Vitamin D status of immigrant and ethnic minority children ages 2 to 5 y in Montréal

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ABSTRACT

Vitamin D (VTD) status is lower in ethnic minorities compared to White Canadians, but this has not been studied in children under 6 y of age. Therefore, the objective of this study was to estimate the prevalence of VTD insufficiency and to identify explanatory variables for 502 children (2 to 5 y) attending Montréal daycares in a cross-sectional study between June 2010 and 2011. Capillary blood sampling demonstrated that 25-hydroxy vitamin D (25(OH)D) concentration was ≤ 50 nmol/L in 10% of White children (n = 262), 14% of Black (n = 36), 0% of Hispanic (n = 15), 16% of Arab (n = 45), 9% of Asian (n = 43) and 14% of Mixed ethnicities (n = 101). The main predictor was sun index for White ($\Delta r^2 = 0.09$, P = 0.263), VTD intake from supplement for Arab ($\Delta r^2 = 0.18$, P < 0.001) and Mixed ($\Delta r^2 = 0.16$, P < 0.001) income for Black ($\Delta r^2 = 0.28$, P = 0.009) and skin type for Asian ($\Delta r^2 = 0.18$, P < 0.001). More than 80% of children attending daycares in Montréal have adequate vitamin D status, regardless of ethnicity, immigration status, years of residence or SES. Since the explanatory variables for VTD status differed among ethnicities, strategies to improve VTD status in those with low status will need to consider the unique needs of each ethnic group.

RÉSUMÉ

Les niveaux sériques de vitamine D des minorités visibles sont inférieures à celui des Blancs, mais n'a pas été étudié auprès des enfants moins de 6 ans. Donc, l'objective de cette étude était d'estimer la prévalence d'insuffisance en vitamine D et d'identifier les paramètres explicatifs pour 502 enfants (2 à 5 ans) fréquentant les garderies de Montréal entre Juin 2010 et 2011. La concentration sérique capillaire de 25-hydroxy vitamine D (25(OH)D) était ≤ 50 nmol/L dans 10% des enfants Blancs (n = 262), 14% des Noirs (n = 36), 0% des Latino-Américains (n = 15) , 16% des Arabes (n = 45), 9% des Asiatiques (n = 43) and 14% des Mélangés (n = 101). Le paramètre explicatif principale était l'exposition au soleil pour les enfants Blancs (r^2 = 0.0.09, P = 0.263), l'apport de supplément en vitamine D pour les Arabes (r^2 = 0.18, P < 0.001) et Mélangés (r^2 = 0.16, P < 0.001), le revenu familiale pour les Noirs (r^2 = 0.28, P = 0.009) et le type de peau pour les Asiatiques (r^2 = 0.18, P<0.001). Plus de 80% des enfants des garderies avaient des taux adéquats de vitamine D peu importe l'ethnicité, le statut d'immigration, le nombre d'année de résidence au Canada ou le statut socioéconomique. Puisque les paramètres explicatifs du statut de vitamine D étaient différents pour chaque ethnicité, les stratégies pour améliorer le statut de vitamine D pour ceux qui présentent de faibles taux devront considérer les besoins unique de chaque groupe ethnique.

AUTHOR'S CONTRIBUTION

T. Pham was the primary author included in this thesis and was a large contributor to the work included. T. Pham was responsible for the planning, organization and recruitment of daycares and preschoolers in the study. T. Pham conducted phone calls for dietary data at home by 24h recalls and food frequency questionnaires, sun exposure and socioeconomic questionnaires at home. T. Pham assisted in the collections of anthropometric data, blood samples and spectrophotometer skin pigmentation measures in preschoolers at visits at the research center and daycares. T. Pham also assisted in dietary data collection by observation and food frequency questionnaires (FFQ) and sun exposure questionnaires at the daycare. T. Pham assisted in the laboratory analysis of 25(OH)D using the chemiluminescence assay (Liaison, Diasorin, Mississauga, Canada) and ionized calcium using the blood gas analyzer (ABL80 FLEX Radiometer Medical A/S, Copenhagen, Denmark). T. Pham entered and analyzed the majority of the 24h recall using Nutritionist Pro and setup the FFQ for data entry and analysis. T. Pham assisted in data entry and audit of data entry for sun exposure and socioeconomic questionnaires, anthropometric measures, FFQ and laboratory results. T. Pham conducted and interpreted statistical analysis, reviewed relevant literature, and drafted the manuscript in this thesis.

C. Vanstone was responsible for the day to day coordination and assisted the development of study documents (i.e. parent's survey, pamphlets, consent forms, contact forms). C. Vanstone was also responsible for establishing the protocol for blood sampling and procurement.

F. Rauch assisted in the conception of the project and was T. Pham's committee member.

C. Rodd contributed to the conception of the project.

S. Agellon was the primary technician for the chemiluminescence assay used for measurement of 25(OH)D.

S. Dell'Elce was responsible for majority of anthropometric measurements and blood procurement. S. Dell'Elce assisted in data entry of the sun exposure survey and parent's surveys.

T. Hazell assisted in the collection of anthropometric measurements, laboratory analysis, dietary data entry of the FFQ and audit of anthropometric data.

S. Jean-Philippe assisted in all data collection at daycares and telephone calls at home, laboratory analysis and 24h recall data entry and analysis.

H. Weiler was laboratory director and principle investigator on this project. H. Weiler was responsible for the conception and overall coordination of all authors involved. H. Weiler was also T. Pham's direct supervisor.

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ABBREVIATIONS

25(OH)D	25-hydroxy vitamin D
7-DHC	7-dehydrocholesterol
BSA	body surface area
CIE	Commission International d'Éclairage
GC	group specific component
DBP	vitamin D binding protein
DV	daily value
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
FFQ	food frequency questionnaire
FGF-23	fibroblast growth factor 23
IOM	Institute of Medicine
ITA	individual typological angle
MED	minimal erythemal dose
preVTD$_3$	previtamin D$_3$
PTH	parathyroid hormone
UL	Upper Level
UVA	ultraviolet A radiation
UVB	ultraviolet B radiation
UVC	ultraviolet C radiation
UVR	ultraviolet radiation
VTD	vitamin D
VTD$_3$	vitamin D$_3$
VTD$_2$	vitamin D$_2$
VDR	vitamin D receptor
SED	standard erythemal dose
SES	socioeconomic status
SPF	sunblock protection factor
RANKL	receptor activator nuclear factor-ΚΒ ligand
RDA	Recommended Dietary Allowance

UNITS

nmol	nanomole
µg	micrograms
cm	centimeter
g	grams
IU	international unit
m	millimeter
mmol	millimole
nm	nanometer
mJ	millijoule
%	percent
m	meter

CONVERSION FACTORS

1 ng/mL of 25(OH)D = 2.5 nmol/L

1 µg of vitamin D = 40 IU

1.0 LITERATURE REVIEW

1.1 INTRODUCTION

Descriptions of rickets were first recorded in the early 17th century[1, 2] but only a century later was it associated with the lack of sun exposure[3]. Rickets continued to rise in children and became an endemic as industrialization increased, making VTD deficiency rickets a public health concern. National food fortification and recommendations for sensible exposure to sunlight[4] were implemented and thought to have eradicated rickets. However, in the 1960's, documentation of rickets cases puts into debate whether it is re-emerging or persisting[3]. Rickets is a bone disease resulting from deficiencies in VTD and calcium during childhood. In addition, VTD is important for maximal bone mass accretion during childhood and young adulthood. VTD deficiency increases the risks of developing osteomalacia, osteoporosis and bone fractures in later years. Bone diseases pose economic burdens on health care systems, yet it is fairly inexpensive to prevent and to treat them in early stages. In addition, VTD sufficiency is recently linked to non-skeletal health outcomes. The main marker of vitamin D status is circulating 25-hydroxy vitamin D (25(OH)D). It is thought that the changing Canadian demographics due to increases in immigration of ethnic minorities with darker skin pigmentation in the last decades may partially explain the incidence of rickets and increased prevalence of VTD deficiency and insufficiency.

1.2 DEFINITIONS

1.2.1 ETHNIC MINORITIES AND IMMIGRANTS

Immigration status is defined as three levels; first generation (who are not born inside Canada), second generation (who are born inside Canada with at least one parent born outside Canada) and third generation (who are born inside Canada with both parents born inside Canada[5]. Very recent immigrants are defined as those with < 5 y and recent immigrants as those with < 15 y of Canadian residence[6].

In Canada, “The Employment Equity Act defines visible minorities as 'persons, other than Aboriginal peoples, who are non-Caucasian in race or non-White in colour”[5]. The visible minorities includes Chinese, South Asian, Black, Arab, West Asian, Filipino, Southeast Asian, Latin American, Japanese, Korean, multiple visible minority and visible minority not included elsewhere (such as Guyanese,' 'West Indian,' 'Kurd,' 'Tibetan,' 'Polynesian,' 'Pacific Islander,' etc.)[5].

1.2.2 VITAMIN D RECOMMENDATIONS FOR HEALTH

1.2.2.1 Indicators for 25(OH)D concentrations

There are 3 terms commonly used to describe vitamin D status: deficiency, insufficiency and sufficiency. The state of deficiency is a 25(OH)D concentration at which consequences to skeletal tissues are clearly obvious while insufficiency is associated with disease outcomes such as cancer, cardiovascular disease or diabetes[7] and sufficiency is set at a concentration where the risks of disease is lowest. In November 2010, the Institute of Medicine (IOM) set new cut offs for 25(OH)D insufficiency to < 50 nmol/L and maintained the definition of deficiency at < 27.5 nmol/L of 25(OH)D[8]. The cut off for insufficiency is lower than that of the Canadian Paediatric Society[9] (Table 1). Despite the new IOM report, there is still a heated debate among the scientific communities regarding the VTD cut offs for high risk groups. The IOM committee highlighted the need for more robust randomized control trials to clarify the role of VTD in extraskeletal health.

1.2.2.2 Dietary Reference Intakes

There are three Dietary Reference Intake (DRI) values for VTD in children age 2 to 5 y; the Estimated Average Requirement (EAR) for the population, the Recommended Dietary Allowance (RDA) for the individual child and the Upper Level (UL). The EAR is 400 IU (10 µg/d) as set by the IOM[8] to meet 50% of

Table 1: Recommended cut offs for vitamin D intake and status for children 2 to 5 y of age

	25(OH)D (nmol/L)	
	Insufficient	**Sufficient**
Institute of Medicine[10]	< 27.5	> 50
American Academy of Pediatrics[11]	< 27.5	> 50
Canadian Paediatric Society[9]	< 25	75 to 225

the population's needs for VTD while the RDA is set higher at 600 IU (15 µg/d)[8] per day to meet 97.5% of the population needs. The UL for intake is 2500 IU (62.5 µg/d) for age 1 to 3 y and 3000 IU (75 µg/d) for 4 to 8 y[8].

1.3 THE CHANGING CANADIAN DEMOGRAPHIC

Although non-White individuals are termed ethnic minorities, their presence in Canada represents a significant proportion of the population and their health has a significant economical impact on our health system. Canada is comprised of over 200 ethnic origins with about 13 million immigrants arriving to Canada in the last 100 years[5]. For the first half of the 20th century, immigrants mostly came from Europe[5]. However, immigration flux changed in the 1970's with half of all immigrants originating from Caribbean nations, Asia and South America[5]. Then in the 1980's growing numbers arrived from Africa[5]. In 2006, 75% of new immigrants belong to an ethnic minority group and the ethnic minorities population in Canada surpassed the 5 million mark (5,068,100) accounting for 16.2% of Canada's population[5]. From 2001 to 2007, ethnic minority population grew 5 times faster than the main population in Canada due to immigration and they make up a greater proportion of the younger age group[5]. Ninety-six percent of ethnic minorities live in census metropolitans and make up 43%, 42% and 26% of the population in Toronto, Vancouver and Montréal, respectively[5]. South Asians, Chinese and Black represent the three largest visible minority group[5]. Among the total population aged 15 y and over, 23.9% are of first generation, 15.6% are of second generation and 60.5% are of third generation immigrant[5]. It is projected that immigration will continue to increase with a quarter of the total Canadian population to be foreign-born or belong to an ethnic minority by 2031[5]. Furthermore, half of Canadians aged 15 y and over will be foreign-born and half of the second-generation Canadians will belong to an ethnic minority group[5].

1.4 BACKGROUND

1.4.1 SOURCES OF VITAMIN D

VTD either comes from exogenous or endogenous sources. Exogenous sources are from food with natural VTD, staple foods with fortified VTD or VTD

supplements. The endogenous source is from VTD photosynthesis in the skin upon ultraviolet B (UVB) radiation (details in Section 1.4.2).

1.4.1.1 Exogenous intake

1.4.1.1.1 Natural Food

Natural VTD exists in two forms vitamin D_2 (VTD_2) and vitamin D_3 (VTD_3). Ergocalciferol (VTD_2) is from plant while cholecalciferol (VTD_3) is from animal based foods. Ergosterol is naturally produced by plankton and mushroom exposed to UVB[12] and cholecalciferol is found in mold ergot and sheep's wool lanolin[13, 14]. UVB-exposed fungoids (mushrooms) can contain up to 100,000 IU per 3.5 ounces, however they are not commercially available[15]. Wild-caught fish contain the most plentiful natural VTD_3 (e.g. salmon, tuna and mackerel)[16, 17] with 500 to 1000 IU (12.5 to 25 µg) of VTD_3 per 100 g while farmed fish contains 100 to 250 IU (2.5 to 6.3 µg) of a mixture of VTD_2 and VTD_3 per 100 g[18]. Baking or boiling preserves the nutritional value of VTD in fish while about 50% of VTD is loss if fried[19]. Table 2 details other natural sources of VTD.

1.4.1.1.2 Fortified Food

Intake of natural food sources of VTD is inadequate to prevent deficiencies because it often provides only 152 to 276 IU (3.5 to 6.9 µg) daily to the diet[20]. Therefore, in the 1930s Health Canada, through the Canadian Food and Drug Regulations, implemented the mandatory fortification of Canadian staple food with VTD_3. Milk is fortified with 35 to 40 IU (0.9 to 1 µg) per 100 ml and margarine with > 530 IU (13.3 µg) per 100 g[21]. Milk includes all infant formula (40 to 80 IU[21] or 1 to 2 µg per 100 ml), plant-based milk substitutes, powdered/evaporated milk and goat milk[22]. Any other food was not eligible for mandatory fortification. However, as of 2005, in recognition of VTD role in health, the Canadian government has permitted the fortification of a variety of new food categories on volunteer basis, for example, cereals, juices and yogurts[23, 24]. Unlike Canada, VTD fortification of milk and margarine in the United States

Table 2: Dietary Sources of vitamin D

Supplements for children	**IUs per servings***
Cod liver oil, 1 tablespoon	1,279
Multivitamin (i.e. Flinstone, Centrum Junior, Kirkland, Life), 1 tablet	400
Flintstone or Iron Kids or Teddy's, 2 gummies	200
Disney, 2 gummies	100
D drops, 5 ml	1,000
PediaVitD, D-visol, Enfamil, 1 ml	400
Pediasure, 240 ml	275
Fish	
Halibut, Greenland (Turbot), cooked, 100 g	1423
Salmon (sockeye), wild, cooked, 100 g	600-1000
Salmon (sockeye), farmed, cooked, 100 g	100-250
Salmon (sockeye), canned, cooked, 100 g	300-600
Mackerel (Jack and pacific), mixed species, cooked, 100 g	462
Seabass, mixed species, cooked, 100 g	290
Mackerel (Jack), canned, 100 g	292
Sardines, canned in oil, drained, 100g	233
Trout, farmed, 100 g	176-600
Tilapia, cooked, 100 g	163
Whitefish, lake, mixed species, cooked, 100 g	142
Cod, 100 g	80-138
Tuna fish (white), canned in water, drained, 100 g	80
Tuna fish, canned, drained, 100 g	64
Grey sole, 100 g	20-92
Fish sticks, frozen, battered, fried, 100 g	20
Other	
Milk (skim/reduced fat/whole), 1 cup	223
Orange juice fortified with vitamin D, 1 cup	100
Margarine, fortified, 1 tablespoon	78
Yogurt, fortified 20% of DV for vitamin D, 100 g	40
Liver, beef, cooked, 3.5 ounces	36
Egg, 1 large (vitamin D is found in yolk)	26
Shitake mushroom, dried, 5 mushrooms	28

*1 IU = 40 µg

Sources: Health Canada, 2009[25]; Holick, 2007[13]; Lu et al., 2007[19]

(US) is voluntary. In addition, the US Food and Drug Administration considered vitamin D as generally recognized as safe (GRAS)[26] and allowed its fortification in diverse food categories (breakfast cereals, grain products and pastas, milk and milk products) since 1983[22] and of juice since 2003[27]. The voluntary US fortification program resulted in inconsistent fortification of VTD, particularly of milk, where 47.7% milk is under fortified compared to label claims[28]. In addition, the various food categories did not contribute significantly to VTD intake of Americans as it represents only 5 to 10% of daily intake[29]. VTD fortification in Canada is carefully regulated because of toxicity risks which include hypercalcemia and hypercalciuria leading to vascular and tissue calcification with symptoms such as anorexia, weight loss, polyuria, heart and arrhythmias[16, 22]. VTD in nutritional labels is expressed as a percent of the daily value (% DV) and the daily value for VTD in Canadian nutrition labels refers to 200 IU (5 µg)[30].

1.4.1.1.3 Supplementation

Fortified food contributes about 260 to 344 IU[31] (6.5 to 8.6 µg) of VTD which is not enough to meet the DRI values (details in Section 1.3.2). Therefore, either in combination or as an alternative to food, multivitamin supplementation is frequently recommended. Most multivitamins and vitamins, in Canada, are made with VTD_3 and are available in various doses, most commonly 100 IU (2.5 µg) to 1000 IU (25 µg)[32], as either over the counter or as a prescription. In Canada, most supplements contains VTD_3, however, prescription forms of VTD are available in VTD_2. Fish oil (e.g. cod liver oil) is a supplement rich in vitamin D, but also in vitamin A[16]. It was once a popular daily practice to take a teaspoon per day among European populations for prevention of rickets; however because fear of VTD toxicity, only Nordic countries now commonly practice daily cod liver oil supplementation[33].

1.4.1.2 Endogenous synthesis

From an evolutionary perspective, ingestion of VTD was not designed to meet human needs. Ninety percent of VTD is from photosynthesis of VTD in the skin with ultraviolet radiation (UVR) from sunlight[1].

1.4.1.2.1 Skin Anatomy and 7-DHC

The skin has two layers: the epidermis and the dermis[34]. Within the epidermis there are 5 strata: corneum, lucidum, granulosum, spinosum and basale[34]. The first four are collectively called the suprabasale layer. The spinosum and basale strata together makes up the Malpighian layer[35] and contains the highest concentration of 7-dehydrocholesterol (or provitamin D_3 or 7-DHC)[23, 24]. 7-DHC is synthesized in the skin and is present in the lipid bilayers of the epidermis[36]. The dermis contains minimal amounts of 7-DHC[35].

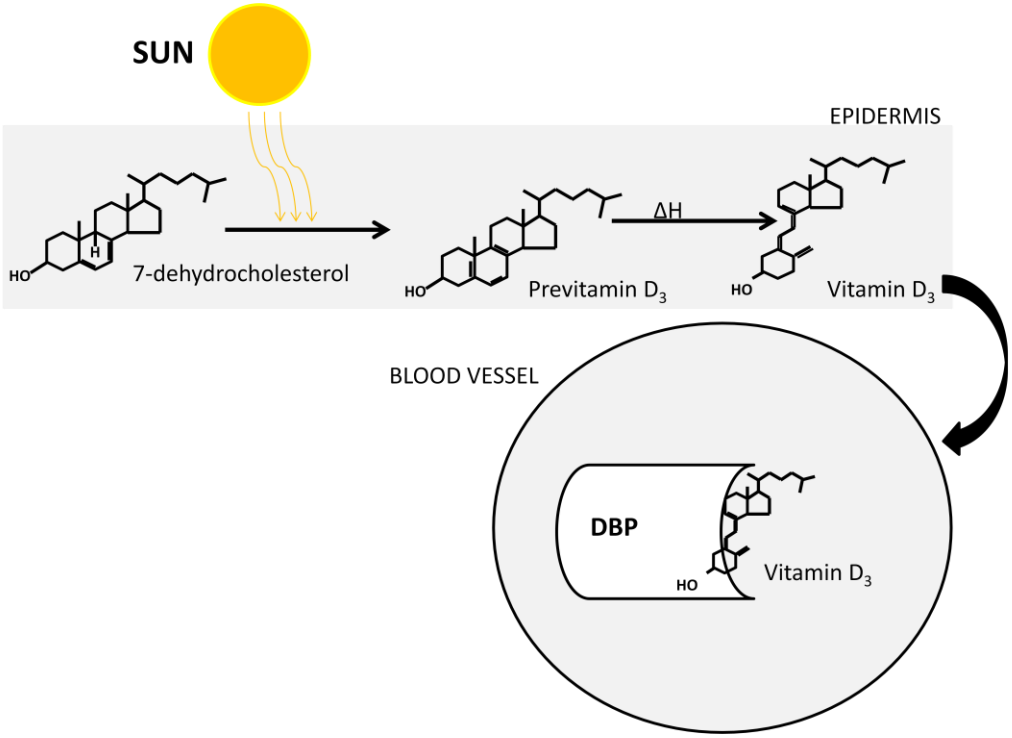
1.4.1.2.2 Cutaneous vitamin D_3 synthesis

When the skin is exposed to UVB radiation, UVB catalyzes the conversion of 7-DHC to previtamin VTD_3 ($preVTD_3$) by breaking the bond at carbon 9 and 10[37]. The heat, then continues to convert $preVTD_3$ to VTD_3 through a 12a photolytic transformation which is followed by a slow, heat-dependent isomerisation[37, 38]. Two hours following the initial formation of $preVTD_3$, VTD_3 exits the dermal capillary bed into the blood stream by binding to vitamin D-binding protein (DBP) also known as transcalciferrin[37] (Figure 1). The binding of VTD_3 to DBP shifts the equilibrium to $preVTD_3$ production[35]. After the last sun exposure, conversion to VTD_3 continues until 95% of the $preVTD_3$ is converted[35]. In blood, photosynthesized VTD_3 peaks 2 d after initial exposure and is maintained up to 4 d later[35, 39]. The Malpighian layer has the greatest capacity for VTD_3 production[34]. Photosynthesized VTD_3 can maintain plasma $25(OH)D_3$ levels for 30 to 60 d versus dietary VTD_2 for only 7 d[40].

1.4.1.2.3 Regulation of vitamin D_3 synthesis

Skin pigmentation regulates the rate while UVB regulates the quantity of VTD_3 photosynthesis[35]. This explains why there has been no known cases of VTD_3 intoxication through sun exposure[35]. All VTD_3 metabolites in the skin are photolabile[41]. This means, just as how sunlight catalyzes 7-DHC conversion to VTD_3, it can also halt the process by converting the metabolites to more inert isomers by photoisomerization. $PreVTD_3$ is converted to $lumisterol_3$ and $tachysterol_3$[35] and VTD_3 is converted to 5, 6-trans-cholecalciferol, suprasterol I

Figure 1: Cutaneous of vitamin D put range nm and solar UVB



and suprasterol II[41]. Photoisomerization to its inert isomers starts when about 15% of 7-DHC concentration is converted[35]. Tachysterol$_3$, like 7-DHC, also plateaus but at 5% of its concentration[35]. In an environment of excessive ultraviolet radiation, only lumisterol continues to increase in concentration regardless of skin pigmentation and, therefore, there is no further increase in VTD$_3$ production once 7-DHC conversion reached plateau[1].

1.4.1.3 VTD$_2$ vs VTD$_3$

VTD$_2$ differs from VTD$_3$ in that the former has an extra double bond between carbons 22 and 23 and an additional 24-methyl group. Some literature suggested VTD$_2$ to be less effective than VTD$_3$ in raising serum 25(OH)D for two reasons: 1) VTD$_2$ is only bound to 60% DBP and the remaining is bound to lipoproteins (40%) and 2) the half-life of VTD$_2$ when bound to DBP versus VTD$_3$ is decreased[42]. VTD$_3$ is metabolized differently, raises 25(OH)D concentration more effectively and maintains concentrations longer due to its superior affinity for DBP[42]. Therefore, VTD$_3$ can be 2 to 9 times more effective than VTD$_2$ in raising serum 25(OH)D concentration, especially at high doses[42-45]. Despite VTD$_2$ is more rapidly metabolized, more recent evidence in adults and children indicates that if administrated at lower regular daily doses, VTD$_2$ is equivalent to VTD$_3$ at maintaining serum 25(OH)D[46, 47]. There is, however, a limitation to all studies comparing VTD$_2$ and VTD$_3$ because, to date, no study has examined vitamin D-specific bioresponse aside from serum 25(OH)D[48].

1.4.1.4 Measurements of sources of VTD

Dietary VTD is measured by various methods: observation, 24h recall, qualitative or semi-quantitative food frequency questionnaire (FFQ), and weighed or non-weighed food records. Cutaneous synthesis of VTD is measured subjectively through sun exposure questionnaires or objectively by a portable UVR measuring device. Sun exposure questionnaires include data on latitude, sunscreen use, percentage of body surface area (% BSA) exposed to direct sunlight and frequency and duration of exposure.

1.4.2 SUN EXPOSURE

1.4.2.1 Electromagnetic spectrum

The sun emits electromagnetic (EM) radiation across most of the EM spectrum ranging between 100 to 10000 nm; the shorter wavelengths the more energy it contains[49]. EM radiations are grouped as following: x-rays, UVR, visible light, infrared and radio waves[49]. In the context of VTD, only UVR are of biological significance[50].

1.4.2.1.1 Types of ultraviolet rays

There are three types of UVR: UVC, UVB, UVA. The wavelengths of UVC are between 100 to 280 nm, UVB is 280 to 320 nm and UVA is 320 to 400 nm[51]. The Earth's atmosphere absorbs most of UVC allowing only UVA and UVB to penetrate to the earth's surface[50]. The shorter the wavelengths the greater the scattering and absorption of the rays through the air mass[52]. Therefore, within the UVB spectrum, only wavelengths > 290 nm reaches the earth's surface and UVB spectrum is dominated by the 295 nm wavelength[53]. Sunlight is composed of 90 to 100% UVA and 0 to 10% UVB[54, 55].

1.4.2.1.2 VTD production spectrum

UVB is responsible for 96% and UVA for 4% of VTD production in the skin[56]. In non-tropical sunshine, UVA wavelength between 315 to 335 nm breaks down VTD_3 in the skin[41, 57]. Based on these, the VTD_3 production spectrum suggested by the Commission International d'Éclairage (CIE) is between 295 to 315 nm. This absorption spectrum was recently challenged by Norval et al.[58]. The authors suggest that the spectrum has only been determined *in vitro*; the shorter spectrum wavelengths may not be relevant *in vivo*[58], however, the mechanism is unclear as to why.

1.4.2.2 Latitude and season

The amount of UVB and the proportion of UVB and UVA that hits earth's surface depends on 1) the length it must travel through the stratospheric ozone layer[38], 2) the length through the atmosphere[38], 3) the angle at which it enters the atmosphere

and 4) the amount of clouds, dust, haze and various organic compounds present in the air[52]. The first three are related to latitude which varies by geographical area and is expressed in reference to the angle (in degrees) north or south from the equator. The equator, latitude 0^o, is the point where UVR travel the shortest distance through air to the sun. As the angle increases, the longer the distance the UVR must travel before entering the atmosphere[59]. Above latitude 40^oN, the UV must travel longer distances and reach the ozone's layers at oblique angles that does not allow UVB rays to penetrate the atmosphere between November and March[57]. At latitude 40^oN, 75% of UVB in the day is within 0900 to 1500 h with a third of it between 1100 to 1300 h[60]. The seasonal variation in temperature characterized by the cool autumn and spring and the freezing winter above latitude 40^oN is also explained by the scattering of the energy-rich short wavelengths from reaching the earth's surface.

1.4.2.3 Dose and rate

UVB radiation is described in terms of dose and rate. Dose refers to the amount of UVB photons per skin surface area and is expressed in mJ/cm^2 while rate refers to the length of UVB exposure and is expressed in min.

1.4.2.4 Erythemal dose

Dose can be expressed in one simple term as either minimal erythemal dose (MED) or standard erythemal dose (SED). With respect to 25(OH)D photosynthesis, one MED is the dose of UVR needed that will increase $25(OH)D_3$ by ~ 50 nmol/L[61] when the full body is exposed for 24 h[1, 62]. For example, 1 MED is about 41 ± 8 for Caucasians and 76 ± 31 mJ/cm^2 for Asians[63]. However, 1 MED is an approximate value that is individualistically based on various characteristics such as age and skin pigmentation. The Commission International d'Eclairage (CIE) "does not consider MED as a standard measure of anything, but rather describes the nature of skin sensitivity to UVR"[64]. MED does not account for the negative feedback of VTD_3 photosynthesis which allow prolonged exposures to UVB without resulting in higher 25(OH)D concentration. Therefore, the CIE adopted Diffey's suggestion of the standard erythemal dose (SED) where 1 SED

is set to 10 mJ/cm^2 at 298 nm wavelength[65]. Hence, 1 MED = 4 SED in White and twice that (1 MED = 8 SED) in Asians.

1.4.2.5 Body Surface Area (BSA) exposed

The percent of body surface area (% BSA) exposed to UVR is determined by using the Lund and Browder chart which is widely used in both dermatology and VTD photosynthesis studies[66]. The % BSA attributed to each body parts are summarized in Table 3. It is lower than those used in the Lund and Browder chart[67] by half to account for half the body facing the sun at one time[68].

1.4.2.6 Relationship between dose, rate, % BSA and latitude to 25(OH)D_3 concentration

It is very difficult to estimate exact sun exposure needs because it is dependent on many variables such as 1) % BSA[69], 2) dose[69], 3) rate[70], 4) frequency[70], 5) time of the day[70], 6) individual 25(OH)D_3 baseline[69], 8) VTD intake, 7) skin thickness[71] and pigmentation[72]. No study, to date, was designed to account for all these variables and the following section summarizes what is known about their inter-relationships. Within this section, all measures or recommendations refer to light skin individuals. Various type of skin pigmentation is discussed in Section 1.4.3.

1.4.6.1 Latitude and season

A dose of at least ~ 1.8 SED to full body is needed to start preVTD$_3$ and significantly increase serum 25(OH)D_3 levels above baseline[73]. At latitude 50^{o}N, Spain, there is a daily average of 1.7 SED in winter, 12 SED in spring/autumn, and 40 SED in summer[60]. However, Spain has an average altitude of 660 m above water compared to Montréal, Canada which has an altitude of 52 m. For every 305 m increase in altitude there is a 4 to 5% increase in SED[74]. This may explain why synthesis in Canada is more limited. Due to this variation in UVB dose, 25(OH)D concentrations in summer and fall are approximated to be 5 to 10 nmol/L higher than in winter and spring in Canadian children age 6 to 11 y[75]. In Canada, summer commences June 21st, Fall on September 23rd, Winter on December 22nd and Spring on March 20th based on solstice and equinox. The relationship between annual UVSED and latitude is shown in Equation 1.

Table 3: Sum of % BSA by body part(s) exposed (age 1 to 4 y)

	Body part(s) exposed								
% BSA	**Face**	**Hands**	**Fore - arms**	**Upper Arms**	**Legs**	**Thighs**	**Feet**	**Upper torso**	**Buttocks**
3%	3%								
6%	3%	+ 3%							
9%	3%	+ 3%	+ 3%						
13%	3%	+ 3%	+ 3%	+ 4%					
18%	3%	+ 3%	+ 3%	+ 4%	+ 5%				
24%	3%	+ 3%	+ 3%	+ 4%	+ 5%	+ 6%			
28%	3%	+ 3%	+ 3%	+ 4%	+ 5%	+ 6%	+ 4%		
41%	3%	+ 3%	+ 3%	+ 4%	+ 5%	+ 6%	+ 4%	+ 13%	
45%	3%	+ 3%	+ 3%	+ 4%	+ 5%	+ 6%	+ 4%	+ 13%	+ 4%

Reference: Lund and Browder chart[67]

Equation 1: Predicted annual SED

$$\text{Annual SED} = 2 \times 10^4 \exp(-\text{latitude}/20)$$

where;
SED = standard erythemal dose in mJ/cm^2
Latitude = the angle away from the equator in degrees
Cited in Diffey 1991[60]

Although autumn and spring may have the minimum SED for $25(OH)D_3$ synthesis, the cool temperature at the end of autumn and early spring often limit BSA exposed to < 6% (full face and hands). A minimum BSA of 6% of UVB exposure is required for > 35 min with a frequency of 4 times per week to raise plasma 25(OH)D concentration above 50 nmol/L[61]. Therefore, in Canada, November 1st through to March 31st is considered as non-synthesizing period[57].

1.4.6.2 Body surface area exposed and UVB doses

The relationship between UVB dose and preVTD3 is linear ($r^2 = 0.17$)[76],however, it is not the same with plasma $25(OH)D_3$ concentration. Circulating $25(OH)D_3$ concentration is positively correlated with SED up to 1.5 SED[69, 77] and between 1.5 to 3 SED there is maximal vitamin D production, independent of % BSA[69]. Similarly, from 6% BSA to 24% BSA exposed, there's a linear relationship with $25(OH)D_3$ but at > 24% BSA, $25(OH)D_3$ production plateau[69]. Sufficient VTD can be synthesized with only 1 SED if 20% BSA is exposed for 10-15 min between 1000 to 1500 h[70] during summer, early autumn and late spring in Canada (Table 4). At 0.75 SED and 6% BSA, no VTD production occurs. In children the time required for UV exposure is shorter because of the higher % BSA of skin relative to body mass[78] and because of thinner skin thickness[71].

1.4.3 SKIN PIGMENTATION

1.4.3.1 Melanogenesis

Within the layers of the skin reside two abundant cells: keratinocytes and melanocytes[34]. Keratinocytes concentration increases from the basale to the corneum stratum while it is the reverse for melanocytes. In other words, stratum basale have the highest number of keratinocytes and stratum corneum have the highest number of melanocytes[34, 79]. Keratinocytes synthesize keratin which strengthens the skin and provides its waterproof outer surface characteristics[34]. Melanocytes synthesize melanin within melanosomes. Melanin is synthesized from tyrosine and its production is mediated by the enzyme tyrosinase[34, 79]. Tyrosine is hydroxylated with dopa to form dopaquinone which is the intermediate compound to various melanin biopolymers[79]. Dopaquinone is then

Table 4: Estimated sun exposure required for vitamin D photosynthesis in adults[1]

SED	%BSA	Frequency (per week)	Rate (min)	Time	VTD_3 equivalence (IU)	Baseline plasma $25(OH)D_3$	Plasma $\Delta 25(OH)D_3$ (nmol/L)	Skin Type	Ref
5.4	100	NR/NM	24h[5]	Sun lamps (280-315nm)	10 000	NR/NM	108-138	Type III	61
>3	>6	2-3	5-10[4]	UVB Lamps	NR	NR/NM	~25	White	61
2[2]	9[2]	2-3	5-10	NR/NM	NR	NR/NM	NR/NM	White	13
1.5	6	4	30[4]	UVB Lamps	NR	34.1	~19	White	61
1[2]	40	NR/NM	10-15	1000 to 1500 h	1000	NR/NM	50[5]	White	1, 70
>0.7	>24	2-3	5-10[4]	UVB Lamps	NR	NR/NM	~20	White	61
0.7	24	2-3	15[4]	UVB Lamps	NR	34.1	~19	White	61
Summer	41[3]	1	30	NR/NM	NR	NR/NM	27.5[5]	White[5]	80
Summer	6[3]	1	120	NR/NM	NR	NR/NM	27.5[5]	White[5]	80

1 SED = 10mJ/cm^2;
NR/NM = not reported or not measured;
[1]For Montréal, Canada (45°N) in light skin individuals between 1000 to 1500 h;
[2]Conversion was done for MED to SED using 1MED = 4SED for White individuals[63];
[3]% BSA based on Lund and Browder chart[67];
[4]Multiply by 1.2 for min needed for sunlight exposure;
[5]Assumptions based on details provided in literature.

further modified to form either eumelanin or pheomelanin[79]. Eumelanin, formed in the absence of cysteine, is a dark brown/black color compound which is alkaline and insoluble[79, 81, 82]. Pheomelanin, formed in the presence of cysteine, is a red-yellow compound and is alkaline soluble[79, 81, 82]. The melanocytes transfer these melanin-rich melanosomes to adjacent keratinocytes through dentritic structures[83]. Keratinocytes eventually migrate upward, bringing along the melanosomes to the upper layers of the epidermis[34, 83]. The migration of keratinocytes from the stratum basale to stratum corneum takes about two weeks. In the stratum corneum, the melanosomes degrade and spread around forming a melanin dust[84]. After two weeks, the stratum corneum sloughs off through the natural process of skin turnover[34].

1.4.3.2 Skin color

Skin color does not depend on density of melanocytes present in the skin, but rather, on the variation in size and aggregation and distribution of melanin granules and melanosomes within skin layers[82]. Dark skin pigmentation is characterized by a higher eumelanin to pheomelanin ratio, high concentration of melanin and larger, nonaggregated melanosomes. In contrast, lighter skin pigmentation is associated with lower eumelanin to pheomelanin ratio, lower concentration of melanin and larger, aggregated melanosomes[85]. Repeated UVB exposure also increases quantity of melanin and size of melanosomes. A dose of 3 SED is needed to induce changes in skin color (tanning)[76].

1.4.3.3 Types of skin

Skin color differs not only between individuals but between body sites within one individual. Normal skin color can be divided into constitutive and facultative pigmentation[85]. Constitutive means an original color determined genetically and facultative refers to “changes due to outer stress such as exposure to sunlight”[85]. Photoexposed facultative sites contain 10% more melanin and larger melanosomes than photoprotected constitutive sites[81]. The forehead is the facultative site that has the highest melanin content[86]. Melanin distribution across the body is more consistent in children than in elderly adults[86]. The buttocks

region is a reliable site to measure constitutive skin pigmentation[87]. It is still controversial as to which sites correlate best with $25(OH)D_3$[88].

1.4.3.4 Melanin function

High melanin content resulting in darker skin pigmentation is theorized by Loomis in 1967 as an evolutionary attribute in the natural selection hypothesis[89]. Melanin protects from skin cancer[83] which is important for longevity, prevents photolysis of folate[90] for neural tube defect prevention[91] and spermatogenesis[92], and damage to sweat glands for thermoregulation[50]. Lighter skin evolved in higher latitudes above 40^oN[35] to adapt to lower UVB exposure. In 1967, Loomis proposed that "the world`s racial distribution by latitude was suggested to be due to the regulation of vitamin D production"[34, 89]. This allowed lighter skin individuals "to avoid a plethora of ill health, reproductive difficulties and early mortality to allow humans to survive"[93]. Skin color is therefore, strongly and negatively correlated ($r = \sim -0.80$) with latitude and geographical area[50]. Equation 2 predicts skin color of the indigenous population based on annual UVB MED.

1.4.3.5 Measurement of skin pigmentation

There are two main ways of measuring skin pigmentation: 1) Fitzpatrick classification or 2) measuring skin reflectance. The Fitzpatrick classification is a measure of erythema sensitivity and tanning ability where a set of characteristics is rated and summed up[94]. These characteristics are based on skin, eye and hair color as well as sensibility to the sun and tanning. The total score is then classified into 6 skin types: I, II, III, IV, V and VI (see details in Table 5). The Fitzpatrick method was widely used in the 1970's [94, 95] but is criticized for its subjective methodology regarding quantification, reliability and ex *vivo* conditions[96], particularly in Asian skin[82, 97]. Recently, technology used in measuring paint color has been applied to skin color measurement. Many instruments were developed to quantify skin reflectance and describe skin color in 3 values, L*, a* and b*. The values increase from black to white for L*, green to red for a* and yellow to blue for b*. Using Equation 3, the L* and b* values are

Equation 2: Predicted skin color based on annual UVMED

Predicted skin color = (annual average UVMED x 0·1088) + 72·7483

where;
Predicted skin color = measured in L value*
Annual average UVMED = average UVR expressed in MED
Cited in Jablonski, 2000[50]

Table 5: Skin classification

ITA^{o}[98]	**Fitzpatrick (1975) Description**[95]	**Chardon (1991) Description**[98]	**Reeder (2006) Description**[99]	**Ethnicity**[39, 81]
≥55^{o} to ≤90^{o}	Type I	Very light	Very Fair	Caucasian[39]
≥41^{o} to ≤55^{o}	Type II	Light	Fair	Caucasian[39]
≥28^{o} to ≤41^{o}	Type III	Intermediate	Medium	Caucasian[39], East Asian[39], Mediterranean[60], Mongoloid[60], Oriental[60], Hispanic[60, 81]
≥10^{o} to ≤28^{o}	Type IV	Tanned	Olive	East Asian[39], Amerindian[60], Hispanic[60], Indian[60]
≥-30^{o} to ≤10^{o}	Type V	Brown	Dark	South Asian[39] (Indian[81], Sri Lankan)
≥-90^{o} to ≤-30^{o}	Type VI	Dark	Very Dark/Black	Black[39] (African[81], South Indian Aborigine[60])

ITA^{o} = individual typological angle (degrees)

Equation 3: Individual typological angle

$$ITA° = (ARCTAN((L*-50)/b*))*180/3.14159$$

where;
ITA° = Individual typological angle measured in degrees
L= light to dark with lightness having higher values*
b = yellow to blue with more yellow having higher values*
Cited in Reeder, 2010[99]

converted to an individual typological angle (ITA^o); a measure of skin color shown to positively correlate (r = 0.70) with melanin content[94]. Based on ITA^o, skin can be classified into groups similar to those of Fitzpatrick.

1.4.4 VITAMIN D METABOLISM

Whether it is ingested or endogenously synthesized, VTD needs to be activated to have significant biological functions. Both photosynthesized and dietary VTD are transported by DBP in blood. Unlike photosynthesized VTD which enters the blood stream directly from the skin, dietary VTD must enter the intestinal-lymphatic system first as a chylomicron/lipoprotein complex[13, 100]. It enters the lymphatic system by passive diffusion with the help of bile salts and free fatty acids in the intestine[100]. The lymphatic system then empties into the subclavian vein[101] where VTD leaves the chylomicron/lipoprotein complex and binds to DBP. From blood, VTD is either carried to target organs for activation or to the adipose tissue for storage[102, 103]. There are two hydroxylation sites in the metabolism of vitamin D: one occurs in the liver and the second in the kidney. DBP also carries 25(OH)D and 1,25$(OH)_2$D and the amount bound to DBP by VTD and its metabolites is dependent on the DBP phenotypes.

1.4.4.1 Metabolic activation of 1,25$(OH)_2D_3$ in the endocrine system

In the liver, the first hydroxylation on carbon 25 forms 25(OH)D (or 25-hydroxyvitamin D or calcidiol) by mitochondrial[104] and microsomal vitamin D-25-hydroxylase enzymes (or P450C25 or CYP27A41)[38, 105-107]. The second hydroxylation on carbon 1 takes place in the proximal convoluted tubule epithelial cell in the kidney where the enzyme 1α-hydroxylase (or P450C1 orCYP27B1) in the mitochondria activates 25(OH)D to calcitriol (or 1,25$(OH)_2$D or 1α,25-dihydroxyvitamin D_3)[38, 106-108]. Hydroxylation in the liver and kidney is the main determinant of the serum concentration of 25(OH)D and 1,25$(OH)_2$D, respectively.

1.4.4.2 Vitamin D binding protein

One other determinant of 25(OH)D and 1,25$(OH)_2$D concentration is DBP phenotypes. DBP is also known as group-specific component (GC)[109] and is a

single polypeptide chain that contains one vitamin D-related sterol binding site for each molecule of protein[110, 111]. It is primarily produced in the liver and circulates in the serum as an apoprotein which is the form free of ligands[109]. GC protein display genetic polymorphism[109]. Polymorphism is genetic variants in the encoding of the genes and differences in the coding of the amino acids results in different phenotypes encoded in alleles[112]. Two common alleles have been identified for DBP: GC*1 and GC*2[113] and within the GC*1 there are two subtypes, 1F and 1S[114]. This gives rise to three common phenotypes: GC-2, GC-1F and GC-1S. There are also other DBP phenotypes and alleles but they are rare.

Since ninety-nine percent of 25(OH)D[115] and 1,25$(OH)_2$D[115] circulate in blood by binding to DBP for transport to its target sites[116], different GC phenotypes relate to serum 25(OH)D and 1,25$(OH)_2$D concentration. It has been found that there is a greater affinity for 25(OH)D in GC-1 versus GC-2[117] and GC-1F versus GC-1S[118] alleles. Hence, GC-2 phenotype is associated with lower 25(OH)D[119-121] and 1,25$(OH)_2$D[119, 120] and GC-1S is only associated with lower 25(OH)D[119]. In addition, the concentration of GC is also higher in GC-1[119]. A randomized control trial of VTD supplementation, in Toronto, Canada, reported that 8.5% of variance in 25(OH)D concentration was explained by GC-1 while 0.2% was by GC-2, respectively[122]. Additionally, DBP polymorphism was shown to be as important as VTD intake where it is reported to explain as much of the variation in circulating 25(OH)D (r^2 = 1.3% for DBP-1 and r^2 = 2.0% for DBP-1) as total vitamin D intake ($r^2 \leq$ 1.2%) during the non-synthesizing period[112]. In the synthesizing period, DBP polymorphism as an explanatory variable accounts for a greater variance in 25(OH)D (r^2 = 1.6% for DBP-1 and r^2 = 3.7% for DBP-1) because of its greater concentration[112]. During this period, DBP polymorphism explains 25(OH)D concentration as much or even more than age, smoking, BMI, calcium intake, VTD intake, energy intake and education[112]. The relationship among GC phenotypes and parathyroid hormone (PTH) does not rely on ethnicity[120].

1.4.4.3 Biomarkers of VTD

The best clinical indicator of overall adequacy of vitamin D intake is 25(OH)D concentration in serum compared to $1,25(OH)_2D_3$[123]. It represents the combined contributions of cutaneous synthesis and oral ingestion of dietary VTD and it has a circulating half-life of 15 days[16]. However, it does not reflect the amount of vitamin D stored in other body tissues[16]. Measurement of serum $1,25(OH)_2D_3$ concentration is inadequate as a biomarker of status and can lead to erroneous conclusions because: 1) it has a short half life of 15 h, 2) its concentration is ~ 0.1% of 25(OH)D, and 3) serum concentrations are not only regulated by calcium but also by PTH and phosphate concentration[16].

1.4.4.4 Blood collection and measurement of 25(OH)D concentration

Blood collection can be either by a capillary or venous sampling. Capillary was reported to inflate values by 18 nmol/L compared to venipuncture[124]. Blood sample are centrifuged and is separated into the plasma and serum aliquots where it can either be measured immediately for 25(OH)D concentration or stored at -80°C for later analysis[125]. There are 4 types of assays: 1) DBP competitive binding protein binding assay 2) radoimmunoassay (RIA), 3) high-performance liquid chromatography (HPLC) and 4) liquid chromatography tandem mass spectroscopy (LS-MC). The DBP and RIA assays recognized $25(OH)D_2$ equally as $25(OH)D_3$ however, it also recognized $24,25(OH)_2D$) and other poplar metabolites[125]. Therefore, the assay overestimates 25(OH)D concentrations by 10 to 20%[125]. To exclude the measurement of these polar metabolites, HPLC assay is developed and is considered the gold standard. However, the assay is very cumbersome because it is labour intensive, technique dependent, require costly equipment and large sample volumes therefore it is not routinely used by reference laboratories for clinical samples[125, 126]. LC-MS measures directly 25(OH)D from human serum and measures both $25(OH)D_2$ equally as $25(OH)D_3$[125]. Recently, an automated chemiluninescence-based assays have become available, utilizing either the DBP or a specific antibody in the measurement of serum 25(OH)D concentration by the LIAISON automated

analyzer. The LIAISON is a rapid, accurate and precise tool for the measurement of 25(OH)D concentration that is more accessible for the general practitioner and has an acceptable correlation to those by the RIA (r = 0.88)[126, 127].

1.4.5 CALCITRIOL FUNCTIONS

Once VTD is hydroxylated to calcitriol, calcitriol circulates in the blood and functions similarly to other steroid hormones by binding to vitamin D receptors (VDR) on target sites[100]. The most well understood function of calcitriol is in the endocrine regulation of plasma ionized calcium (Ca^{2+}) and phosphate homeostasis[100]. In addition, calcitriol has a role in skeletal health by having an indirect effect through PTH. Calcitriol is tightly up or down regulated by circulating concentrations of PTH, fibroblast growth factor 23 (FGF-23), plasma calcium and phosphorus[4, 128]. In recent years, calcitriol has been studied for its extra-skeletal functions which to date is not yet fully well understood.

1.4.5.1 Calcium homeostasis

Calcium homeostasis begins in the parathyroid glands of the endocrine system[128]. The calcium-sensing proteins in these glands detect plasma calcium concentration and when low, stimulate PTH secretion[129, 130]. PTH then stimulates calcitriol production by activating 1α-hydroxylase in the convoluted tubules cells of the kidney. In addition, PTH also mobilizes calcium from bone[128, 130, 131]. Low plasma phosphate also stimulates calcitriol synthesis.

Calcitriol increases serum plasma calcium concentration back to 2.5 mmol/L[100, 132] by acting on various organs with PTH in three ways[100]. First, in response to PTH, calcitriol binds to vitamin D receptors (VDR) within osteoblasts to produce receptor activator nuclear factor-KB ligand (RANKL)[13, 133]. RANKL then stimulates osteoclastogenesis through activation of osteoclasts and induces bone resorption[100, 128]. The result is calcium and phosphorus resorption from bone to normalize the blood level[38, 106, 107]. This occurs solely in the absence of calcium in the environment (e.g. diet)[128]. Calcitriol has not been found to be anabolic on bone by itself but acts only in the presence of PTH[128]. Hence having adequate

VTD status is a preventive measure to reduce bone loss otherwise used to support calcium homeostasis. Second, calcitriol affects gene transcription and expression. Calcitriol binds to the vitamin D receptor protein in the nucleus of a cell and forms the VDR-calcitriol complex. The complex heterodimerises with RXR within cells such as osteoblasts, enterocytes and parathyroid, to form the VDR-RXR complex. This VDR-RXR complex when bound to vitamin D-responsive elements (VDREs) situated at a specific promoter region of a gene, allows for transcription of specific messenger ribonucleic acids (mRNAs) to enhance or inhibit specific proteins[38, 100, 106, 107, 128, 134]. For example, these mRNA are eventually translated for synthesis of proteins that make up the epithelial calcium channels and the calcium-binding protein (calbindin) in the mucosal brush border of the intestine. This results in active transport of calcium across the duodenum[38, 106, 107]. Simultaneously, phosphate absorption is also increased through cleavage of phosphate esters by acid phosphatase thereby increasing phosphorus absorption in the jejunum and ileum[135]. There is also an alternative mechanism where it is thought that there is a non-gene related mechanism through opening of the voltage-gated calcium channels[100]. The full mechanism is not well understood[1]. Without calcitriol, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed and with VTD this is increased to 30-40% and 80%, respectively[1, 13, 136]. In periods of active growth, calcium absorption efficiency can increase to 60% to 80%[1]. In adolescents who have less than recommended intake of calcium, calcitriol increases the efficiency of intestinal calcium absorption[100]. Thirdly, calcitriol binds to VDRs in the cells of the distal tubule of the kidney and increases reabsorption of calcium and phosphorus. Calcium reabsorption is increased by 1% which is an equivalent to about 7 g/day[128]. This addition to the calcium pool is a major contribution.

Once calcium concentration is reinstated, through negative inhibition, calcitriol and PTH are down regulated. Increased serum calcium decreases PTH and therefore removes the stimulation on calcitriol synthesis. Increased calcitriol inhibits 1α-hydroxylase and slows down calcitriol synthesis[137] and stimulates 24-

hydroxylase (or P450C24 or CYP24R)[138]. 24-hydroxylase converts calcitriol to 24,25-dihydroxyvitamin D ($24,25(OH)_2D_3$)[139] which is then further hydroxylated to calcitroic acid (1,24,25-trihydroxyvitamin D)[139], an inactive metabolite[134] that is excreted in bile[100, 137]. 24-hydroxylase is also increased in response fibroblast growth factor 23 (FGF-23) produced by osteocytes in bone matrices[137, 140]. Like 1α-hydroxylase, the main site of 24-hydroxylase activity is at the proximal tubule of the kidney[137]. Calcitriol is also inhibited by increased serum phosphorus[139]. Calcitriol, mediated by the VDR receptor, also inhibits PTH[139].

1.4.5.2 Skeletal health

The role of vitamin D in calcium and phosphorus homeostasis is clear; however, its role in bone mineralization at typical physiological concentrations needs further research. One of the postulates is proposed as followed. Calcitriol may enhances bone formation, inhibit resorption[141] and influences the balance of calcium between bone and blood[142] by indirectly affecting PTH regulation. It may suppress the gene expression of the preproparathyroid hormone and therefore prevent the proliferation of parathyroid gland cells[128, 134]. In VTD deficiency, unsuppressed proliferation leads to hyperparathyroidism, a condition known to mobilize calcium from bone and over time resorbs bone[128]. These mechanisms give insight as to why VTD deficiency rickets results in children[143, 144], along with failure to attain peak bone mass in adolescence, osteomalacia[145] in adults and falls, fractures[146] and osteoporosis[147] in elderly.

1.4.5.3 Non-skeletal health

It is known that VDR exists not only in the target cells of enterocytes, osteoblasts, and distal renal tubule cells but also in greater than 30 other tissues [134, 148-153]. Hence, each tissue is capable of converting calcidiol to calcitriol by producing its own 1α-hydroxylase locally[154]. With a known role in gene expression in calcium homeostasis, calcitriol is thought to also be involved in greater than 900 other genes regulation and expression[155]. Thus, VTD metabolism may play an important role in muscle weakness[156, 157], skin disorders [158], immune function [159-161], cardiovascular disease [162-165], cancer [166-168], fertility and pregnancy outcomes

[7, 169], mental health [170, 171], and all cause mortalities [172]. In children, VTD may reduce the risk for type 1 diabetes mellitus[173], respiratory infection, non-Hodgkin's lymphoma [174], bronchiolitis[175], pneumonia[175], allergies[176], eczema[177], wheezing[178], and asthma[179]. However, the strongest evidence only supports VTD in the prevention of bone fracture, falls and osteoporosis. The evidence for extra-skeletal roles of VTD is weak and/or conflicting[7, 13, 169, 180-185].

1.5 VITAMIN D STATUS

In this section, unless stated, prevalence refers to the IOM's VTD insufficiency definition of < 50 nmol/L of 25(OH)D and deficiency of < 27.5nmol/L of 25(OH)D for children.

The 2007-2009 Canadian Health Measures Survey (CHMS) reported 25.7% of VTD insufficiency among Canadians ages 6 to 79 y[75]. The CHMS also identified non-White Canadians to have higher risk of not achieving adequate levels of VTD status[75]. This national survey has a few limitations: 1) children under the age of 6 years were not included, 2) skin pigmentation was not measured and 3) the non-white group included aboriginal population. To date, limited studies on VTD status have been conducted in Canadian preschool age children particularly those in ethnic minority and immigrant populations. It remains to be seen whether the self identified ethnicities and immigration status in the national survey explain vitamin D status in school age children. Canadian specific studies are important because VTD status is dependent on latitude (details in Section 1.4.2) and VTD food fortification programs (details in Section 1.4.1). This section will summarize Canadian studies and complement the gap in knowledge with international studies regarding VTD status among preschoolers, ethnic minorities and immigrants.

1.5.1 CANADIAN STUDIES

To date, there are 5 Canadian specific studies on preschoolers (2 to 5 y) (Table 6). These have reported an insufficiency range between 17 to 73%[186-190] which is higher than the CHMS prevalence for 6 to79 y. Many of these studies have the

Table 6: Vitamin D status of preschoolers (2 to 5 y) in Canada

Studies	Location	Latitude (°N)	Population studied	Month-Year of measurement	Age (y)	n	25(OH)D (nmol/L) Mean/ Median	25(OH)D (nmol/L) % < 50
El Hayek, Egeland et al. 2010[186]	16 Canadian Arctic communities (IPYIAHS)	56 to 72	Healthy aboriginal preschoolers	Aug to Nov 2007 Aug to Sept 2008	3 to 5	334	48 39	52 73
Maguire, Birken et al. 2011[188]	Toronto, Canada	43	Preschoolers attending well-child visit center	Nov 2007 to May 2008	2 to 2.5	91	60	32
Stoian, Lyon et al. 2011[189]	Calgary, Canada	51	Children scheduled for elective surgery during 12-mths period	NA[4]	2 to 13	1442	86	39[1]
Roth, Martz et al. 2005[190]	Edmonton, Canada	52	Children presenting at paediatric Emergency Department	April 2003	2 to 8	35	52	17
Newhook, Sloka et al. 2009[187]	Labrador and Newfoundland, Canada	46 53	Children presenting for blood work at the hospital	Sept 2005 to Mar 2006	0 to 14	48	53 68	35

[1]Based on <75 nmol/L;
[2]Values converted to nmol/L using 2.5 nmol/L=1 ng/ml;
[3]NR = not reported;
[4]NA= only able to access abstract

same limitations as the CHMS in addition to: 1) sampled population is not random and 2) the population studied was a high risk population. The CHMS reported that children age 6 to 11 y had a 14.1% prevalence of insufficiency which is lower than the whole population (25.7%)[75]. The lower prevalence rate may be ascribed to age where younger children are reported to be have better VTD status[33, 180, 189-191] because of higher intake of fortified food and UV exposure. Hence, perhaps young children may be able to achieve and maintain VTD status better than adults. The youngest healthy Canadian age group in which the relationship between ethnicity and VTD status is reported was by Gozdzik et al. They studied a group of students 18 to 30 y of various ethnicities in Toronto, Ontario and reported a 73.6% VTD insufficiency[192]. Whether this is true in children less than 6 y of age remained to be demonstrated.

1.5.2 NORTHERN UNITED STATES STUDIES

To have an insight into the VTD status of ethnic minority and immigrant preschoolers, selected studies from other countries with similar population demographics and cultures are shown. As in Canada, the United States also has a national survey, the 2001-2006 National Health and Nutrition Examination Survey (NHANES). The NHANES reported a prevalence of 14% of insufficiency in children age 1 to 5 y[193]. In addition, within the same age group, a higher prevalence (20 to 31%) was reported for the non-White population[193]. However, because it is a national study, the NHANES includes many southern US states and cities which have higher UV exposure access year round. In addition, the measurements of vitamin D status in northern US were done during summer months. Other than NHANES, there was only one other study that looked at preschoolers in the US. The study was conducted in northern USA (49^{o}N) with children under 6 y. Similarly to NHANES, they found 29% of VTD insufficiency in low-income immigrants who are Hispanic, Somalis and East Africans[194]. There are several factors that differ between United States and Canada that affects VTD status of the population: 1) the largest ethnic minorities in the USA are Hispanic or Black, 2) the USDA fortification program differs from the Canadian (as

endogenous synthesis of VTD due to latitude and possibly greater food variety in support of higher VTD status than Canadians.

1.5.3 NORTHERN EUROPEAN STUDIES

Similar to the USA, the VTD insufficiency prevalence is of 17% in England. For the same reasons as USA, factors affecting VTD status in Canadians differs from the British (Table 7). There are two additional studies conducted involving preschool age children in Northern Europe, however, these studies are done in non-healthy children[195, 196].

1.5.4 CIRCULATING 25(OH)D CONCENTRATION IN ETHNIC MINORITIES COMPARED TO WHITES

National surveys in several countries report lower VTD status of ethnic minorities compared to White. CHMS estimates non-White age 6 to 11 y to have a mean 25(OH)D concentration of 63.3 nmol/L which is 15.2 nmol/L lower than White children[197]. While in England, the difference is found to be of 25, 25 and 31 nmol/L in Bangladeshi, Indian and Pakistani, respectively, compared to White. The higher difference observed in the British population may be partially explained by the lack of a VTD fortification program[198]. NHANES 2001-2006 found a difference of 16 and 7 nmol/L lower in plasma 25(OH)D in Blacks and Hispanics, respectively, compared to White preschoolers[193]. New Zealand's national survey reported a 10 to 17 nmol/L difference between White and darker-skinned ethnic children and adolescents[191] (Table 8). Plasma 25(OH)D concentration in specific ethnic minority groups are not all consistently lower than Whites. For example, Blacks and Asian Indians are consistently reported to have lower 25(OH)D status while status in Orientals is controversial[39] suggesting that darker skin pigmentation's role on 25(OH)D photosynthesis (details in Section 2.5.1) effect on 25(OH)D concentration is relative to ethnicity. Generally, 25(OH)D concentration is higher in Japanese, Latin American, Southeast Asian populations compared to those in China, India, Tibet, Mongolia, Arabs, and Africa[33]. In Europe, Sweden and Norway have higher vitamin D status than Mediterranean countries because of their higher intake of fish oil and fatty fish

Table 7: Vitamin D status of preschoolers (2 to 5 y) in North American and Europe

Studies	Location	Latitude (^{o}N)	Population studied	Month-Year of measurement	Age (y)	n	25(OH)D (nmol/L) Mean/ Median	25(OH)D (nmol/L) % < 50
NORTHERN US STUDIES								
Mansbach, Ginde et al. 2009 [193]	Nationally representative US sample (NHANES)	25 to 50	Healthy preschoolers	2003 to 2006	1 to 5	1799	70	14
Kersey, Chi et al. 2011 [194]	Minneapolis, USA	45	Low-income ethnic minority immigrant children attending outpatient clinic	May 2008 to Jun 2009	0.5 to 6	253	37	29
NORTHERN EUROPEAN STUDIES								
Lawson, Thomas et al. 1999 [199]	Nationally representative England sample (National Survey 1994-1996)	50 to 60	Healthy preschoolers	Oct to Nov 1996	1.5 to 2.5	831	NR[3]	17
Prusa, Cepova et al. 2011 [195]	Prague, Czech Republic	50	Overweight and obese children	Oct to Nov 2010	4 to 18	113	29	92
Hoyland, Rees et al. 2011 [1964]	London, UK	42	Sickle cell anaemia children staying in hospital	2006-2009	1 to 21	276	NA[4]	93[1]

[1]Based on <75 nmol/L; [2]Values converted to nmol/L using 2.5 nmol/L=1 ng/ml; [3]NR = not reported; [4]NA= only able to access abstract

Table 8: Difference in circulating 25(OH)D concentration between ethnic minorities and Whites in national surveys

	Location	n	Age (y)	Details	Ethnic	Difference (nmol/L)
Langlois, Greene-Finestone et al. 2010[197]	Canada	903	6-11	Nationally representative Canadian sample (CHMS)	Non-White	15
Lawson, Thomas et al. 1999 [199]	England	831	1.5-2.5	Nationally representative England sample (National Survey)	Bangladeshi Pakistani Indian	25 31 25
Mansbach, Ginde et al. 2009[193]	USA	1799	1-5	Nationally representative US sample (NHANES)	Non-Hispanic Black Hispanic Other	16 7 5
Rockell, Green et al. 2005[191]	New Zealand	1585	5-14	National Survey	Maori Pacific	10 17
Gutierrez, Farwell et al. 2011[200]	USA	8415	>12	National Sample (NHANES 2001-2006)	Black Mexican	27 15
Moreno-Reyes, Carpentier et al. 2009[201]	Brussel, Belgium	401	40-60	National Survey	Moroccan Turkish Congolese	22 18 11

[1]Based on <75 nmol/L;
[2]Values converted to nmol/L using 2.5 nmol/L=1 ng/ml;
[3]NR = not reported;
[4]NA= only able to access abstract

described in Section 1.4.1) and 3) the country is closer to the equator aside from Alaska. Hence, it is thought that Americans have greater opportunity for consumption[33]. However, there is a study in London with sickle cell anaemia in children (1 to 5 y) that demonstrated no association between 25(OH)D and ethnic background (east/west African and Caribbean's)[196].

1.5.5 STUDIES WITH IMMIGRANTS

Numerous studies have reported lower VTD status of immigrants compared to White, however, most are in the adult population[202-205]. The difference is reported to be between 7 to 23 nmol/L[203, 205] and the prevalence of insufficiency is reported between 78 to 90% (Table 9) which is higher than White and ethnic minorities.

1.5.6 ETHNIC MINORITIES, IMMIGRANTS, AND RISK OF VITAMIN D-DEFICIENCY RICKETS

The Canadian fortification program includes milk, a high source of calcium, as a vehicle to VTD to prevent rickets. However, many studies show that rickets is either still persisting or resurfacing due to increased immigration from countries near the equator. Most cases of rickets are either due to low calcium intake or low VTD plasma concentration or both. In 2004, 104 rickets cases are found across Canada[2]. Seventy of these are aged between 1 to 7 y (mean age of 1.4 y) and 89% were of intermediate or dark-skinned color (33% were Black, 14% were Middle Eastern, 1% were Latin American and 1% were Asian)[2]. Studies in other western countries also found most cases of rickets and osteomalacia to be present mostly in ethnic minorities and immigrants (Table 10). Hence, although rickets and osteomalacia is not prevalent in North-America and in European countries, it is still highly prevalent in ethnic minorities and immigrants population[145, 206].

1.6 PREDICTORS OF VITAMIN D STATUS OF ETHNIC MINORITY AND IMMIGRANT PRESCHOOLERS

As highlighted, there is a lack of Canadian research on VTD status in ethnic minority immigrant preschoolers. In 2006, Canada has a population of approximately 32 million with ~ 6% in preschool age and among preschoolers ~ 5% lives in Montréal, Canada[207]. The percentage of Canadian preschoolers who

Table 9: Vitamin D status of immigrants

Studies	Location	Latitude (^{o}N)	Population studied	Month-Year of measurement	n	Age (y)	Ethnic	25(OH)D(nmol/L) Mean	25(OH)D(nmol/L) % < 50
Berg et al. 2010[203]	Oslo, Norway	59	HUBRO 2000-2001	2000 to 2001	177	16 to 65	Asian	25	NR[3]
Madar et al. 2009[208]	Oslo, Norway	59	Routine check-up infant and mothers	Mar 2004 to Feb 2006	86	NA[4]	Pakistan Turkish Somali	25 41	91 60
Holvik, Meyer et al. 2005[209]	Oslo, Norway	59	HUBRO 2000-2001	2000 to 2001	1000	31 to 60	Turkey Sri Lanka Iran Pakistan Vietnam	28	90
Grootjans-Geerts and Wielders 2002[210]	Netherlands	52	Veiled, Turkish women	Mar 2001	51	14 to 63	Turkish	NA[4]	82
van der Meer, Boeke et al. 2008[205]	Netherlands	52	General practices	Sept 2003 to June 2005	613	18 to 64	Turkish Morrocan Surinam Creole African	27 30 24 27 33	84
Meulmeester, van den Berg et al. 1990 [211]	Hague and Rotterdam, Netherlands	52	Immigrant children	Feb or April	80	8	Turkish Moroccan	23 30	42 23
Erkal, Wilde et al. 2006[204]	Germany	52	NR[3]	NR[3]	994	16 to 69	German Turkish	72 38	29 78

[1]Based on <75 nmol/L; [2]Values converted to nmol/L using 2.5 nmol/L=1 ng/ml; [3]NR = not reported; [4]NA= only able to access abstract

Table 10: Vitamin D deficiency, osteomalacia and rickets cases

Study	Age range	Time	Location	# cases	Details
Ward, Gaboury et al. 2007[2]	2 wk - 6.3 y	July 2002-June 2004	Canada	104	89% has intermediate or darker skin
Ahmed, Franey et al. 2011[212]	2 wk-14 y	2002-2008	West Scotland	42	n = 3: White n = 39: South Asian, Middle Eastern or Sub-Saharan
Modgil, Williams et al. 2010[213]	0-16 y	2003-2007	Bristol, UK	127	n = 90 Somali, n = 37 non-somali
Mytton, Frater et al. 2007[214]	All ages	Jan 2003-Sept 2005	Bristol, UK	272	182 = Black, 134 = Somali, 14 = Asian
Callaghan, Moy et al. 2006[215]	< 5y	May 2000-April 2001	UK	24	All Asian

are ethnic minority and immigrant is not available but if the total population proportion (~ 26%) is applied (details in Section 1.3), this sums up to more than 25 000 preschoolers of ethnic minority immigrant status therefore representing a significant proportion of the population living in Montréal Island. Yet, no studies to date have been designed to measure VTD status and its predictors in this group. Skin pigmentation, season, sun exposure, dietary intake of calcium and VTD are identified to be strong predictors in older age groups and BMI, sex and socioeconomic status are variables that are known to influence VTD status. How these variables affects plasma 25(OH)D are discussed in the this section.

1.6.1 DARK-SKINNED INDIVIDUALS LIVING AT HIGH LATITUDES

1.6.1.1 Latitudes

Evolution ensured a balance between VTD_3 synthesis and protection of the skin from UVR by creating a skin pigmentation gradient correlated with latitude (details in 1.4.3.4). The advantages of the distribution of skin pigmentation by latitude is, however, disrupted in respect to VTD photosynthesis when dark skin pigmented individuals started to migrate to northern latitudes.

1.6.1.1.1 Dose and rate

At latitudes > 40^{o}N, studies showed darker skinned individuals have more difficulties maintaining VTD status throughout the year[216] because they produce $preVTD_3$ less efficiently than lighter skinned individuals[18, 72] due to higher concentration of melanin and eumelanin dust in the stratum corneum of their skin. Hence, while melanin protects the skin from damages by absorbing and scattering[217] UVB rays from penetrating the spinosum and basale strata, it also prevents $preVTD_3$ production[39, 50, 84]. Melanin competes for UVB photons with 7-DHC and therefore, a higher dose and longer rate of UVB exposure is needed in dark skin pigmentation. For example, an Asian Indian person requires 2 to 3 times and a Black person requires 6 to10 times more UVB exposure to synthesize equal amount of VTD_3 compared to Whites[35, 61, 73, 218, 219]. UVB dose is also increased. A sub-Saharan African (L*=35) needs twice the dose (8 SED) than a typical

Equation 4: UVB dose and L* value

$$z = 0.010904*x*y$$

where;
z = Δ25(OH)D3 from baseline (nmol/L)
x = is UVB dose (mJ/cm2)
*y = skin lightness in L**
reference: cited in Armas, 2007[72]

northern European (L*=70)[72]. Equation 4 shows the relationships between change in plasma 25(OH)D, skin pigmentation and UVB dose. There are a few proposed mechanisms as to why darker skinned pigmentation has lower preVTD$_3$ synthesizing capacity in countries of higher latitudes: 1) Canadian UVB exposure year round cannot provide the dose and rate required, 2) exposed % BSA cannot be met during cold climate, and 3) the proportion of shorter wavelengths in the UVB spectrum reaching northern countries is lower than southern countries. The first two were discussed in detail previously in Section 1.4.2. To summarize, the dark skin pigmentation is not adapted to the weather and latitude of Canada and therefore VTD$_3$ photosynthesis is insufficient during non-synthesizing periods. This is not only evidenced by the difference in 25(OH)D of Canadian ethnic minorities compared to Whites Canadians but also compared to ethnic minorities living in their home land[206]. The difference can be observed in all ethnic minorities (i.e. Turkish, Moroccan, Indian, sub-Saharan African) and may be as high as 60 nmol/L[206].

1.6.1.1.2 UVB wavelengths

The other difference between Northern and Southern latitudes may be explained by dose and rate of shorter wavelengths within the UVB spectrum. Recently, a RCT trial published contradictory results to the positive relationship between 25(OH)D and skin pigmentation seen in epidemiological studies. The mechanism is hypothesized to lie in shorter wavelengths emitted in UVB lamps compared to sunlight. These shorter wavelengths (280 to 295 nm) are able to penetrate to the strata granulosum, independent of melanin concentration in stratum corneum, where there is a small but significant concentration of 7-DHC present[55]. Strata granulosum has the highest proportion of 7-DHC to melanin content. Therefore, efficiency of preVTD$_3$ synthesis is highest in the stratum granulosum and at shorter wavelengths[58]. These two characteristics, together, are hypothesized to be capable of preVTD$_3$ synthesis regardless of skin pigmentation[54, 55]. Bogh et al. showed that four replicates of skin irradiation for 10 min at 3 SED and at 2-3 days apart, can increase VTD$_3$ by 27 nmol/L, independently from skin pigmentation[54].

Spectrum of wavelengths present in sunlight that is capable of doing the same conversion seen in UVB lamps in skin is between 290 to 295 nm. This suggests that possibly, at lower latitude, the UVB rays between 290 to 295 nm may be available at a higher dose and longer rate and therefore is able to raise 25(OH)D above the deficiency level regardless of skin pigmentation and only VTD sufficiency and insufficiency differences are associated with skin pigmentation.

1.6.2 LIGHT-SKINNED ETHNIC MINORITIES

As mentioned, ethnic minority is defined as those with darker skin pigmentation and darker skin pigmentation is associated with lower VTD_3 photosynthesis efficiency. However, not all ethnic minorities defined in the Employment Equity Act have significantly darker skin compared to Whites. For example, constitutive skin ITA^o values in Asians but not in Blacks is not significantly different to Whites[81]. Examples of ethnicities with darker skin pigmentation are given in Table 4. In addition, the way lighter skinned ethnic minorities, such as Asians, tan and the relationship with VTD_3 photosynthesis is unclear which may explain why there are controversial studies on the VTD status of Asians.

1.6.2.1 Tanning

Theoretically, based on similar constitutive skin pigmentation, light skinned ethnic minorities should photosynthesize VTD_3 as efficiently as Whites; however, there is another difference in skin anatomy that sets light skin ethnic minorities apart from Whites. In the Fitzpatrick assessment of skin phototype, light skinned ethnic minorities (Type III-IV) are often described as those which tan easier and burn less[220]. The one study which looked at skin tanning between ethnicities by Tadokoro et al. does not support the observation by Fitzpatrick. They did not report a difference in melanin distribution between light skinned Asians and Whites upon UVR exposure, like they do in Blacks[221]. The change in melanin was only measured for 7 days while it is known that it takes a total of 4 weeks from the beginning of melanin migration to skin slough off. Therefore, the question remains about how melanin synthesis and degradation differ between light skinned ethnic minorities compared Whites regarding present and past UV

exposure and how the difference impact on 25(OH)D concentration. In Whites, tanning of the skin resulting in a change of -5 ITA^{o} or -0.95 L* between constitutive and facultative is associated with an increase in 15[222] or 27[72] nmol/L of 25(OH)D concentration, respectively. However, melanin content in dark skin increases quicker than in light skin and there is a greater increase in melanin migration to upper layers of the skin[221] when they are exposed to an equal number of repeated UVR exposures as compared to Whites. This suggests that for the same change in ITA there is a lower change in 25(OH)D in darker-skinned people compared to Whites.

1.6.3 SUNSCREEN VERSUS CANCER

Sunscreen, like melanin, is also a competitor for UVB photons. There is a debate as to whether sunscreen recommendations should be different in darker skin. The following arguments support sunscreen recommendations based on skin type: 1) exposure of < 0.5 SED provides the best risk-benefit ratios 2) no markers of skin damage associated with skin cancer was found in brown and Black skin pigmentation when exposed to large dose of UVR (2 SED and 3 SED) in the basale and dermal layers[94] and further evidenced by the lower association of basal and squamous cell carcinoma with African-Americans than with Caucasians[223-227], 3) damage to the basale and not the suprabasale cells are suspected to be the origin of epidermal carcinoma development[94, 228], 4) cancer mortality is suggested to be reduced by moderate unprotected UV exposure[229], 5) only 5 to 7 billion dollars is spend on health care resulting in UVR damages while 40 to 53 billion is spent on VTD deficiency related health issues[230] and 6) black epidermis protects the skin against sunburn equivalent to a sunblock protection factor (SPF) of 13.4[84] and a SPF of 8 blocks out 95% of UVR[231]. In addition, current recommendation is slightly flawed. Exposure to sunlight in mid-day is less likely to cause skin cancers than early or late in the day because basale carcinoma is more likely to occur after exposure to excessive UVA rather than UVB[232-234]. Sunscreens have more protection against UVB rather than UVA[1]. In adults, sunscreen use in relation to 25(OH)D concentration is controversial[235-237], however, the

relationship may be clearer and more positive in children. Unlike adults where sunscreen application does not follow guidelines in quantity (2 mg/cm^2), in length prior to exposure (15 min) and in reapplication (2 h)[237],children who attend daycare are subjected to these directives by policies. More than 50% of preschool age children attend daycares in Canada where sunscreen application recommended by Health Canada[238] is strictly followed by daycares providers. Daycare providers apply sunscreen 15 min prior to going outdoors and outdoor time in daycares rarely exceeds 2 h. Chronic sunscreen use is present in children and it is associated with lower 25(OH)D concentration[235]. Therefore, sunscreen increases risks for VTD deficiency in darker skinned ethnic minorities and may be more exaggerated in preschool age children.

1.6.4 SUN AVOIDANCE PRACTICES

Adding to the challenge of achieving adequate VTD status through photosynthesis, ethnic minority immigrants are known to have sun avoidance behaviours (covering of skin with clothes or avoidance of direct sun exposure)[205, 206, 239-243204, 205 242, 244 243 245] and this is highly dependent on their values and cultural beliefs[33]. It is observed that the more recent the immigration, the stronger the adherence to these beliefs[5] and immigration status is associated with lower VTD status[203-205, 208-211] (details in Section 1.6.7). Clothes covering behaviours do not affect children however the avoidance of direct sun exposure (staying indoors or in the shade) does. Shade reduces UVB by 60%[246]. Southeast Asians[247] and Africans[33] are two known cultures to avoid sun and prefer to stay indoors or in the shade[33]. In contrast, Nordic countries who have sun seeking behaviours have better VTD status than their southern neighbour, the Mediterranean countries[33]. Casual sun exposure can increase serum 25(OH)D by 25 to 37.5 nmol/L during the summer[248]. According to the CHMS, people who spend less than 1 h outside per day have lower VTD status[197]. Ethnic minority children are reported to spend less time outdoors because their parents value strongly academic performance and when given the choice, they prefer enrolling their children in more academic than sport based extracurricular activities. In addition, living in crowded household[186]

in metropolitan cities[246] with increased pollution[249] is associated with lower VTD status. Ninety-five percent of ethnic minorities live in the 3 largest metropolitan census (Montréal, Toronto, Vancouver)[5]. Decreased urbanization and increased time spent indoors decrease VTD synthesis[63, 246]. It is, therefore, argued that inadequate sun exposure is not a factor in the relationship between skin pigmentation and latitude but a "product of industrialization, urbanization and over population"[50, 250].

1.6.5 BMI AND SOCIOECONOMIC STATUS

Robins et al. state that during summer months, it is possible to synthesized adequate 25(OH)D_3 and accumulate sufficient VTD stores in fat tissue for use during winter months. The argument is however not supported by studies done in overweight and obese individuals where they are reported to have lower serum 25(OH)D concentrations than healthy weight individuals[251]. Although VTD is stored in fat[102, 103], this storage pool is not readily mobilized in time of need as it is dependent on turn-over of fat tissue. In fact, body fat is suggested to sequester VTD because of its solubility and renders it unavailable for metabolic use[139]. Ethnic minority immigrants in Canada consume more energy and are heavier in weight due to poor dietary intake and low physical activities[252-254] which are associated with their lower SES and education status[255]. Adiposity is negatively correlated with serum 25(OH)D ($r=-0.55$)[251] and potentially 1,25$(OH)_2D$[119].

1.6.6 MILK CONSUMPTION

One of the characteristics of poor dietary habits is high consumption of sweetened beverages and low consumption of fortified milk[197]. Like sun exposure, the degree of adherence to cultural and religious beliefs of new immigrants influences the integration of fortified cow's milk in their diet. Culturally, cow's milk is not a staple food for many ethnic minorities. This is because, prior to refrigeration, northern countries were able to consume fresh milk regularly because of cold temperature acting as a preservation method[256]. In equatorial (i.e. Vietnam, Africa, Haiti) and even in temperate countries (i.e. Italy, France, Belgium), due to warm year-round climate, fresh milk was converted to cheeses and yogurts or a

substitute was consumed such as soy products[256]. Therefore, in Canada, unless informed, fortified cow's milk is not consumed on a regular basis by some new immigrants[256] and seeking dietary sources of VTD may not be a conscious activity among ethnic minorities and immigrants.

For these reasons, both VTD and calcium intake are reported to be lower in ethnic minorities compared to White. VTD, phosphorus and calcium intake is correlated because milk is an excellent source of these nutrients. Asians have the lowest consumption of fortified milk[192] with 60% less calcium[257] and 35% less VTD[192] than Whites. Lower intake of calcium and VTD is also reported in Blacks[258, 259] and North and Southern Indians[197]. Milk intake in Canada is important because it has been repeatedly shown to positively associate with improved VTD status[188, 197]. Cow's milk provides 45% to 69% and 29% to 51% of VTD and calcium, respectively[260], intake in the diet of Canadian children and teens. The proportion of VTD intake from milk in preschool age children tends to be at the higher end of the normal range of intakes because natural sources of VTD are not commonly consumed in this age group[1]. Thus milk is the main contributor to VTD intake. Among Canadians of all ages, milk intake (greater than once per day) is associated with higher serum 25(OH)D by 12 nmol/L[197]. In addition, milk intake is suggested to be a stronger predictor of VTD status than skin color and sun exposure in children[180]. Thus the question as to how much VTD is consumed from milk and other sources by ethnic minority immigrants preschool age children is unknown.

1.6.7 GENETICS AND METABOLISM DIFFERENCES

1.6.7.1 Vitamin D endocrine system adaptation

The consequences of lower VTD status due to lower intake and photosynthesis in ethnic minorities may not be of the same severity as for other risk groups. It is suggested that there is a vitamin D endocrine system adaptation to chronic VTD deficiency due to several years of diminished exposure to sunlight[139]. The adapted system metabolizes 25(OH)D, absorbs and excretes calcium differently than in

others such as those of Caucasian race[139]. For example, Blacks[261], Hispanics[262] and Asian Indians[263] who are VTD deficient is presented with secondary hyperparathyroidism and Blacks and Asian Indians have higher renal calcium retention[139]. Hyperparathyroidism increases PTH concentration and therefore stimulates increases in calcitriol production. This is evidenced by observations in Blacks with lower 25(OH)D compared to Whites, but higher serum calcitriol and calcium absorption[119, 261, 264]. The differences persist even when after accounting for BMI, age, gender and 25(OH)D[119]. In addition, the relationship between PTH and 25(OH)D is also altered[139]. Observed 24,25 hydroxylase enzymes activities are also higher[139, 263] and the PTH plateau is set at lower 25(OH)D cut off (<30 nmol/L)[265] than Whites. However, this adaptation does not seem sufficient to decrease risk to VTD deficiency, rickets and osteomalacia[263]. More studies are needed to investigate the differences in circulating concentration of 25(OH)D and calcitriol between ethnicities.

1.6.7.2 Polymorphism and ethnic minorities

The observed differences seen in $1,25(OH)_2D$ can also be explained by DBP polymorphism. GC*2 allele is present in a greater percentage in Caucasians (20 to 40%) than in Blacks (0 to 15%) while the difference is not seen in Asians (5 to 31%)[109]. Caucasians and Oriental Asians can be distinguished by differences in frequencies of GC-1F and GC-1S. There is 20 to 80% of GC-IF in Orientals versus 0 to 30% in Caucasians[109]. Figure 2 illustrates the differences in GC-2 and GC-1F between ethnicities. The association between skin pigmentation and intensity of the sun with geographic areas seems to be partially explained by the variation in GC allele[109]. This suggests the GC*2 and GC*1F allele codes for phenotypes that are associated with the blackness and yellowness of the skin, respectively[109]. South Asians (i.e. Indians) are unique in that their phenotypes of DBP are similar to White while their skin pigmentation is markedly darker[109]. Hence, although darker skin pigmented ethnic minorities is less efficient at photosynthesizing VTD, they compensate by having DBP phenotypes that

Figure 2: GC-2 and GC-1F by ethnicity

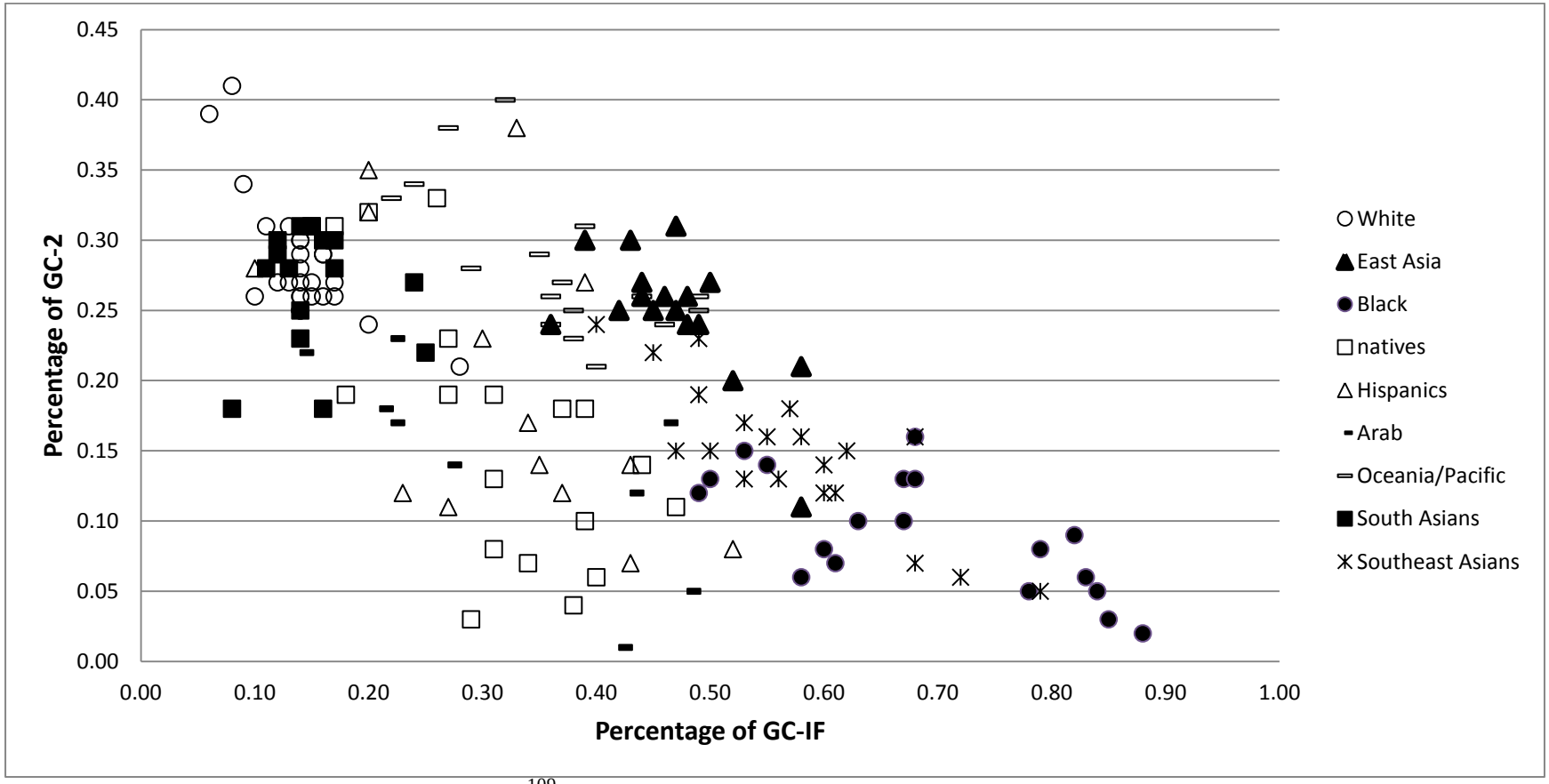


Figure created using data from Kamboh 1984[109].

increase efficiency in VTD conversion to 25(OH)D and 1,25$(OH)_2D$[109]. These differences put into question whether the cut offs used to assess VTD adequacy and the recommendation for VTD intake currently in use is adequate for use in ethnic minorities. Beyond VTD metabolism, there seems to be also differences explained by genetics regarding higher peak bone mass attainment in childhood and better maintenance of bone mass in adulthood and lower fracture risk in old age in Blacks and Hispanics but not in Asians compared to Whites[139]. Bone mass is outside of the scope of this review and will not be discussed. There is no VDR genotypes relationship with bone mass or ethnicities[266, 267].

1.6.8 IMMIGRATION STATUS

In a previous Section 1.6.2 and Section 1.6.5, the adherence to ethnic cultures and religions has been shown to affect sun exposure and dietary VTD intake. This section will discuss how education and socioeconomic status (SES) limit and delay integration to Canadian cultures and increase food insecurity because of transnational ties.

1.6.8.1 Transnational ties

In addition to ethnicity and dark skin pigmentation, there is one other factor which effects both photosynthesis and intake of VTD; immigration status. Immigration status explains, in part, the degree to which ethnic minorities settling into Canada adhere to the lifestyles and dietary habits brought over from their homeland which is influenced by their culture, values and religion. The ongoing ties they keep with their communities of origins while being in Canada is termed transnational ties[268]. They are described to "have come to "imagine" and belong to a community that is no longer "either-or" but "in between" the home land and the host land"[268]. Hence, not only are ethnic minorities adhering to a culture and religion they brought along, but they also are continually living in a place that is no longer of immediate relevance to daily living conditions[268]. Transnational ties prevent/delay integration into Canadian culture. In addition, improved communication and transportation in recent years may prolong and strengthen these ties further[268]. To quantify the degree of these ties, immigrants were questioned on their identity in a

Canadian survey concerning whether their ancestral origins are important to their identity. Positive response were in 71% of 1st generation compared to 57% and 44% in 2nd generation and 3rd generation immigrants, respectively[269].

1.6.8.1 Socioeconomic status of ethnic minority immigrants

The period of highest adherence and vulnerability is in the first 5 years of arrival to Canada. Within the first 10 years of arrival, about 65% of immigrants enter low income and of these, two-thirds did so during their first year[270]. The low-income rates among immigrants during their first full year is 3.5 times and for the following 4 consequent years is 2 times higher than those who are Canadian born[270-272]. This disparity in income between immigrants and Canadian-born continues to persist for at least 20 years[208, 243]. In 1993, Canadian immigration changed their criteria to increase immigration of educated and skilled migrants into Canada in the hope to decrease low income rates among migrants. However, this change did not improve the economic situation of the new flux of immigrants in the consequent years[270]. Therefore, although they are observed to have better education status[208, 243], income levels are independent of education. Instead length of time in Canada is proportionally related to income in recent immigrants[273]. There is a disparity in income among all immigrants in general, but if ethnic minorities are compared to United States, Western Europe and Great Britain migrants, ethnic minorities from South Asia and South/Central America earn up to 50% less[273]. More specifically, migrants from India and China earn only 75% of the income earned by those from Vietnam, Hong Kong and Philippines. Low income is associated with food insecurity defined as insufficient quantities of food available on a consistent basis[274]. Food insecurity[275] and lower SES is associated with lower milk intake (OR=0.6)[254] and lower VTD status. Although, in children, food security is less closely tied to income[276]. Therefore, the immigration status of ethnic minority Canadians can give insight into their SES status and the adherence to their cultures and religions which affects their VTD status.

1.7 RATIONALE AND OBJECTIVES

The ethnic minority immigrant preschooler is an at risk population for low vitamin D status on the basis of lower socioeconomic status, lower education, lower sun exposure, lower consumption of fortified milk, lower VTD photosynthesis efficiency and higher sunscreen use. Their immigration status and that of their care givers affects the degree in which they practice their cultural and religious beliefs and these beliefs influence their dietary habits and lifestyles. In Canada, ethnic minority immigrants are forecasted to increase at a rapid rate such that it may redefine their status between majority and minority. They make up a large and significant proportion (~25%) of Canadian preschoolers in Montréal and their presence and impact on Canadian health system cannot be ignored. Yet studies, on ethnic minorities are few in proportion to those conducted in Whites. Among existing reports, most are not designed to identify the predictors to the VTD status. Specifically, skin pigmentation and dietary intake are less well integrated into study designs. Canadian-specific studies are important because of uniqueness in latitude and food fortification guidelines. To date, no study has been conducted among Canadian ethnic minority groups in children of preschool age regarding VTD status. Therefore, the objective of this study was to determine: 1) the prevalence of low VTD status in ethnic minority preschoolers compared to White children and 2) identify what socioeconomic, sun exposure and dietary characteristics were most related to VTD status.to VTD status.

2.0 MANUSCRIPT

Vitamin D status of immigrant and ethnic minority children ages 2 through 5 y in Montréal

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2.1 ABSTRACT

Ethnic minorities are often characterized by darker skin pigmentation, lower socioeconomic status (SES) and recent immigration status. These attributes may relate to lower vitamin D (VTD) intake and photosynthesis resulting in lower vitamin D status compared to White populations. This study aimed to evaluate the prevalence of VTD deficiency and identify explanatory variables of VTD status of 502 children age 2 to 5 y (n = 139 ethnic minorities, n = 101 Mixed ethnicity, n = 276 immigrant Whites and ethnic minorities) attending Montréal daycares in a cross-sectional study between June 2010 and 2011. Total plasma 25(OH)D from a capillary blood sample was measured using a chemiluminescence assay (Liaison, Diasorin). In multiple linear regression, VTD intake assessed by 24h intake and food frequency questionnaires, ultraviolet (UV) exposure and socioeconomic status (SES) assessed by questionnaires, skin pigmentation measured by spectrophotometer and anthropometry were used to explain the variation in 25(OH)D concentration for each ethnic group. Plasma values were ≤ 50 nmol/L in 10% of White (n = 262), 14% of Black (n = 36), 0% of Hispanic (n = 15), 16% of Arab (n = 45), 9% of Asian (n = 43) and 14% of Mixed ethnicity (n=101). Plasma 25(OH)D ≤ 50 nmol/L was not significantly different among ethnic groups or according to immigration status, recent immigration status, skin type or SES. The main predictor was sun index for White (Δr^2 = 0.09, P = 0.263), VTD intake from supplement for Arab (Δr^2 = 0.18, P < 0.001) and Mixed (Δr^2 = 0.16, P < 0.001) income for Black (Δr^2 = 0.28, P = 0.009) and skin type for Asian (Δr^2 = 0.18, P < 0.001). More than 80% of children attending daycares in Montréal have adequate vitamin D status. Since the explanatory variables for VTD status differed among ethnicities, strategies to improve VTD status in those with low status will need to consider the unique needs of each ethnic group.

2.2 INTRODUCTION

During childhood the immediate consequences of vitamin D (VTD) deficiencies on bone may include rickets[143, 144] and potentially failure to achieve a normal peak bone mass in the long term. Recent evidence, suggests type 1 diabetes mellitus[173], non-Hodgkin's lymphoma [174], bronchiolitis[175], pneumonia[175], allergies[176], eczema[177], wheezing[178], and asthma[179] are also associated to VTD deficiency.

National surveys in all ethnic minority children (6 to 11 y) in Canada[197] and New Zealand[191] and preschoolers (2 to 5 y) in the USA[193] and England[199] reported that mean 25(OH)D concentration was 7 to 31 nmol/L lower than in Whites[191, 193, 197]. In Canada, people living at latitudes between 42 to 52^{o}N can synthesize vitamin D between April 1st to Oct 31st[57]. Ethnic minorities living in Canada face an additional challenge due to darker skin pigmentation of type IV to VI, according to Fitzpatrick classification, which acts like a sunblock with sun protection factor (SPF) 13[84] blocking 95% of ultraviolet radiation (UVR)[231]. This results in 3 to 10 times more ultraviolet B (UVB) exposure[35, 61, 73, 218, 219] required to synthesize an equal amount of VTD compared to White.

Lower income, lower education and more recent immigration status are also reported to be more common in minority populations in Canada[270] and are often associated with lower VTD status[254, 275]. Recent immigration status may affect VTD intake and sun exposure due to adherence to religious beliefs, homeland societal/cultural values and traditional diets[269, 270]. The main source of VTD intake in Canada is fluid milk because of mandatory fortification with VTD[21]. In children (1 to 18 y), cow's milk provides 45% to 69% and 29% to 51% of VTD and calcium, respectively[260]. However, milk is not usually a staple food for ethnic minorities[192, 256], consequently their intake of calcium has been reported to be 40% lower in children (8 to 10 y)[257] and VTD is 35% lower in young adults (18 to 30 y)[192] compared to White. Despite lower vitamin D status, ethnic minorities are reported to have increased calcium absorption[139], renal calcium retention[139], PTH

concentrations[139] and have vitamin D binding protein (DBP) phenotypes with higher affinity for 25(OH)D[109]. These differences are considered by some as a reflection of adaptation of the vitamin D endocrine system to the environment and genetic polymorphisms in DBP[109, 139].

In Canada, between 2001 and 2007 the population of ethnic minorities grew 5 times faster than the main population as a function of the increase in immigration flux from Caribbean nations, Asia, Africa and South America[5]. This growth is forecasted to continue to increase at a rapid rate with ethnic minorities making up to 25% of the Canadian population by 2031[5] compared to 16% in 2006. The proportion is reported to be even higher in metropolitan census areas such as Montréal (~25% in 2006) where 95% of ethnic minorities live[5]. Their presence and impact on the Canadian health system is important considering the cost of health issues related to VTD deficiency which is 5 to 7 times more than that of ultraviolet radiation damage[230]. Studies are needed to understand how ethnic minorities' sun exposure behaviour, dietary lifestyle and SES status impact on 25(OH)D concentration in order to help prevent VTD deficiency. The last national survey was the Canadian Health Measures Survey (CHMS) in 2007 to 2009 that included children 6 y of age and older and assessed ethnic minorities' VTD status briefly as a group of non-White including Canadian aboriginal status[75]. To date, no studies have examined vitamin D status of ethnic minority and immigrant preschoolers (2 to 5 y) in Canada. Thus the objective of this study was to determine: 1) the prevalence of low VTD status in ethnic minority preschoolers compared to White children and 2) identify what socioeconomic, sun exposure and dietary characteristics were most related to VTD status.

2.3 MATERIALS AND METHODS

Design - A total of 502 children (2 to 5 y) were sampled in randomly selected daycares (n=77) in a cross-sectional study between June 2010 and 2011 to represent 10% of all daycares (Figure 1). Based on the home address postal code,

the sample represented 31 (91%) regions of Montréal, Canada (45°N, 73°W, 52 m) and 18 regions within a 100 km radius of the Montréal city center.

Daycare recruitment – Daycares registered with the Ministère de la Famille et des Ainés were randomly selected based on regions. For each region, the targeted number of children to be studied reflected the proportion of children in that region relative to the whole encatchment area of Montréal and our sample size of 500. For example if a region accounted for 1% of the children attending daycare, 5 would be recruited into the study (1% of 500 = 5). Daycares were recruited per area until the number of children in each area was achieved. A minimum of 74 daycares was needed to represent 10% of the total number of daycares (n=733). An average of 7 children per daycare was recruited. Children were sampled proportionally per season.

Ethics - This study was approved by the McGill Faculty of Medicine Institutional Review Board. Informed consent was obtained from parents/primary caregivers prior to inclusion in the study. This was facilitated by the daycare directors.

Inclusion criteria - Children ages 2 through 5 y, term born, not taking medications and without health conditions known to affect vitamin D metabolism or bone were included. 1 participant did not speak English or French and was excluded.

Anthropometry - Height was measured with a portable stadiometer (Seca 213, Seca Medical Scales and Measuring Systems, Hamburg, Germany) and body weight was measured with a digital scale (Home Collection 63-8711-0, Trileaf Distribution, Toronto, ON, Canada) with the child wearing light clothing and no shoes. Z-scores for age were calculated using the World Health Organization (WHO) 2007 reference data (WHO AnthroPlus, Geneva, Switzerland) for height (HAZ), weight (WAZ) and BMI (BAZ) [277].

Skin pigmentation – Skin type was established by measuring pigmentation three times at each site for constitutive pigmentation at the inner upper arm and facultative pigmentation at the forehead, mid-forearm and lower leg using a spectrophotometer (CM-700d/600d, Konica Minolta, Ramsey, NJ, USA). Individual typological angle (ITA^{o}) was calculated with the L* and b* values using the equation from the Commission D'Éclairage (1986)[98] and were classified into 6 skin types based on Fitzpatrick descriptions[94, 99].

Vitamin D status – A non-fasted 1 mL capillary blood sample (via a finger lance) was collected in a heparinized microtainer at daycares. Samples were transported on ice to the research unit where all samples were centrifuged at 3000 g and 4°C for 20 min. Plasma aliquots were stored at -80°C for subsequent analysis of total 25(OH)D concentration using a chemiluminescence assay (Liaison, Diasorin, Mississauga, ON, Canada) at McGill University. The 25(OH)D inter-assay and intra-assay CV% were 7.3 and 5.1% for the low 25(OH)D control (39.8 nmol/L) and 7.1 and 2.8% for the high 25(OH)D control (130.3 nmol/L). According to the Institute of Medicine, 25(OH)D concentrations > 50 nmol/L are consistent with the Recommended Dietary Allowance (RDA) of 600 to 800 IU for those aged ≥ 1 y^{8} while the Canadian Paediatric Society (CPS) suggests 75 nmol/L as the target concentration[11].

Dietary Data – Food intake over 24 h was collected for each child by observation at daycare and caregivers recall for time before and after daycare attendance using a telephone administered multiple pass technique. VTD intake from supplements was collected using a validated semi-quantitative food frequency questionnaire (FFQ). Both food intake assessments were analyzed using Nutritionist Pro™ (Axxya Systems LLC, Stafford, TX, US), the Canadian Nutrient File (v2010b) and/or the percentage daily value (% DV) on nutrition labels. A second 24h intake was possible within the next 7 days for only 15% of children (VTD intake did not differ ($p > 0.05$) between day 1 and day 2 of collection) to adjust VTD intake following the guidelines set by the Institute of Medicine[278] using the National

Research Council method[279]. The adjusted data were used to compare intakes against the EAR value.

Other questionnaires- Child's sun exposure was assessed while at daycares by interviewing the daycare directors (sun exposure survey) and at home with caregivers (parent survey). Data regarding sun exposure during the previous month was collected as a percentage of body surface area (% of BSA) exposed, frequency of sunscreen use (%) and total time spent in direct sunlight per day (min/d) between 1000 and 1500 h. Sun index was then calculated for each child by multiplying % of BSA with time spent outside per day where % of BSA was calculated based on the Lund and Browder chart[67]. Date of collection was also used as a measure of UV exposure where it is classified into season (summer, fall, winter, spring) or as synthesizing (April 1st to Oct 31st[57]) and non-synthesizing period (November 1st to March 31st[57]). Summer starts on June 21st, fall on September 23rd, winter on December 22nd and spring on March 20th in Canada. To reflect possible differences in longer term UVB exposure, the number of days between April 1st and sampling date (synthesizing days) were calculated for each child. The parent survey also collected information regarding SES (education of primary caregivers and household income), immigration status, date of arrival to Canada and ethnicity. Ethnicity of the child was classified using 5 groups (White, Asian, Arab, Hispanic and Black) according to Statistics Canada[280]. An additional group was added for children with multiple ethnicities 'Mixed' with two subgroups; those of multiple ethnicities excluding white (Mixed minorities) and those including at least one White ethnicity (Mixed White). Mixed subgroups are used as explanatory variables. Immigration status of the child was classified as non-immigrant if they were of 3rd generation and immigrant if they were 2nd or 1st generation based on Statistics Canada[280]. Recent immigration status was based on the mother's number of years of residence and if born in Canada, it equals to mother's age. Maternal recent immigration status is divided into three categories based on Immigration Canada: very recent (< 5 y), recent (5 to 15 y) and non-recent (>15 y)[6]. Household annual incomes, non-specific to family size, before

taxes were classified as high (> $75 000/y) or low income (< $30 000/y) based on Statistics Canada[281].

Statistical analysis – All statistical analysis was conducted using SAS (v9.2, Cary, USA). Differences between 25(OH)D < 50 nmol/L and median plasma 25(OH)D concentration were tested according to ethnic group, immigration status, skin type, recent immigration status, income and maternal education using ANCOVA or Fisher's Exact Test for proportions. Multiple linear regression was originally used to predict 25(OH)D concentration for the whole sample. However, 24 significant two-level interactions ($p<0.05$) were associated with ethnicity (n=5), sun index (n=7), season (n=7), immigration status (n=2), skin type (n=2) and VTD intake (n=2). Even with these interactions, the model including all children was only able to predict 30% of the variance in 25(OH)D concentration. Therefore, the sample was divided into ethnic groups to minimize interactions, and individual regression models were performed for all ethnic groups; except for the Hispanic group due to small sample size. Independent variables included in final regression model were tested in univariate and 2 level interactions in multivariate regression model. Diagnostics were performed to test for homogeneity, heteroscedasticity, multi-colinearity and residual normality in all models. Residuals were not normal therefore 25(OH)D was log transformed for all groups except Asians.

2.4 RESULTS

2.4.1 STUDY POPULATION

Of the 502 children included in the study, 28% were ethnic minorities coming from 42 countries and 55% were immigrants of 1st or 2nd generation. There is also diversity in White children where they represent 27 countries. Based on ITA^{o} from the inner upper arm, 92% of White ethnicity had skin type I or II, 86% of Arab and Hispanic had type II or III, 86% of Mixed ethnicity had type II-IV, 91% of Asian had type II-IV and 92% of Black ethnicity had type V-VI.

VTD intake was not significantly different between ethnic groups including supplement intake with 93% of all ethnic minorities drinking milk everyday (> 250 ml/d). VTD intake was above the EAR in 5% (n = 7) of ethnic minorities. UV exposure between 1000 h and 1500 h was on average > 60 min per d during summer and 18 to 36 min per d during fall and spring with 95% ethnic minorities having UV exposure everyday (> 10 min per d) during synthesizing period. All year round, the percentage of children who went on vacation in the preceding month was 4.9% of White, 4.4% of Arab, 4.6% of Asian, 2.9% in Mixed while none in Black and Hispanic children. Ethnic minorities had significantly lower SES and darker skin pigmentation compared to White ($p < 0.05$) (Table 1).

2.4.2 VITAMIN D STATUS

Plasma 25(OH)D concentration ≤ 50 nmol/L was prevalent in 10% of White, 14% of Black, 16% of Arab, 9% of Asian and 14% of Mixed ethnicity children, while none of the Hispanic children had 25(OH)D concentration ≤ 50 nmol/L (Figure 2). Concentration of 25(OH)D ≤ 50 or 75 nmol/L or mean plasma 25(OH)D concentration was not significantly different among ethnic groups. In addition, plasma 25(OH)D concentration ≤ 50 nmol/L were not significantly different according immigration status, skin type, recent immigration status, income, synthesizing period or maternal education. Overall, 25(OH)D concentration was negatively correlated with non-White ethnicity, increased age, higher BMI and darker skin pigmentation but positively with higher VTD intake, and higher sun index.

Multiple linear regressions (CI = 95%, power > 0.80) were used to identify the factors that explain VTD status within each ethnic minority. The key explanatory variables for Mixed and Arab is VTD intake from supplements, for Asian is skin type, for Black is income, and for White is sun index (Table 2). Sex was only a explanatory variable in Blacks, BMI was in none and age was in all except for Asian.

2.5 DISCUSSION

This is the first report regarding vitamin D status of preschool children from a large urban centre in Canada taking into account ethnic and immigration status. Our study showed that more than 80% of preschoolers had vitamin D status well above the cut-off suggested by the IOM (50 nmol/L) regardless of ethnicity, immigration status, recent immigration status and skin pigmentation. The healthy vitamin D status may be ascribed to the daycare environment where, in the province of Québec, daycares are heavily funded by the provincial government ensuring an affordable daycare system for all Québec residents. Daycares have regular inspections verifying the compliance of strict governmental guidelines including the amount of time spent outside daily and menus are encouraged to be cross-checked with a registered dietitian provided by the government.

Median 25(OH)D concentration for ethnic minorities in the current study was 74.4 nmol/L which was not different from our White group (Δ25(OH)D = 1.2 nmol/L). Our results showed higher mean plasma 25(OH)D and/or lower differences when compared to White than previously reported among Canadian non-White children of 6 to 11 y (63.3 nmol/L; Δ25(OH)D = 15.2 nmol/L)[197], Inuit children of 3 to 5 y (48.3 nmol/L[186]), toddlers of 2 y in Toronto (60 nmol/L)[188] and American Black (58 nmol/L; Δ25(OH)D = 16.1 nmol/L) and Hispanic children (67 nmol/L; Δ25(OH)D = 7.2 nmol/L) of 1 to 5 y[193]. To date, there were no studies reporting on Asian and Arab children, however based on adult studies, reported plasma 25(OH)D concentration were lower for Asian adults living in Toronto (48.2 nmol/L; Δ25(OH)D = 25 nmol/L)[216] and for Moroccan adults living in Belgium (21.7 nmol/L; Δ25(OH)D = 27.2 nmol/L)[201]. However, many of these studies were limited since skin pigmentation was not measured, immigration status was not assessed, ethnic minorities were grouped together with aboriginals as non-White, sampling was not done across all season, or population were sampled in high risk groups, by convenience or in non-Canadians.

Vitamin D intake and/or UV exposure in our study were the main factors related to vitamin D status except for Black and Asian children. Adjusted daily intake of vitamin D by ethnic minorities (5.9 µg/d) is comparable to our White group (5.8 µg/d) and is similar to those reported for Inuit children (6.3 µg/d)[186] and Canadian children of 1 to 3 y (6.3 µg/d)[282] and 4 to 8 y (5.6 µg/d)[282]. Dietary intake was below the current RDA (15 µg/d) and the EAR (10 µg/d)[10] and was similar to the recent nationally reported values for Canadian children of preschool age[282]. Milk intake (1.7 servings/d) was similar to toddlers in Toronto (1.4 servings/d)[188], Quebec preschoolers (2 servings/d)[254] children in Alberta (2.1 servings/d)[190] and Canadian children of 1 to 8 y (1.5 servings/d)[260]. VTD intake from supplement by dose and frequency were not statistically different between groups except for Arab children where the VTD dose is significantly higher than White children. On average 95% of ethnic minority preschoolers in our study receive direct UV exposure everyday between 1000 h and 1500 h averaging 60 min per d in summer which is less than those reported for children in Alberta (20 h per week)[190] but higher than toddlers in Toronto (41 to 50% spend > 4 hours per week)[188]. However, sunscreen was always used during summer (100%) in ethnic minorities and less frequently during fall and spring (40 to 92%) which is higher than that reported by toddlers in Toronto (53-63%)[188].

This is also the first report where variation in 25(OH)D concentration was explored using ethnic minority groups. Our models have identified that key explanatory variables differ by ethnicity. Our White group was fairly homogenous for vitamin D status, thus the maximum variation accounted for was only 23 %. Sun index in our White model accounted for 9 to 13 % and total VTD intake for 5 % of variance in plasma 25(OH)D concentration. The variance but not the direction of the relationship is similar to those reported for UV exposure and VTD intake in non-Hispanic White adults accounting for 8% each[68]. Arab are predicted to have 47.4 nmol/L and Mixed 42.4 nmol/L higher plasma 25(OH)D concentration than White children when they have a total VTD intake of 10 µg/d which is consistent with the IOM recommendation where an intake of 400 IU/d is

consistent with plasma 25(OH)D concentration of > 50 nmol/L[8]. Black children living in household with income > $75 000/y are predicted to have 28.1 nmol/L higher plasma 25(OH)D concentration than those in household with income < $30 000/y. In Asian, children with lighter skin (type I to II) living in household income < $30 000/y are predicted to have an average of 57.9 nmol/L higher plasma 25(OH)D concentration than children with darker skin (type II to IV). However, at higher income brackets (> 75 000$/y), the relationship between skin type and plasma 25(OH)D reverses where lighter skin Asian children are predicted to have a lower rather than higher plasma 25(OH)D concentration by 12.8 nmol/L. The interactions are observed as well in White, however this accounts for much less variance in plasma 25(OH)D concentration (3% vs. 14%). Children living in households of higher income have lower plasma 25(OH)D concentration in White and Asian ethnicity, however, the relationship is reversed in Black, Arab and Mixed.

The major strength of this study was the comprehensive assessment of vitamin D status of ethnic minority groups including dietary and sun behaviours, SES status and anthropometric measurements across season. Additionally, skin pigmentation was measured objectively and dietary intake by observation by a registered dietitian combined with recall by caregivers. Our regression analysis within ethnic groups combined with testing of interactions was able to explain the majority of the variation in 25(OH)D concentration on the basis of UV exposure and VTD intake. The present study also has several limitations. Being cross-sectional in design, causality cannot be inferred. Additionally, our results can only be generalized to all children attending daycare in Montréal and not those staying at home or residing in other regions in Canada. Our small sample from each ethnic group allowed us to identify only a few key factors. Based on our preliminary analyses, approximately 50 children (CI=95%, power >0.80) per group per season should be sought in future study designs. Although only our Mixed, Black and White ethnic group was sampled proportionally by season, our Hispanic, Asians and Arab were sampled mostly in spring and fall which has an intermediate UV

index relative to winter and summer and their median 25(OH)D concentration were shown to not differ by synthesizing periods. Lastly, 25(OH)D was measured by capillary sampling and may resulted in inflated values by 18 nmol/L compared to venipuncture[124].

In summary, ethnic minority children attending daycare have adequate VTD status particularly in children who take VTD supplements and have high UV exposure. Further studies are needed to investigate in depth the adaptation process of recent immigrants in Canada regarding their VTD status and how daycare environment benefits, facilitates or accelerates immigrants and ethnic minorities' integration. Interactions between variables are important explanatory variables in explaining variance in 25(OH)D concentration and since the explanatory variables for VTD status differed among ethnicities, strategies to improve VTD status in those with low status will need to consider the unique needs of ethnic groups. For example, promotion or screening programs among low income Black ethnicities can be designed regarding VTD intakes while in Asians it would be in those with darker skin type.

TABLES

Table 1: Characteristics of preschool age children and according to ethnicity[1]

	White	Black	Hispanic	Arab	Asian	Mixed
Sample size	262	36	15	45	43	101
Age, mo[a]	46.3 [35.8,54.7]	39.7 [31.9,55.4]	46.3 [33.5,60.6]	41.6 [33.6,56.6]	47.5 [36.6,54.1]	44.2 [34.6,53.6]
HAZ[a]	-0.0 [-0.7,0.5]	0.5 [-0.2,1.1]	-0.7 [-1.0,1.0]	-0.3 [-0.9,0.5]	-0.0 [-0.7,0.5]	0.1 [-0.8,0.7]
WAZ[a]	0.3 [-0.40,0.8]	0.2 [0.0,1.1]	0.4 [0.0,1.2]	0.1 [-0.5,0.8]	0.0 [-0.4,0.5]	0.4[-0.1,0.9]
BAZ [a]	0.4 [-0.1,1.0]	0.2 [-0.4,1.2]	1.0 [0.3,1.8]	0.4 [-0.1,1.1]	0.2 [-0.3,0.7]	0.4 [-0.1,1.2]
Males, %[b]	53.1	44.2	53.4	47.1	51.0	55.6
Food VTD intake[2] (unadjusted), µg/d[a]	5.8 [3.6,7.7]	6.3 [3.1,8.2]	6.9 [3.4,8.1]	5.7 [4.4,8.5]	6.0 [4.1,8.9]	5.4 [3.7,7.6]
At Home, %[b]	31.0	20.6	14.4	31.5	20.0*	37.0
Milk servings[2], per 250 ml[a]	1.7 [0.9,2.3]	2.0 [0.8,2.5]*	2.0 [1.1,2.6]	1.7 [1.0,2.2]	1.8 [1.1,2.3]	1.5 [1.0,2.2]
Supplement[3] Frequency, % yes[b]	26.5	16.6	33.3	20.0	37.2	32.3
Dose, µg/d[a]	5.7 [3.5,10.0]	4.6 [2.5,10.0]	7.1 [5.0,10.0]	10.0 [10.0,10.0]*	5.0[2.5,10.0]	8.6 [3.7,10.0]
Sampled in Summer, %[b]	26	27	7	11*	5*	21
Fall, %[b]	29	27	13	22	7*	27
Winter, %[b]	20	11	40	44*	18	27
Spring, %[b]	25	33	40	22	69*	25
Synthesizing, %[b]	58	80	47	35	79	52
Sun index Summer[a]	11.7 [10.3,12.2]	11.6 [11.1,13.3]	-	11.9 [10.9,12.9]	12.2 [11.3,13.2]	12.8 [11.1,14.0]
Fall[a]	3.5 [2.3,9.6]	9.0 [3.0, 9.6]	5.5 [1.4,9.6]	0.8 [0.6,1.0]	8.4 [7.8,8.9]	3.2 [2.1,8.8]
Spring[a]	4.0 [1.4,9.9]	4.8 [3.1,6.4]	5.5 [1.7,6.6]	2.3 [1.5,9.9]	4.2 [2.1,8.8]	3.0 [1.7,7.2]
Sunscreen use, % yes Summer[b]	100.0	100.0	100.0	100.0	100.0	100.0
Fall[b]	71.0	90.0	50.0	40.0	100.0	67.8
Spring[b]	59.0	91.6*	66.6	50.0	70.0	48.0
Low household income, %[b]	23.2	72.2*	66.6*	51.1*	44.1*	34.6*
Low maternal education, %[6, b]	3.4	28.5*	7.1	6.6	30.9*	6.0
Immigrant children, %[b]	31.6	94.4*	93.3*	95.5*	95.3*	61.3*
Recent immigrant mothers, %[b]	6.2	19.4*	20.0	15.5	20.9*	5

[1]Values are percent, median [IQR]; [2]data from 24h intake analysis; [3] data from food frequency analysis; - not reported due to small sample size (n<2); *P<0.05 significantly different from White; [a]Tested using Wilcoxon Two-sample Test for continuous variables; [b] Tested using Fisher's Exact Test.

Table 2: Multivariate regression models to explain 25(OH)D concentration (nmol/L) in preschool age children according to ethnicity

	Variables	β	SE	P	Δr^2
White	Intercept	4.857039	0.190215	<0.001	-
Log 25(OH)D	Age, y	-0.052591	0.022477	0.020	0.02
R^2= 0.23	Synthesizing, d	-0.000593	0.000317	0.062	0.00
P < 0.001	VTD intake (total), µg/d	0.012039	0.004157	0.004	0.05
n = 203	Skin type[a]	-0.341126	0.137577	0.014	0.00
	Income[b]	-0.103891	0.033048	0.001	0.02
	Sun index (total), min/d*% BSA[1]	-0.014146	0.012624	0.263	0.09
	Synthesizing*Sun index	0.000243	0.000075	0.001	0.04
	Skin type*income	0.084987	0.031817	0.008	0.03
Arab	Intercept	4.292413	0.172465	<0.001	-
Log 25(OH)D	Age, y	-0.137022	0.039412	0.001	0.12
R^2= 0.65	Sun index (daycare)	0.056379	0.015094	<0.001	0.14
P <0.001	VTD intake (supplement), µg/d	0.046918	0.011312	<0.001	0.18
n = 39	Income[b]	0.082897	0.025624	0.002	0.14
Asian	Intercept	198.34	18.58	<0.001	-
25(OH)D	Synthesizing days, d	-0.14	0.03	<0.001	0.16
R^2= 0.60	Sun index (home)	-2.57	0.73	0.001	016
P < 0.001	Skin type[a]	-32.58	6.58	<0.001	0.18
n = 39	Income[b]	-22.50	4.87	<0.001	0.20
	Income*skin type	7.53	2.23	0.002	0.14
Mixed	Intercept	5.278096	0.236975	<0.001	-
Log 25(OH)D	Age, y	-0.097798	0.033762	0.004	0.09
R^2= 0.44	Mixed White, vs Mixed minorities	-0.443304	0.171808	0.011	0.00
P < 0.001	Synthesizing, d	-0.002207	0.000790	0.006	0.03
n = 87	VTD intake (food), µg/d	-0.016236	0.007060	0.024	0.04
	VTD intake (supplement), µg/d	0.036058	0.00832	<0.001	0.16
	Skin type[a]	-0.063450	0.034276	0.068	0.01
	CAN residence, y	-0.017721	0.006190	0.005	0.02
	Income[b]	0.047275	0.021405	0.030	0.10
	Synthesizing*residence	0.000056	0.000026	0.035	0.03
	Mixed white*skin type	0.255941	0.098770	0.011	0.05
Black	Intercept	4.019229	0.197453	<0.001	-
Log 25(OH)D	Age, y	0.022883	0.197453	0.695	0.14
R^2= 0.54	Sex[c]	0.925118	0.057833	0.004	0.00
P < 0.001	Income[b]	0.082565	0.030225	0.009	0.28
n = 33	Sex*age	-0.254873	0.029468	0.004	0.16

P = p-value; Δr^2 = change in r^2 when the variable was removed from the model; β = estimate; SE = standard error;[1]BSA exposed;[a]0 = type I, 1 = type II, 2 = Type III, 3 = Type IV, 4 = Type V;
[b]0 = <15 000$, 1 = 15-30 000$, 2 = 30-45 000$, 3 = 45-60 000$, 4 = 60-75 000$, 5 = ≥ 75 000;
[c]0 = male, 1 = female.

FIGURES

Figure 1: Daycare and participant recruitment

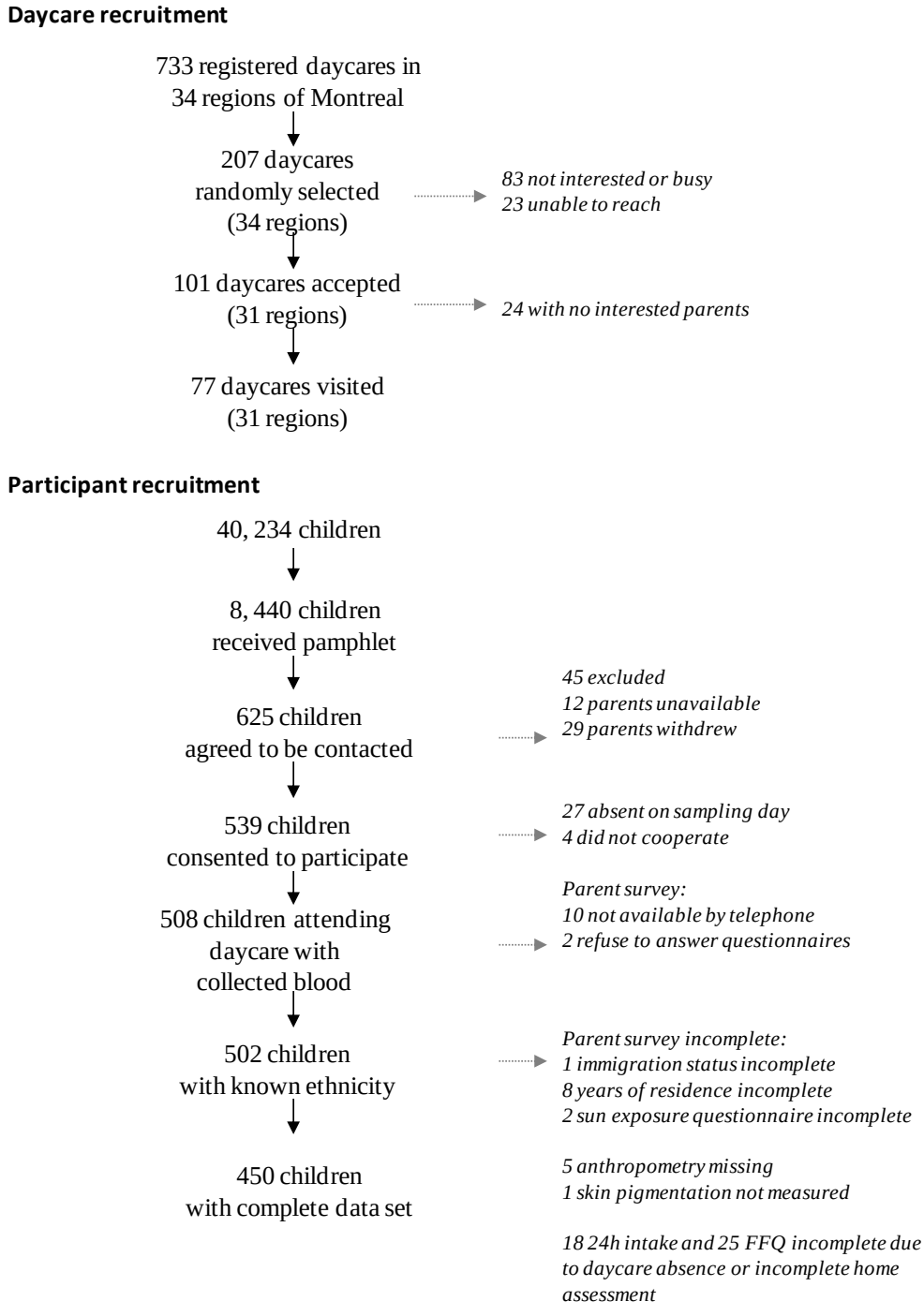
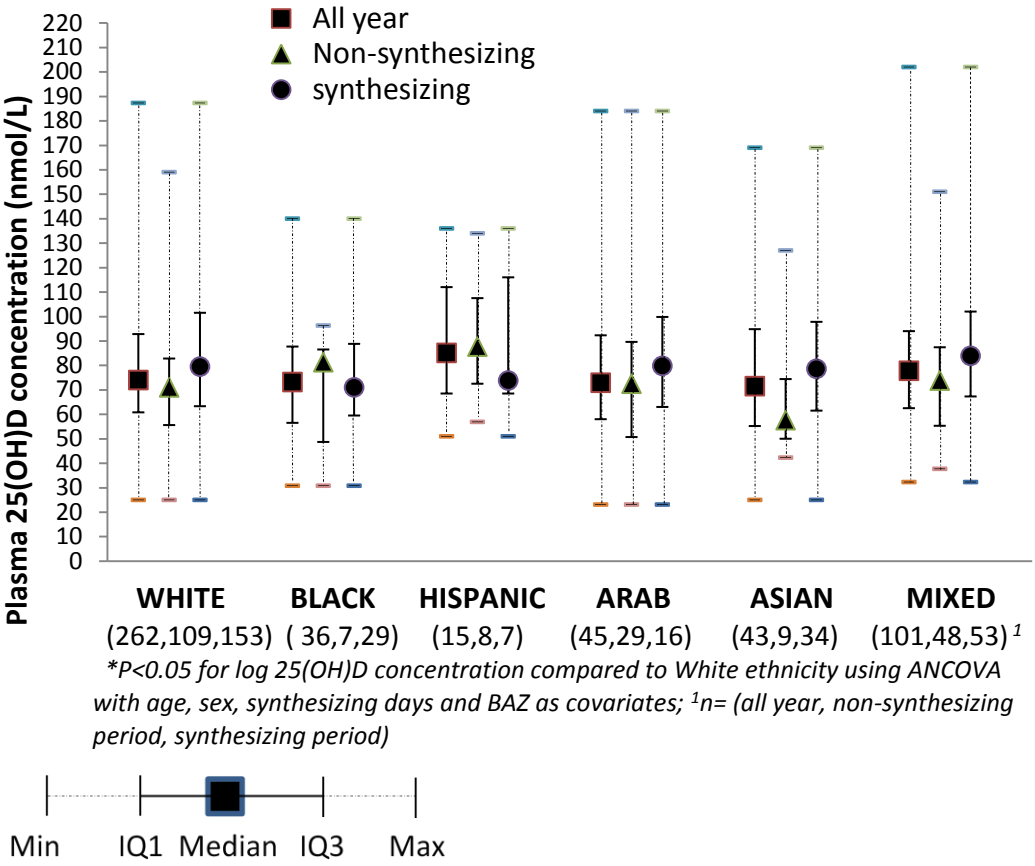


Figure 2: Median 25(OH)D concentration (nmol/L) in preschool age children and according to ethnicity and synthesizing period



**P<0.05 for log 25(OH)D concentration compared to White ethnicity using ANCOVA with age, sex, synthesizing days and BAZ as covariates; [1]n= (all year, non-synthesizing period, synthesizing period)*

3.0 GENERAL DISCUSSION

3.1 FINDINGS

This thesis is designed to study the prevalence of low vitamin D status of ethnic minorities preschoolers and hypothesized a lower mean plasma 25(OH)D concentration in ethnic minority when compared to White preschoolers. The main findings showed more than 80% of preschoolers had vitamin D status well above the cut-off suggested by the IOM (50 nmol/L) regardless of ethnicity, immigration status, recent immigration status, synthesizing period and skin pigmentation. Median plasma 25(OH)D concentration for ethnic minorities in the current study was 74.4 nmol/L which were not different from the White group. Adjusted daily intake of vitamin D and sun index in the ethnic minorities groups were comparable to the White group explaining their comparable vitamin D status. The models for individual ethnic group identified that key explanatory variables differ by ethnicity with sun index for White, total VTD intake for Arab, VTD intake from supplement for Mixed, skin type for Asian and income for Black ethnicity. Interactions between variables (i.e. years of residence, immigrations status and ethnicity) were shown to account for a significant proportion of variance in plasma 25(OH)D concentration and its explanation allowed preliminary understandings into adaptation and integration into to Canadian lifestyle by ethnic minorities. The data from the interactions between income and skin type suggests that the general concept of darker skin pigmentation associated with lower VTD status is true when differences between skin pigmentation reflects differences in ethnic background. However, within an ethnic group, darker skin pigmentation may be a measure of differences in UV exposure where UV exposure may be related to income level.

In regression analysis, there are a total of 24 two-level interactions in univariate analysis for the whole sample. Most interactions are associated with ethnicity (n = 5), quartiles of sun index (n = 7) or season (n = 7) (Appendix: Table 1). There were also 3 level and 4 level interactions; however these were not explored in

depth due to limitation of sample size. When 16 variables were included in the regression model for the whole sample, they were found to predict only 19% of the variation in plasma 25(OH)D ($p < 0.001$). Six two-level were found to be significant ($p < 0.05$) in the model but together with all 16 variables, they were only able to better explain the variation by an extra 11% ($r^2 = 0.30$, $p < 0.001$). The low prediction is also seen when analyses were done by skin type and season with the exception of skin type V to VI (n = 47). However, when models were done for each ethnic group, the prediction increased. In Black, Arab and Asians, all variables and two-level interactions can explain up to 85 to 87% of the variation in plasma 25(OH)D concentration and 53% in Mixed groups while in White this is only 33%. The r^2 coefficient and the number of total significant interactions found for individual ethnic groups are summarized in Appendix: Table 2. This showed that testing for interactions is important when trying to achieve for maximal explanation in plasma 25(OH)D variance. For these reasons, the regression analyses were chosen to separate by ethnicity. Due to the small sample size, the ethnic minority groups were limited in variables in the expense for power > 0.80 therefore other variables were excluded reducing the variance explanation, however still higher than those predicted by season and skin type analyses. Nevertheless, the reduction in variance highlight the importance of the contribution of variables excluded and a necessity for larger sample size to include all significant explanatory variables.

Table 4 in the appendix is the regression model for the whole sample. Overall, 25(OH)D concentration was negatively correlated with non-White ethnicity, increased age, higher BMI and darker skin pigmentation but positively with higher VTD intake, and higher sun index which is in line with previous reports[18, 72, 191, 251]. Black ethnicity explained only a 1% change compared to White ethnicity which is lower than previously reported difference of 24 to 41% lower 25(OH)D concentration in Black compared to White[283]. The different observations may be ascribed to homogenous VTD status and intake in the study. Skin pigmentation was associated with 3% lower plasma 25(OH)D concentration

which is in line with the variance reported in Asian adults (1%)[216]. Higher maternal education and years of residence were positively correlated with 25(OH)D while higher income and immigration status were negatively correlated. The model, predicts children with mothers who achieved less than a high school degree have plasma 25(OH)D concentration of 40.5 nmol/L lower than those with mothers who achieved a master or PhD degree, children living in household with income < $15 000/y have 50.4 nmol/L higher concentrations than those in household of income > $75 000/y. Immigrant children have an average 18.5 nmol/L lower concentration than non-immigrant with an additional 10.9 nmol/L lower value if their mother is a very recent immigrant (<5 y). Together, immigrant children living in low income household with uneducated, very recent immigrant mothers, have 19.5 nmol/L lower plasma 25(OH)D concentration than non-immigrant living in high income household with educated mothers. To date, the relationships between SES and plasma 25(OH)D have not been previously reported but have been suggested indirectly by studies reporting lower VTD intake (OR=0.6)[254] or high prevalence of VTD insufficiency (32%) in low income children[284].

The interactions allowed for preliminary insights into the relationship between SES or immigration status and direct measures (intake and UV exposure) of plasma 25(OH)D. Skin types interaction with income as shown in White (Appendix: Figure 1c) and Asian (Appendix: Figure 1a) models describes that higher plasma 25(OH)D concentration in darker skinned children living in household of higher income compared to lighter skinned children. In all children, longer years of residence is shown to have lower plasma 25(OH)D in spring only (Appendix: Figure 2a) and in Mixed children longer years of residence is shown to have lower values in those of 1st generation immigrant (Appendix: Figure 2c).

The interaction with skin type seen in White may be explained by higher sun index in darker skinned children living in higher income household (Appendix: Figure 1c-2). All other interactions were not explained by differences between

UV exposure or VTD intake (P>0.05) however, trend were seen for higher sunscreen use in proportion to sun index in darker skinned Asian children living in high income household (refer to interaction in Appendix: Figure 1a), in non-recent 2nd generation immigrant children of mixed ethnicity (refer to interaction in Appendix: Figure 2a) and in all children of non-recent immigrant status in spring (refer to interaction in Appendix: Figure 2c) (data not shown for explanation of interactions).

3.2 STRENGTHS AND LIMITATIONS

The major strength of this study was the comprehensive assessment of vitamin D status of ethnic minority groups including dietary and sun behaviours, SES status and anthropometric measurements across seasons. In addition, many of the data points were measured using an objective and more accurate methodology than previously conducted studies. 24h intakes were measured in combination with an assessment of usual intake using a FFQ. The studies conducted on VTD intake of children such as the Quebec preschoolers[254], toddlers living in Toronto[188], Asian adults living in Toronto, Inuit children[186], and the Canadian Community Health Survey (CCHS)[285] were done through indirect measures of intake either using a full 24h recall or a FFQ but not both with the exception for the one done by preschoolers living in Quebec[286]. However, the study with Quebec preschoolers relied on second-hand data regarding VTD intake at daycare by interviewing caregivers whereas we directly observed snacks and lunch. In both dietary collection methods, VTD and calcium content of food were collected and analyzed using brand name specific for yogurt, cheese, VTD supplement, ice cream and beverages (juice and soy drinks). Caregivers were asked to read VTD content on labels of supplements. The analyses were conducted using Nutritionist Pro software, however, VTD content of Canadian brand name specific products were limited in the Canadian Nutrient File (CNF) database. To complement the CNF, % DV were computed on nutrition labels and estimated VTD content using 200 IU as the RNI from 1983 after consultation by telephone with Health Canada and manually entered it in Nutritionist Pro or incorporated it in the FFQ analysis

spreadsheet. The limitation in the dietary collection was the limited assessment of inter-observer reliability[287, 288]. The 3 dietitians were not trained accordingly to the suggested 5 phases that was proven to ensure reliability and accuracy: 1) protocol development, 2) training of field staff, 3) certification of field staff in laboratory setting, 4) implementation in a child-care setting and 5) certification of field staff in a child-care setting[289]. However, the dietitians were all registered with an order where two of them are with the Ordre professionnel des diététistes du Québec[290] and all were trained by the dietetics internships from McGill University. The lack of inter-observer reliability was mainly due to time restraints. A few of the records for food eaten outside of daycares were not done on the same day as the dietary observations (30%) due to unavailability of caregivers. However, paired t-tests were performed to compare the two methodologies and VTD intake between dietary data collected at home on the same day or on separate day as observation was not statistically different from one another ($P>0.05$). Reliability of dietary collection method were assessed by a 2nd 24h intake and FFQ in 15% of the children. FFQ were validated against the 24h intake data, however it remained that the best validation method is against a biomarker[291]. In general, 24h recall dietary data collection method underestimates daily VTD intakes of children and FFQ overestimate usual VTD intake[287]. Since the data collection method was a combination of direct observation and recall for the 24h intake, the underestimation was minimized. Intake reported by caregivers for children was previously reported to have good agreement with observed intake[292].

Another example of an objective assessment is skin pigmentation with the spectrophotometer. The one only other Canadian study on ethnic minority and VTD status using this method was in young adults (18 to 30 y)[192] while most studies used the Fitzpatrick methodology which were reported to be highly subjective[96] especially in Asian skins[82, 97]. Fitzpatrick skin classification was used mainly as a categorical variable while those with the spectrophotometer can quantify skin pigmentation as a continuous or categorical variable. In addition, the objective measure was able to differentiate between constitutive and facultative

skin pigmentation, an important differentiation that allowed measurement of sun exposure (tanning). The use of the spectrophotometer was reported to have good reliability and validity in skin pigmentation measurement while self-reporting had a bias toward overestimation of skin color[99]. Prior to the commencement of the study, the instrument were validated by taking 5 repeated measures of 7 skin sites on 12 subjects and the CV was 3% with no differences between and within groups ($P > 0.05$). There were 4 researchers performing skin pigmentation measurements however only 2 were assessed for reliability and accuracy of measurement between researchers.

Plasma 25(OH)D concentration was measured using capillary blood for ease of blood sampling in young children however it is reported to inflate values by 18 nmol/L in adults[124]. Measurement of plasma 25(OH)D is highly variable among methods and therefore interpretation and comparison among studies should proceed with caution[293, 294]. The plasma 25(OH)D concentration was measured using a chemiluminescence assay (Liason, Diasorin, Mississauga, ON, Canada); the same equipment as the Canadian national survey (CHMS). The choice of similar laboratory assessment tools allowed the comparison and complement the results published by the CHMS.

Sun exposure questionnaires were subjective and therefore may have low reliability and reproducibility[295]. The questionnaires captured by a single question on time spent outdoor on average per day but did not capture separately exposure weekdays and weekend at home. Since during the weekdays at home, children did not spend time in direct sunlight between 1000 and 1500 h due to daycare attendance, averaging the time reduces accuracy regarding frequency. Sunscreen questionnaires did not capture the details regarding the proper use of sunscreen such as frequency of re-application and time delay between application before UV exposure. The proper use of sunscreen affects circulating plasma 25(OH)D concentration more so than the frequency and the use of sunscreen on daily basis[237]. When caregivers were asked what body parts were exposed to direct

sunlight, they answered using the types of clothes worn by children therefore required some interpretation from the interviewers to fill out the questionnaires. Although, objective measures such as portable UVR measuring device are available, the use of these devices may have low compliance and complex instructions to follow therefore may not be conducive for this age group in all settings. Hence, sun exposure questionnaires may still be the most effective of methods known to date for measurement of UV exposure.

The demographic questionnaires not only capture ethnicity but as well immigration status and years of residence in Canada. Years of residence in Canada provided a continuous variable which allowed for a preliminary assessment into adaptation and integration. Ethnicity is subjective because it relies on self-reported data. However, skin pigmentation were cross checked with reported ethnicities and the data corresponds to previously reported associations with ethnic minorities living in their homeland[39, 60, 81]. Ethnicity were categorized based on both caregivers and had additional groups for Mixed ethnicities. This allowed groups of ethnic minorities without children of mixed ethnicities. Inter-cultural marriages between parents of Mixed children suggest weaker ethnic ties than those from ethnic minorities and also 86% of mixed children included White ethnicities. Hence, their dietary and sun behaviours may differ from White and ethnic minorities groups and by categorizing them separately, it allowed the understanding and the chance to explore and compare a child with ethnic minority origins only or with part White ethnic origins. A large group of Mixed ethnicity (n = 101) was captured in the sampling. The Mixed as well as ethnic minority children are growing in numbers and, to date, have not been reported regarding their dietary and sun behaviours. Prevalence of plasma 25(OH)D < 50 nmol/L among ethnic minorities were assessed according to various other explanatory variables associated with low vitamin D status in ethnic minorities including immigration status, recent immigration status, skin pigmentation, SES and between synthesizing and non-synthesizing period.

The two main limitations for the results regarding prevalence included sample size and disproportionate seasonal sampling. Using the correlation coefficient between vitamin D intake per kg body weight to plasma 25(OH)D concentration ($r = 0.452$) from the study by Roth et al.[190], a calculated sample size of 48 per group per season is needed (CI = 95%; $\beta = 0.010$) based on the method by Hulley et al.[296]. The individual ethnic minority group had a sample size of <50 and when combined, the ethnic minority sample size was 139 which both were below the target sample size of 200.

Analysis by ethnic subgroups was also explored but due to small sample size they were not reported. Appendix: Table 1 defines ethnic subgroups. East Asians and Mixed (with White) ($P < 0.05$) did not have significantly different skin pigmentation to White while South and Southeast Asians did ($P < 0.05$). A trend of higher mean plasma 25(OH)D was seen in Southeast Asian (93.3 nmol/L) and lower in South Asians (63.9 nmol/L) than East Asians (74.4 nmol/L). Even within White subgroups, a trend of lower mean plasma 25(OH)D was seen in Mixed (white only) (74.9 nmol/L) compared to northern White (80.6 nmol/L). Within Mixed subgroups, although the mean plasma 25(OH)D is similar, prevalence of plasma 25(OH)D < 50 nmol/L was higher in Mixed (with White) (2%) compared to Mixed (non-White) (12%). These differences within subgroups were reflected by differences in SES, VTD intake and sun behaviours. Future analysis should include ethnic subgroups when categorizing ethnicity when sample size permits.

3.3 CONCLUSION

Ethnic minority children attending daycare have healthy VTD status particularly those who take a VTD supplement and/or have frequent UV exposure. VTD status of ethnic minorities did not differ from that of White children. For all children, age, indices of adiposity, endogenous synthesis, exogenous intakes of VTD and education were explanatory variables. Ethnicity was a significant explanatory variable in those who take a VTD supplement versus those who do not. Within

individual ethnic groups, the variance in vitamin D status was explained by differences in economic indices in Black children, endogenous synthesis in White, exogenous intakes of VTD intake in Mixed and Arab and skin type in Asians. Dietary intake was below the current RDA (600 µg/d) and the EAR (400 µg/d)[10] and was similar to the recent nationally reported values for Canadian children of preschool age[282]. Whether similar observations regarding predictors of vitamin D status in preschool age of White and ethnic minority groups in other regions parallels that observed within the current study remains to be established.

3.4 FUTURE DIRECTIONS

It is hypothesized that the explanation of the comparable vitamin D status and dietary and sun behaviours in ethnic minorities to White children are ascribed to the daycare environment which may play a role in facilitating the integration and adaptation of immigrant and ethnic minorities' families. Future studies should thus compare the vitamin D status of ethnic minority preschoolers who do and do not attend daycare and measure the degree of daycare environment impacts on vitamin D status. A minimum sample size of 50 should be sought per ethnic group per season to obtain a 95% confidence interval and a power of >0.80. If daycare environment does facilitate integration and adaptation to Canadian lifestyles, governmental programs can be designed to promote/encourage ethnic minority caregivers to place their children in such daycare settings. Governmental programs can also create easier and more affordable access to daycares for ethnic minorities, especially for those from low SES. Since the explanatory variables for VTD status differed among ethnicities, strategies to improve VTD status in those with low status will need to consider the unique needs of each ethnic group. For example, promotion or screening programs among low income Black ethnicities can be done regarding VTD while in Asians these promotions can be done in those with darker skin type.

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5.0 APPRENDIX

TABLES

Table 1: Classification of ethnic groups and subgroups by country of origins*

Groups (n)	Subgroups (n)	Specific ethnic group
White (262)	Northern (226)	Canadian, British, French, German, Hungarian, Ukrainian, Irish, Scottish, Ashkenazy, Austrian, Belgian, Berbers, Bulgarian, Czech, Dutch, Danish, Polish, Latvian, Russian, Romanian, Moldavian, Swiss, Yugoslavian
	Southern (15)	Italian, American, Greek, Spain
	Mixed (White only) (21)	Mixed between Northern and Southern White
Mixed (101)	Mixed (non-White) (13)	Child of multiple ethnicities excluding White ethnicity
	Mixed (with White) (88)	Child of multiple ethnicities including at least one White ethnicity
Asian (43)	East (15)	Japanese, Chinese, Korean, Taiwanese
	South East (12)	Cambodian, Filipinos, Indonesian, Vietnamese
	South (16)	Bangladeshi, Indian, Sri Lankan
Hispanic (15)	-	Chilean, Colombian, Cuban, Honduran, Dominican, Mexican, Peruvian, Portuguese, Salvadorian, Venezuelan
Arab (45)	-	Moroccan, Algerian, Tunisian, Afghanistan, Armenian, Egyptian, Iranian, Israeli, Turkish, Lebanese, Saudi Arabian, Serbian
Black (36)	-	Barbados, African, Caribbean, Costa Rican, Ethiopian, Guinean, Haitian, Jamaican, Trinidad

** all ethnicities listed are those reported by the participants in the sample; 12 participants have unreported ethnicity*

Table 2: Interactions associated with ethnicity, VTD intake and season

	Season	**Ethnicity**	**Sun index**	**Immigration Status**	**Skin type**	**VTD**
	p=	**p=**	**p=**	**p=**	**p=**	**p=**
Sample size						
Age	0.283	0.671	0.833	0.953	0.228	0.066
Sex	0.861	0.173	0.580	0.377	0.804	0.172
BAZ	0.413	0.937	0.024	0.535	0.883	0.822
ITA^{o}	0.016	0.315	0.024	0.899	0.278	0.105
Maternal education	0.021	0.543	0.580	0.709	0.963	0.089
Immigration status	<0.001	0.490	<0.001	-	0.143	0.960
Years of residence	<0.001	0.354	0.016	0.702	0.670	0.014
Income	<0.001	0.005	0.029	0.765	0.033	0.574
Synthesizing days	<0.001	0.891	0.014	0.573	0.780	0.026
Sun index daycare	0.177	0.001	0.308	<0.001	0.000	0.246
Sun index home	0.030	0.015	0.008	0.005	0.099	0.232
VTD home	0.177	0.002	0.286	0.237	0.922	0.830
VTD daycare	0.372	0.788	0.791	0.191	0.676	0.135
VTD supplement	0.250	0.012	0.204	0.164	0.851	0.182

Table 3: Model development

		All	White	Black	Arab	Asian	Mixed
Number of variables	Number of variables =	16	16	15	15	15	16
	r^2=	0.19	0.33	0.77	0.74	0.67	0.41
	P=	<0.001	<0.001	0.008	<0.001	0.008	0.001
	number of variables or interactions with power =	5	2	0	0	0	0
Model including all variables and two-level interactions	Number of variables =	16	16	15	15	15	16
	Number of interactions =	24	5	3	3	4	3
	r^2=	0.34	0.32	0.87	0.84	0.84	0.53
	P=	<0.001	<0.001	0.005	<0.001	0.003	<0.001
	number of variables or interactions with power =	5	2	0	1	0	1
Selected model	Number of variables =	12	7	3	4	4	7
	Number of interactions =	4	4	1	0	1	3
	r^2=	0.26	0.25	0.49	0.64	0.60	0.36
	P=	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	number of variables or interactions with power =	9	2	3	4	3	3

r^2= correlation coefficient; p = p-value; power>0.80

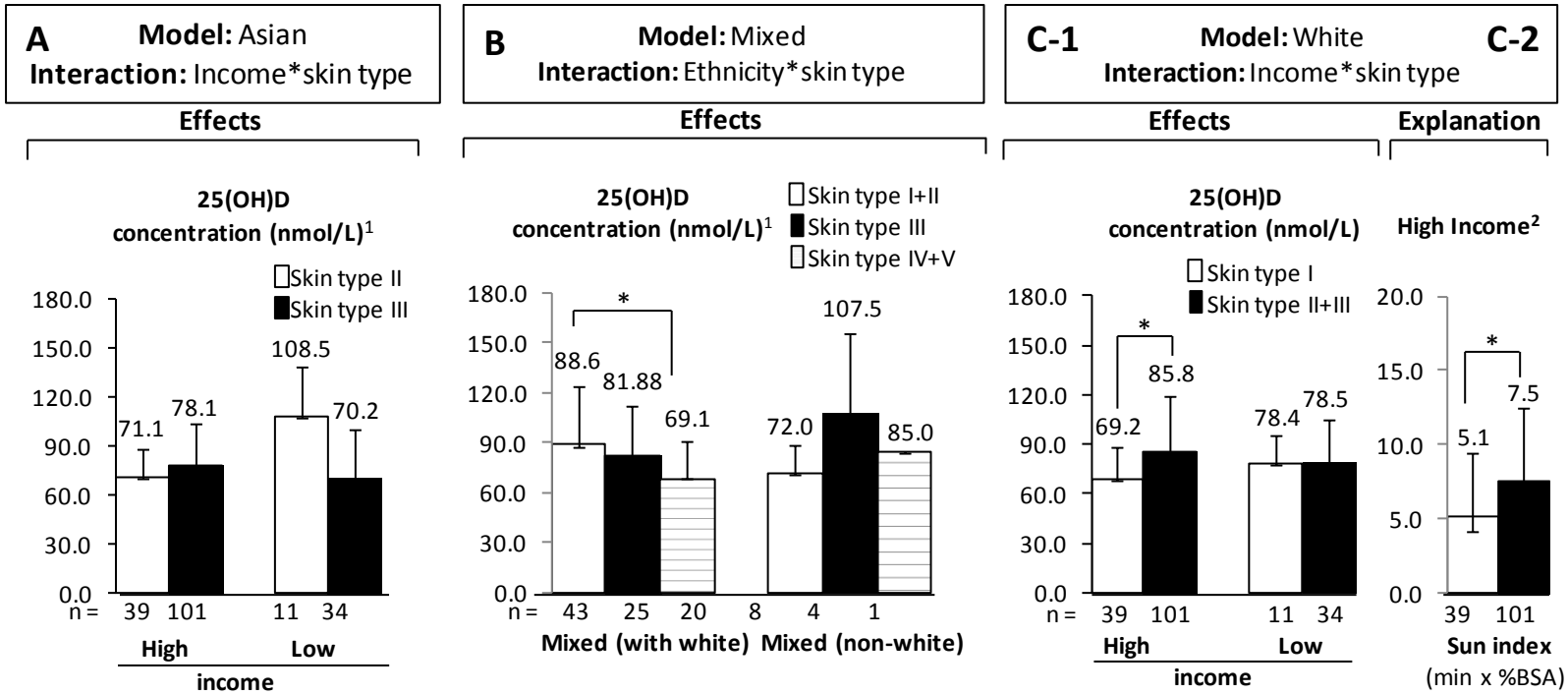
Table 4: Multivariate regression model to explain log 25(OH)D concentration (nmol/L) in preschool age children in the whole cohort

	Variables	β	SE	P	Δr^2
All	Intercept	5.295810	0.162171	<0.001	-
$R^2 = 0.30$	White	-0.227324	0.100030	0.023	0.01
P < 0.001	Black	-0.174948	0.125912	0.165	0.01
n = 400	Asian	-0.960788	0.240367	<0.001	0.02
	Mixed	-0.221926	0.101628	0.029	0.01
	Arab	-0.577254	0.141297	<0.001	0.02
	Age, y	-0.078905	0.016557	<0.001	0.04
	BAZ, z-score	-0.061103	0.016819	<0.001	0.02
	Synthesizing, d	-0.001560	0.003118	<0.001	0.01
	Sun index (total), min/d * % BSA[1]	-0.008382	0.009392	0.372	0.02
	VTD intake (food at home), µg/d	0.014286	0.005321	0.007	0.03
	VTD intake (supplement), µg/d	0.012533	0.004180	0.002	0.02
	Maternal education[a]	0.056666	0.022428	0.011	0.01
	CAN residence, y	-0.011218	0.002677	<0.001	0.01
	Skin type[b]	-0.128977	0.035612	<0.001	0.00
	Income[c]	-0.052506	0.018340	0.004	0.01
	Immigration status[d]	-0.059245	0.023668	0.012	0.01
	Asian*immstat	0.308108	0.103697	0.003	0.02
	Asian*CAN residence	0.010714	0.005539	0.053	0.01
	Synthesizing*CAN residence	0.000032	0.000108	0.003	0.02
	Synthesizing*sun index	0.000157	0.000056	0.005	0.01
	Skin type*income	0.033352	0.008463	<0.001	0.03
	Arab*income	0.116107	0.003300	<0.001	0.02

P = p-value; Δr^2= change in r^2 when the variable was removed from the model; β = estimate; SE = standard error; [1]BSA exposed;[a]0 = elementary or High School, 1 = vocational/apprentice/CEGEP, 2 = university, 3 = master or PHD; [b]0 = type I, 1 = type II, 2 = Type III, 3 = Type IV, 4 = Type V;
[c]0 = <15 000$, 1 = 15-30 000$, 2 = 30-45 000$, 3 = 45-60 000$, 4 = 60-75 000$, 5 = ≥ 75 000$;
[d]0 = 3rd generation, 1 = 1st generation, 2 = 2nd generation.

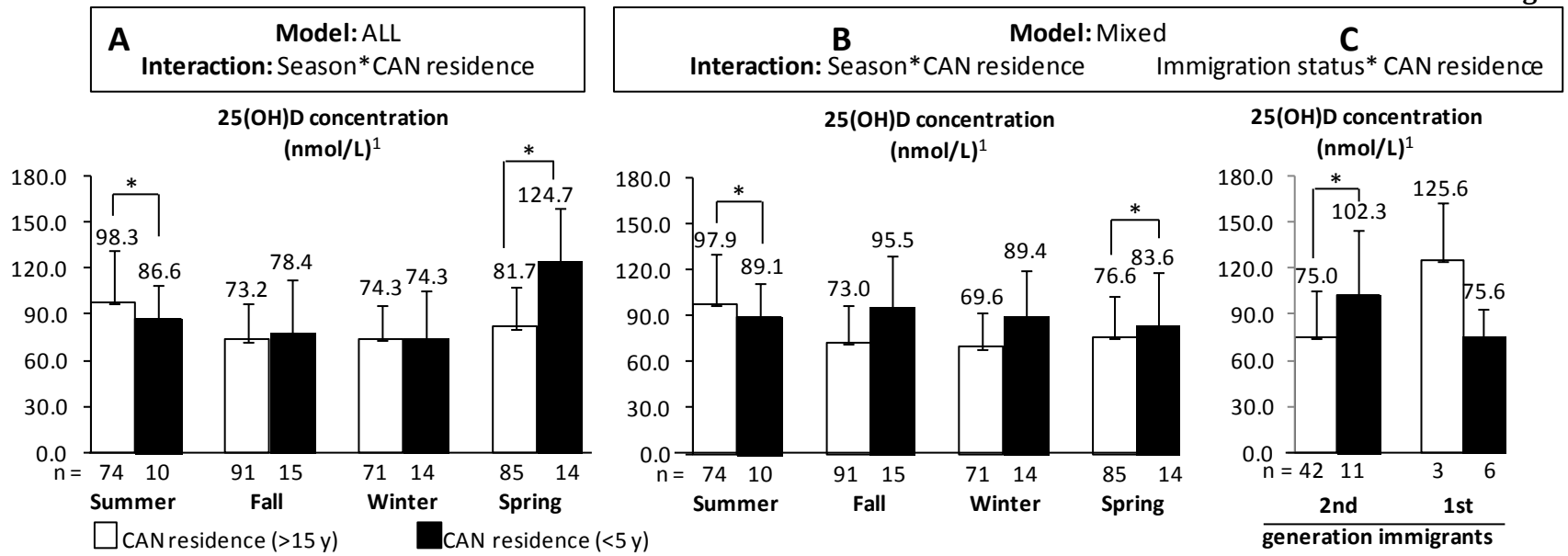
FIGURES

Figure 1: Effect of interactions with skin type on 25(OH)D concentration by selected ethnic group



*[1]values are in means±SD and the values above bars are means; *P<0.05 tested using Wilcoxon Two-sample Test;[2]P>0.05 for vacation, BSA>24% or VTD intake (data not shown).*

Figure 2: Effect of interactions with years of residence on 25(OH)D concentration and explanation for interactions by selected ethnic group



*1values are in means±SD and the values above bars are means;*P<0.05 tested using Wilcoxon Two-sample Test.*

Figure 3: Food Frequency Questionnaires

Nutrition Questionnaire: Vitamin D Intake

GENERAL INSTRUCTIONS: Do your best to answer each question, but if you are uncomfortable with any question, just ask us to leave it blank.

State how often (if ever) your child ate the following vitamin D-containing foods during the last month, and then indicate the number and average portion size.

Food Item	Frequency and # of servings: Never	Monthly	Weekly	Daily	Check Serving Size: (mark one only)
EXAMPLE: Milk for drinking (incl. Choc milk/hot cocoa with milk)			**10**		☒ 125 ml (.5 cup) ☐ 250 ml (1 cup) ☐ 375 ml (1.5 cup)
Milk for drinking (incl. Choc milk/hot cocoa with milk)					☐ 125 ml (.5 cup) ☐ 250 ml (1 cup) ☐ 375 ml (1.5 cup)
Milk on cereal or in soups					☐ 60 ml (.25 cup) ☐ 125 ml (.5 cup) ☐ 250 ml (1 cup)
Baby food cereal a) made with milk Quantity milk_____ b) made with water					☐ 60 ml (.25 cup) ☐ 125 ml (.5 cup) ☐ 250 ml (1 cup) ☐ 60 ml (.25 cup) ☐ 125 ml (.5 cup) ☐ 250 ml (1 cup)
Soy or rice beverage or orange juice with added calcium and vitamin D					☐ 125 ml (.5 cup) ☐ 250 ml (1 cup) ☐ 375 ml (1.5 cup) Soy or rice beverage ☐ orange juice ☐
Eggs and egg dishes (including yolk) (ex. Fried, hard boiled, omelettes, quiche).					☐ 1 (large) ☐ 1 (medium) ☐ 1 (small)
Fish: Including salmon (canned & fresh), mackerel, herring, oysters or tuna (fresh) Specify Types:					☐ 75 g (2 ½ oz) ☐ 150 g (5 oz) ☐ 225 g (7 ½ oz)
Fish: Including fish sticks, shrimp, sole/flounder or tuna (canned) Specify Types:					

Margarine Brand:_____					☐ 5 ml (1 tsp) ☐ 15 ml (1 tbsp) ☐ 45 ml (3 tbsp)
Yogurt Specify brands:					☐ 60 ml (.25 cup) ☐ 125 ml (.5 cup) ☐ 250 ml (1 cup) ☐ 30 g (1 oz) ☐ 60 g (2 oz) ☐ 90g (3 oz)
Cheeses (Including cheddar, mozzarella, Kraft Singles®, Cheez Wizz®, parmesan, gouda, edam, brie, havarti, feta, blue, and chèver)* Specify brands:					☐ 60 ml (.25 cup) ☐ 125 ml (.5 cup) ☐ 250 ml (1 cup) ☐ 30 g (1 oz) ☐ 60 g (2 oz) ☐ 90g (3 oz)
Ice cream and frozen desserts (including sundae, ice cream cone, Fudgesicle®) Specify brands:					☐ 60 ml (.25 cup) ☐ 125 ml (.5 cup) ☐ 250 ml (1 cup)
For breastfeeding and formula fed children enter daily total # of 1 oz feeds and frequency below. For weaned children enter "0".					
Breast milk					☐ 30 ml (1 oz) OR ☐ # feed/day
Infant formula Specify brand (if not mixed according to manufactures instructions please describe):					☐ 30 ml (1 oz) OR ☐ # feed/day
Additional sources of vitamin D					
Cod or Halibut Liver Oil					☐ 15 ml (1 tbsp) ☐ 30 ml (2 tbsp) ☐ 45 ml (3 tbsp)
Vitamin D or multivitamin supplement					☐ 200 IU ☐ 400 IU ☐ 800 IU Specify brands:
*excluding cream cheese and cottage cheese.					