□ Rarely or never
□ Don't know

**Appendix B: Equations for Calculating State and Estimated Time Spent Outdoors and Indoors.**

| Activity | | Equation |
|---|---|---|
| Outdoor Stated | Weekday | [(4+6c+8+10+12+14d+16)*Q1] +(18*Q1) |
| | Non-Weekday | (24*Q2) |
| | **Total** | **Weekday + Non-Weekday** |
| Outdoor Estimate | Weekday | [(4+6c+8+10+12+14d+16)*Q1] +[(18a+18b+18c)*Q1] |
| | Non-Weekday | [(25a+25b+25c)*Q2] |
| | **Total** | **Weekday + Non-Weekday** |
| Indoor Stated | Weekday | **{**[(5-3)-4]+6a+6b+[(9-7)-8]+[(11-9)-10]+[(13-11)-12]+6a+6b+[(15-2)-16]*Q2**}** +(17a+17b+17c+17d+17e+17f)*Q1 |
| | Non-Weekday | (22*Q2) |
| | **Total** | **Weekday + Non-Weekday** |
| Indoor Estimated | Weekday | **{**[(5-3)-4]+6a+6b+[(9-7)-8]+[(11-9)-10]+[(13-11)-12]+6a+6b+[(15-2)-16]*Q2**}** +(17a+17b+17c+17d+17e+17f)*Q1 |
| | Non-Weekday | [(19+23a+23b+23c+23d+23e+23f)*Q2] |
| | **Total** | **Weekday + Non-Weekday** |

## Appendix C: Equations to Calculation Hours per Week Spent on Total Reading, Sports and Other

| Activity | Day | Location | Equation |
|---|---|---|---|
| Total Reading | Weekday | Indoor | [(17a+17b+17c+17d)*Q1] |
| | | Outdoor | 18a*Q1 |
| | | **Total** | **Indoor + Outdoor** |
| | Non-Weekday | Indoor | [(23a+23b+23c+23d)*Q2] |
| | | Outdoor | 25a*Q2 |
| | | **Total** | **Indoor + Outdoor** |
| | **Total** | | **Weekday + Non-Weekday** |
| | | | |
| Total Sports | Weekday | Indoor | (17e*Q1) |
| | | Outdoor | (18b*Q1) |
| | | **Total** | **Indoor + Outdoor** |
| | Non-Weekday | Indoor | (23e*Q2) |
| | | Outdoor | (25b*Q2) |
| | | **Total** | **Indoor + Outdoor** |
| | **Total** | | **Weekday + Non-Weekday** |
| | | | |
| Total Other | Weekday | Indoor | (17f*Q1) |
| | | Outdoor | (18c*Q1) |
| | | **Total** | **Indoor + Outdoor** |
| | Non-Weekday | Indoor | (23f*Q2) |
| | | Outdoor | (25c*Q2) |
| | | **Total** | **Indoor + Outdoor** |
| | **Total** | | **Weekday + Non-Weekday** |

## Appendix D: Summarized Data for Each Subject

| SID | Total Outdoor Stated | Total Outdoor Estimated | Total Indoor Stated | Total Indoor Estimated | Total Reading | Total Sports | Total Other |
|---|---|---|---|---|---|---|---|
| 1 | 24.00 | 14.00 | 100.36 | 107.36 | 22.50 | 15.00 | 14.00 |
| 2 | 12.00 | 12.00 | 108.89 | 108.89 | 43.50 | 7.00 | 2.00 |
| 3 | 5.00 | 5.00 | 120.67 | 104.67 | 30.50 | 0.00 | 0.00 |
| 4 | 6.00 | 9.00 | 117.92 | 120.92 | 41.50 | 3.00 | 0.00 |
| 5 | 30.00 | 15.00 | 121.27 | 121.27 | 40.50 | 11.00 | 10.50 |
| 6 | 21.50 | 12.50 | 92.33 | 110.33 | 45.50 | 2.00 | 7.00 |
| 7 | 16.00 | 14.00 | 117.83 | 124.83 | 44.00 | 2.00 | 17.00 |
| 8 | 11.00 | 12.50 | 127.85 | 101.85 | 16.50 | 12.00 | 7.50 |
| 9 | 13.50 | 30.50 | 112.60 | 98.60 | 25.50 | 12.50 | 15.00 |
| 10 | 9.00 | 10.50 | 112.50 | 108.50 | 33.50 | 0.00 | 8.00 |
| 11 | 7.00 | 5.00 | 126.00 | 112.00 | 47.00 | 0.00 | 0.00 |
| 12 | 14.50 | 16.00 | 104.58 | 111.58 | 43.00 | 5.50 | 7.00 |
| 13 | 7.50 | 7.50 | 134.49 | 138.49 | 47.50 | 11.50 | 9.00 |
| 14 | 14.50 | 15.50 | 99.14 | 89.14 | 21.00 | 0.00 | 15.00 |
| 15 | 12.00 | 9.68 | 107.00 | 75.50 | 14.68 | 5.50 | 3.00 |
| 16 | 12.00 | 10.00 | 129.00 | 139.00 | 69.00 | 0.00 | 0.00 |

continued

Appendix D continued

| SID | Total Outdoor Stated | Total Outdoor Estimated | Total Indoor Stated | Total Indoor Estimated | Total Reading | Total Sports | Total Other |
|---|---|---|---|---|---|---|---|
| 17 | 3.00 | 0.00 | 124.00 | 121.00 | 48.00 | 5.00 | 0.00 |
| 18 | 8.50 | 7.50 | 142.03 | 146.03 | 68.00 | 2.50 | 3.00 |
| 19 | 16.50 | 13.35 | 129.67 | 144.67 | 62.85 | 8.50 | 0.00 |
| 20 | 14.00 | 12.00 | 117.97 | 114.97 | 40.50 | 6.00 | 0.00 |
| 21 | 14.00 | 19.00 | 126.58 | 120.58 | 44.00 | 9.00 | 9.00 |
| 22 | 28.00 | 19.50 | 113.22 | 100.22 | 28.50 | 5.00 | 7.00 |
| 23 | 16.50 | 12.50 | 121.58 | 107.58 | 25.00 | 5.00 | 2.50 |
| 24 | 16.33 | 5.83 | 116.14 | 111.14 | 26.00 | 5.00 | 3.00 |
| 25 | 22.67 | 20.33 | 105.35 | 110.35 | 37.17 | 10.50 | 0.00 |
| 26 | 22.33 | 15.83 | 124.33 | 117.33 | 39.50 | 9.50 | 0.00 |
| 27 | 18.17 | 14.17 | 117.33 | 117.33 | 40.00 | 14.00 | 0.00 |
| 28 | 22.00 | 20.00 | 111.00 | 102.00 | 28.00 | 19.00 | 0.00 |
| 29 | 25.00 | 19.00 | 107.00 | 93.50 | 29.50 | 4.00 | 12.00 |
| 30 | 22.50 | 16.00 | 145.75 | 138.75 | 52.50 | 8.00 | 3.00 |
| 31 | 11.75 | 7.50 | 117.42 | 117.42 | 37.50 | 5.00 | 0.00 |
| 32 | 19.00 | 20.00 | 110.00 | 111.00 | 33.00 | 12.00 | 6.00 |

## Appendix E: Legend of Dietary Supplements with units or intake per day from Block Food Frequency Questionnaire.

| Abbreviation | Actual Name | Units | Abbreviation | Actual Name | Units |
|---|---|---|---|---|---|
| DtKcal | Food energy | kcals | DtBcaro | Beta-carotene | mcg |
| DtProt | Protein | gms | DtCrypt | Cryptoxanthin, beta | mcg |
| DtTFat | Fat | gms | DtLutein | Lutein-Zeaxanthin | mcg |
| DtCarb | Carbohydrate | gms | DtLycope | Lycopene | mcg |
| DtCalc | Calcium | mg | DtRetinol | Retinol | mcg |
| DtPhos | Phosphorus | mg | DtCarote | Pro-vitamin A carotenoids | mcg |
| DtIron | Iron | mg | DtVitAR | Vitamin A (RAE) | mcg |
| DtSodi | Sodium | mg | DtVitEAt | VitaminE | mcg |
| DtPota | Potassium | mg | DtVit B12 | Vitam B-12 | mcg |
| DtThia | Thiamin –Vitamin B1 | mg | DtCopper | Copper | mg |
| DtRibo | Riboflavin—Vitamin B2 | mg | DtSelnium | Selenium | mcg |
| DtNiac | Niacin | mg | DtFolfort | Fortified Dietary Folic Acid | mcg |
| DtVitC | Vitamin C | mg | DtFolFood | Natural Dietary Folate | mcg |
| DtSFat | Saturated Fats | gms | DtFolDFE | Total folate/folic acid | mcg |
| DtMFat | Monounsaturated fatty acids | gms | DtVitK | Vitamin K | mcg |
| DtPFat | Polyunsaturated fat acids | gms | DtTheoBr | Theobromin | mg |
| DtChol | Cholesterol | mg | DtTotSug | Sugars—Total | gms |
| DtFibe | Dietary Fiber | gms | DtOme3 | Omega-3 Fatty Acids | gms |
| DtFolFD | Food Folate | mcg | DtOme6 | Omega-6 Fatty Acids | gms |
| DtZinc | Zinc-Total | mg | DtVit D | Vitamin D | IU |
| DtAnZin | Zinc-Animal Sources | mg | DtTranFat | Trans fat—Total | gms |
| DtVitB6 | Vitamin B6 | mg | GrpSoldTo | Grams of solid food/day | |
| DtMagn | Magnesium | mg | TotalVitD | Total Vitamin D | IU/wk |
| DtAcaro | Alpha Carotene | mcg | Cutenthmg | Copper (tenth mg) | mg |
| | | | BloodVitD | Blood Vitamin D | ng/ml |