The Relationship between Insulin Resistance and Vitamin D Deficiency: A Cross-sectional Study among Employees at a Private University in Lebanon

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Lea Majdalani

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Faculty of Nursing and Health Sciences

Department of Nursing and Health Sciences

We hereby approve the thesis of

Lea Majdalani

Candidate for the degree of MS in Human Nutrition

Dr. Jessy El Hayek	Supervisor, Chair
Dr. Antoine Aoun	Committee Member
Ms Samar Merhi	Committee Member

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Abstract

Insulin resistance plays a fundamental role in the pathophysiology of many chronic diseases including Type II diabetes, cardiovascular and metabolic abnormalities. There is an increasing interest in assessing the association between insulin resistance and vitamin D status. Vitamin D deficiency predominates in the Middle East where more than half of the population is deficient. This cross-sectional study aims to explore the association between vitamin D status and insulin resistance among university employees. In September 2016, an email was sent to all the employees of Notre Dame University (NDU) to invite them to participate in our cross-sectional study. The participants completed a 20-minute face-to-face interview questionnaire. They were then requested to pass by the Nutrition Laboratory-NDU to acquire anthropometric, clinical and biochemical measurements. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was performed to assess insulin resistance (IR); a food frequency questionnaire was conducted to assess vitamin D intake; enzyme-linked immunosorbent assay (ELISA) was used to measure serum 25 hydroxyvitamin D (25(OH)D) and a 24h recall multiple pass method recall was filled to estimate energy and nutrient intakes. Statistical analyses were performed using SPSS version 23. The study population consisted of 318 adult participants (48.1% male, 51.9% female) with an average age of 41.5 ± 11.1 years. Among the participants, 33.3% and 61.9% had serum 25(OH) D < 20ng/mL and < 30ng/mL, respectively. A positive proportional association between HOMA-IR and BMI categories in both genders ($p<0.001$) was observed. Mean glucose level was also significantly higher among participants with IR for both genders ($p<0.001$). Body fat percentage was higher and risky waist circumference was more

prevalent among participants with IR for both genders ($p<0.001$). A significant inverse association was observed with serum 25(OH)D < 20ng/mL ($p=0.028$) and HOMA-IR; however, this association was not significant when cutoff of 30ng/mL was used. Stratification based on gender showed a significant inverse association among females ($p=0.031$) but not males at 25(OH)D < 20ng/mL. After adjusting for confounding factors using multiple logistic regression, the association between vitamin D status and HOMA-IR was no longer significant for both genders. However, there was a significant independent association between HOMA-IR and waist circumference among males ($p=0.008$); and between HOMA-IR and marital status ($p=0.006$), hypertriglyceridemia ($p=0.010$) and body fat percentage ($p=0.045$) among females. This study contributes towards the limited literature on vitamin D and IR in a Middle-Eastern sample. Further research is needed to better understand the relationship between vitamin D and IR to direct future public health or clinical applications.

I. Literature Review

1. Insulin Resistance

Insulin resistance (IR) is a state where there is an increase in insulin secretion in the pancreas due to the fact that target cells like the muscles and adipose tissue no longer respond to normal amounts of insulin secretions (Salaroli et al., 2017). Therefore, insulin is unable to adequately take up glucose from the blood into the cells (Tam et al., 2012). An emerging body of evidence has suggested that IR can play a fundamental role in the pathophysiology of many chronic conditions including Type II diabetes mellitus (T2DM), cardiovascular and metabolic abnormalities including the metabolic syndrome, polycystic ovarian syndrome (PCOS), nonalcoholic fatty liver disease, inflammation, cancer and Alzheimer's disease (Freeman & Pennings, 2018; Frozza, Lourenco, & Felice, 2018). Many of these conditions represent leading causes of death in adults in developed and developing countries, with alarming projections (Baena-Díez et al., 2016). Accordingly, an increasing number of studies are focusing on the understanding of IR.

2. Assessment of IR

Two approaches exist to measure IR: the measurement could be taken by examining fasting blood levels of glucose, insulin and triglycerides; or by measuring glucose and insulin levels 2 hours after ingesting 75g of glucose in a test called oral glucose tolerance test (OGTT). The choice of the method to assess IR also depends whether it is a research or a clinical setting (Gutch, Kumar, Razi, Gupta, & Gupta, 2015).

a- Assessment Methods Used in Research Settings

Hyperinsulinemic euglycemic clamp (HEC) is the gold standard for measuring insulin sensitivity. In HEC, insulin is infused intravenously in a peripheral vein to reach a certain target range. Then, glucose is infused and the glucose level is measured every 3 to 5 minutes until a constant level of glucose is reached. The amount of glucose infused needed to maintain glucose homeostasis reflects the person's insulin sensitivity (Wile & Wilding, 2014). Table 1 lists the methods used in research settings for the measurement of insulin sensitivity, as well as their advantages, disadvantages and correlation with HEC.

Table 1: Methods to assess insulin sensitivity in research settings [Retrieved from (Gutch, Kumar, Razi, Gupta, & Gupta, 2015)]

Method	Normal level	Advantage	Disadvantage	Correlation coefficients with HEC
Hyperinsulinemic euglycemic glucose clamp	Clamp performed at 80 mU/m^2 min, a cutoff of 5.3 mg/kg FFM+17.7 z min (98% prediction probability) for IR	Direct measure of insulin under steady-state conditions	Laborious, involves intra venous infusion of insulin, frequent blood sampling	Gold standard method for quantifying insulin sensitivity
McAuley index	<5.8	The combination of fasting insulin (mIU/l) and triglycerides (TAG, mmol/l) showed the best pre-diction of IR	Robust method, suitable for epidemiological studies	≤0.63 in diabetic patients
Belfiore index	Values above 1.27 indicate pathological IR	Showed normal value for basal glucose and insulin concentrations and for mean normal value for glucose and insulin areas during OGTT	Multiple blood sampling	0.65; P<0.01 in subjects with normal glucose tolerance, 0.54; P<0.01 in subjects with impaired glucose tolerance, and 0.48; P<0.01 in subjects with diabetes type 2
Avignon index	-	Determines glucose tolerance and insulin sensitivity in single test	Its correlation is very weak in diabetic patients	Normal glucose tolerance (0.89; P≤0.0001), with impaired glucose tolerance

				(0.96; P≤0.0001), and in patients with diabetes mellitus type 2 (0.69-0.83; P≤0.05)
Stumvoll index	-	Utilizes demographic data like age, sex and BMI along with plasma glucose and insulin to predict insulin sensitivity	Very robust and weakly correlate in diabetic patients	Correlation coefficients with HEC were in the range between 0.62 and 0.79 (P<0.001)
Gutt index	<45 predict IR	Good to predict onset of type 2 diabetes	Suitable for epidemiological studies	Correlation coefficients with HEC 0.63; P<0.001

BW: Body weight, HEC: Hyperinsulinemic euglycemic clamp, BMI: Basal metabolic rate, OGTT: Oral glucose tolerance test, TAG: Triglycerides, IR: Insulin resistance, FFM: Fat-free mass, ISI: Insulin sensitivity index, MCR: Metabolic clearance rate. HEC – I mean: Average steady state plasma insulin response (µIU/ml), Mmean: Metabolized glucose expressed as average steady state glucose infusion rate per kg of BW (mg/kg/min), Gmean: Average steady state blood glucose concentration (mmol/l), 0.18: Conversion factor to transform blood glucose concentration from mmol/l into mg/ml. HOMA-IR – I 0 : Fasting insulin (mIU/l), G0 : Fasting glucose (mmol/l) concentration. QUICKI – I 0 : Fasting insulin (mIU/l), G0 : Fasting glucose (mmol/l) concentration. McAuley index – I 0 : Fasting insulin (mIU/l), TAG: Fasting triglyceride concentration. Matsuda index – I 0 : Fasting plasma insulin concentration (mIU/l), G0 : Fasting plasma glucose concentration (mg/dl), Gmean: Mean plasma glucose concentration during OGTT (mg/dl), Imean: Mean plasma insulin concentration during OGTT (mU/l), 10,000: Simplifying constant to get numbers from 0 to 12. Belfiore index – GS, GN: Plasma glucose concentrations expressed as fasting values or as areas obtained during a standard OGTT at 0 and 2 h (0–2h areas are equal to GS,N=G0 +G120) or at 0, 1 and 2 h (0-2h areas are equal to GS,N). Avignon index: I and G represent the plasma concentrations of insulin (mIU/l) and glucose (mmol/l) respectively, VD is the glucose distribution volume calculated using a mono compartmental model: VD=150 ml/kg of BW. Stumvoll index: Fasting insulin (mIU/l), G0 : Fasting glucose (mmol/l) concentration. Gutt index – I 0 : Fasting plasma insulin concentration (mIU/l), G0 : Fasting plasma glucose concentration (mg/dl), Gmean: Mean plasma glucose concentration during OGTT (mg/dl), Imean: Mean plasma insulin concentration during OGTT (mU/l)

b- Assessment Methods Used in Clinical Settings

Multiple methods exist for the assessment of insulin sensitivity in clinical settings. Table 2 lists these methods, as well as their advantages, disadvantages, formula and correlation with HEC, the gold standard for measuring insulin sensitivity.

Table 2: Methods to assess insulin sensitivity in clinical settings [Retrieved from (Gutch, Kumar, Razi, Gupta, & Gupta, 2015)]

Method	Normal level	Advantage	Disadvantage	Correlation coefficients with HEC
HOMA-IR	<2.5	Simple, minimally invasive, predicts fasting steady-state G and I levels	Insulin sensitivity in subjects treated with insulin needs further validation	Normal glucose tolerance (0.65; P<0.0001), impaired glucose tolerance (0.56; p<0.0001) and with type 2 diabetes (0.51; p<0.0001)
QUICKI	0.382±0.007 for nonobese, 0.331±0.010 for obese and 0.304±0.007 for diabetic individuals	Consistent, precise index of insulin sensitivity, minimally invasive	Normal range to be established for each laboratory due to significant inter laboratory variations in insulin assay	Correlation coefficient 0.78; $P<2 \times 10^{-12}$
Matsuda index	<4.3 predict IR	Represents both hepatic and peripheral tissue sensitivity to insulin	Its correlation is very weak in diabetic patients	0.73 (P<0.0001) in subjects with normal glucose tolerance, 0.66 (P<0.0001) in subjects with impaired glucose tolerance, and 0.60 (P<0.0005) in nondiabetic subjects, and in subjects with type 2 diabetes mellitus the correlation proved to be weaker 0.54 (P<0.0001)

HEC: Hyperinsulinemic euglycemic clamp, HOMA-IR: Homeostasis model assessment-insulin resistance, QUICKI: Quantitative insulin sensitivity check index

c- Cutoff values of HOMA-IR

According to the World Health Organization, IR is defined as “a value greater than the 75th percentile value for non-diabetic subjects” (Alberti & Zimmet, 1998). However the cutoff values adopted in the literature vary between races, age groups and medical conditions (Yamada, Moriyama & Takahashi, 2012). In the literature, instead of relying on the percentile distribution of the population, criteria of the metabolic syndrome were taken into consideration. (Gayoso-Diz et al., 2013). Table 3 shows the different cutoff values of HOMA-IR used in the literature.

Table 3: Main HOMA-IR cut-off values in the literature [sample size ≥ 1000; adapted from (Tang, Li, Song & Xu, 2015)]

Country	Sample size	Population characteristics	Threshold value	Criteria	References
Sweden	*n* = 4,816	Age: 74-93 years; Healthy population	2.0	75th percentile	(Hedblad, Nilsson, Janzon & Berglund, 2000)
France	*n* = 1,153	Age: 35-64 years; Healthy population	3.8	75th percentile	(Marques-Vidal et al., 2002)
Caucasus	*n* = 1,156	Age: 18-78 years; Rural population; non-diabetic	2.29	75th percentile	(Radikova et al., 2006)
Brazil	*n* = 1,317	Age: 40 ± 12 years; BMI: 34 ± 10 kg/m^2	2.77	90th percentile	(Geloneze et al., 2009)
U.S.	*n* = 2,804	Age ≥ 20 years; normal BMI and fasting glucose	2.73	66th percentile	(Sumner & Cowie, 2008)
Iran	*n* = 3,071	Age: 25-64 years Adult individuals;	3.875	ROC curve	(Esteghamati et al., 2010)
Iran	*n* = 1,036	Age 18-45 years Women of reproductive	2.63	95th percentile	(Zadeh-Vakili, Tehrani & Hosseinpanah, 2011)
Japan	*n* = 6,868	Age: 49.7 ± 12.1 years; non-diabetic subjects	1.7	ROC	(Yamada, Moriyama & Takahashi, 2012)
China	*n* = 3,203	Age: 6-18 years (children and adolescents)	3.0	95th percentile	(Yin et al., 2013)
Portugal	*n* = 1,784	Age ≥ 18 years ; non-diabetic individuals in a Cardiology ward; BMI < 25 Kg/m^2; Fasting Plasma Glucose < 100	2.33	90th percentile	(Timóteo, Miranda, Carmo & Ferreira, 2014)

		mg/dL			

3. Prevalence of IR

The prevalence of IR varies across populations. For instance, the lowest prevalence of IR (15.5%) was reported among Danish adults (n=3354, age: 19-72 years), using a HOMA-IR cutoff ≥ 2.5 (Friedrich et al., 2012). A higher prevalence rate of IR (39.1%) was observed in Cameron County, Texas, USA among 1854 Hispanic adults (>18 years old) using a higher cutoff of HOMA-IR of 3.8 to define IR (Qu et al., 2011). Using a lower cutoff of HOMA-IR ≥2 to indicate IR, the highest prevalence rate of 46.5% was reported among Venezuelan adults (n=2026, mean age: 39.7 ± 15.4 years) in Maracaibo city (Bermudez & Salazar, 2016). Using a higher cutoff of HOMA-IR (≥2.5), a similar prevalence of IR (44.6%) was reported in a national sample of randomly selected adults in Lebanon (n=308 and mean age: 41.0 ±15.5) (Naja et al., 2012). Despite the high prevalence shown in the previously mentioned studies, there is a lack of international data regarding the global prevalence of IR, supporting the fact that more studies should be done to assess the prevalence of IR.

4. Risk Factors of IR

IR could be mediated by biological, demographic, and lifestyle factors (Salaroli et al., 2017) as highlighted in the section below.

- Age: Aging is positively associated with HOMA-IR in healthy individuals; as age increases, HOMA-IR value increases in both men and women (Oya et al., 2014). However, some studies showed that the increase of IR with aging is due to the

increase in adiposity with the aging process rather than aging itself (Karakelides, Irving, Short, O'Brien & Nair, 2009).

- Gender: Men are at higher risk of IR compared to women; this could be due to the higher amount of visceral and hepatic fat in men, in addition to the lack of the potential protective effect of estrogen (Geer & Shen, 2009).
- Ethnicity: Insulin sensitivity among 4 different ethnic groups was compared, and the results showed that Caucasians had the highest insulin sensitivity, followed by Asian-American and African-American and then by Mexican-American who showed the least insulin sensitivity (Chiu et al., 2000). Mexican-American showed higher insulin sensitivity compared to Arabs with similar BMI (Hassoun et al., 2017). When comparing insulin sensitivity among Asian men, Chinese had the highest insulin sensitivity followed by Malays and then Asian-Indian (Tan et al., 2015). These results could be explained by the fact that different ethnicities have genetically different body composition, BMI, fat, muscle and bone mass (Kodama et al., 2013). Even though a person might speculate that visceral fat level should be associated with higher IR and lower insulin sensitivity, but a study showed that Caucasians, who are more insulin sensitive than Africans, have a higher visceral fat level and lower bone and mass level than Africans (Lovejoy, de la Bretonne, Klemperer & Tulley, 1996). This suggests that other factors may be involved like less insulin clearance and more insulin secretion at baseline needed to build muscle and bone mass (Kodama et al., 2013).
- Smoking: Smokers are at higher risk of IR compared to non-smokers (Haj Mouhamed et al., 2016). This effect could be explained by the fact that smoking

leads to the production of large amounts of insulin antagonist hormones (catecholamines and cortisol) (Lager et al., 1986). In addition, smoking may lead to the release of free radicals which in turn can decrease insulin sensitivity (Paolisso & Giugliano, 1996). Moreover, nicotine itself may be associated with increasing IR (Eliasson et al., 1996).

- Physical inactivity: Physical activity improved insulin sensitivity in both sexes. The intensity of the exercise did not have any significant effect on insulin sensitivity (Balkau et al., 2008). On the other hand, physical inactivity was positively associated with IR (Hamburg et al., 2007). This association may be due to the decrease in fat mass and increase in muscle mass accompanying physical activity, in addition to enhanced glucose transport on the level of the muscles (Holloszy, 2005; Joseph et al., 2006).
- Obesity: Obesity is positively associated with IR. This could be due to the accumulation of visceral fat and thus the accumulation of fat in the liver which impairs insulin signaling (Hardy, Czech & Corvera, 2012).

Weight reduction, diet as well as management of hyperglycemia can improve IR but total recovery is rare (ADA, 2010). Accordingly, a large body of evidence is addressing the prevention or alleviation of IR since its prevalence appeared to be relatively high in many countries. From this perspective, there is an increasing interest in assessing the association between IR and dietary factors, one of which is vitamin D. This vitamin is gaining more attention in research due to its potential association with IR.

5. Vitamin D

Vitamin D is a micronutrient, and is part of the fat-soluble vitamins family. The term "vitamin D" refers to either "ergocalciferol" or vitamin D_2 (formed from ergosterol) and "cholecalciferol" or vitamin D_3 (formed from 7-dehydrocholesterol) (Ross, Taylor, Yaktine & Cook, 2011). Figure 1 shows the chemical structure of ergocalciferol and cholecalciferol.

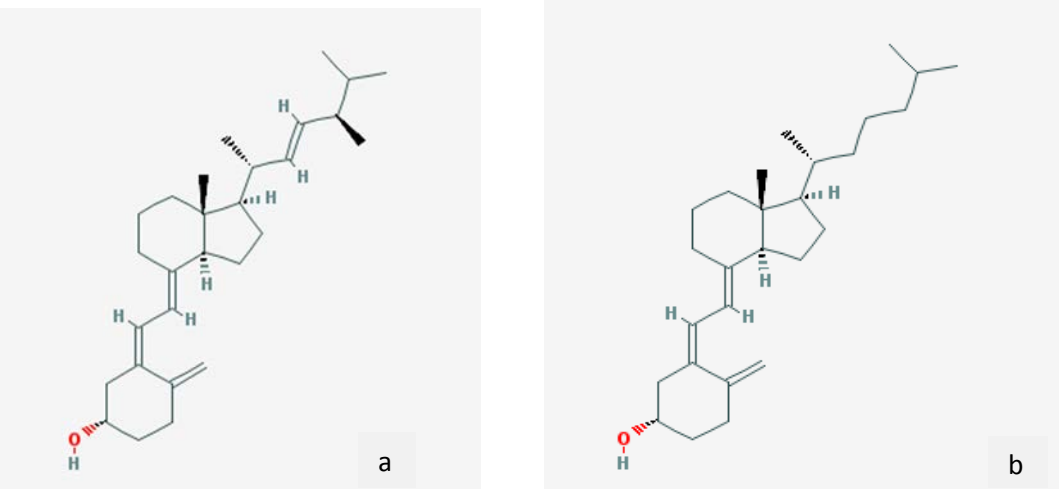

Figure 1: Ergocalciferol (a) and Cholecalciferol (b) chemical structure [Retrieved from (Cholecalciferol, 2018; Ergocalciferol, 2018)].

6. Vitamin D Sources

The sources of vitamin D can be either endogenous or exogenous. Vitamin D can be produced endogenously in the body through dermal synthesis, or obtained through the consumption of vitamin D rich foods and supplements (Pludowski, et al., 2018).

6.1. Endogenous Synthesis of Vitamin D

The main natural source of vitamin D is vitamin D_3; it's produced in the skin in a 2 step procedure involving 7-dehydrocholesterol (7-DHC) as a precursor and ultraviolet B irradiation from the sun (Pludowski, et al., 2018). 7-DHC, present in the plasma membrane of keratinocytes, is first converted to previtamin D_3 and then converted to vitamin D_3 through thermal isomerization (Pludowski, et al., 2018; Mostafa & Hegazy, 2015). Vitamin D-binding protein (DBP) is then used to transport vitamin D_3 synthesized in the skin in order to enter the systemic circulation. The concentration of vitamin D_3 in the blood peaks at 24h-48h after exposure to the sun (Mostafa & Hegazy, 2015). If the whole body is exposed to the sun for a period of 15 to 20 minutes, up to 250 µg (10 000IU) of vitamin D will be produced (Mostafa & Hegazy, 2015). Vitamin D synthesis in the skin can be influenced by several environmental or personal factors. The environmental factors include time of the day (day versus night) (Holick, 2007), weather (cloudiness) (Estupiñán, Raman, Crescenti, Streicher & Barnard, 1996) and season (summer versus winter) (Greene-Finestone et al., 2010); all these factors could affect the amount of sun rays and UVB reaching the skin. In addition, personal factors such as age (Tsiaras & Weinstock, 2011), skin color (Engelsen, 2010) and lifestyle practices, including clothing habits and sun protection practices (i.e sunblock use) (Greene-Finestone et al., 2010) can also influence vitamin D synthesis. For instance, the skin of elderly people becomes thinner with age and thus its capacity of producing vitamin D decreases (Tsiaras & Weinstock, 2011). In addition to that, the color of the skin also plays a role; light skins (type I) produce 6 times more vitamin D than dark skins (type VI) (Engelsen, 2010).

6.2. Dietary Sources of Vitamin D

The availability of vitamin D in foods is very limited. Some food sources of vitamin D include fish liver oil, fatty fish, or egg yolks. Nevertheless, the diet, even if varied, is not considered a reliable source to obtain the recommended daily doses of vitamin D (Pludowski, et al., 2018). Accordingly, fortified foods and supplements are becoming significant sources of vitamin D. Table 4 shows the different dietary sources of vitamin D.

Table 4: Dietary sources of vitamin D [Retrieved from (Office of Dietary Supplements - Vitamin D)]

Food	**IUs per serving***
Cod liver oil, 1 tablespoon	1,360
Swordfish, cooked, 3 ounces	566
Salmon (sockeye), cooked, 3 ounces	447
Tuna fish, canned in water, drained, 3 ounces	154
Orange juice fortified with vitamin D, 1 cup (check product labels, as amount of added vitamin D varies)	137
Milk, nonfat, reduced fat, and whole, vitamin D-fortified, 1 cup	115-124
Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces (more heavily fortified yogurts provide more of the DV)	80
Margarine, fortified, 1 tablespoon	60
Sardines, canned in oil, drained, 2 sardines	46
Liver, beef, cooked, 3 ounces	42
Egg, 1 large (vitamin D is found in yolk)	41
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 0.75-1 cup (more heavily fortified cereals might provide more of the DV)	40
Cheese, Swiss, 1 ounce	6

* IUs = International Units; 1 µg= 40 IU

7. Vitamin D Dietary Recommendations

In 2010, the National Academy of Medicine (NAM) formerly called - the Institute of Medicine (IOM) - published the dietary recommendations for vitamin D based on age (Ross, Taylor, Yaktine & Cook, 2011). For the first year of life, an adequate intake (AI) and tolerable upper intake level (UL) were recommended. The AI is 400 IU (10 µg) for ages 0 to 12 months; while the UL is 1000 IU (25 µg) from 0 to 6 months and 1500 IU (38 µg) from 6 to 12 months. For ages older than 1 year, the recommended dietary

allowance (RDA) was suggested. Table 5 presents the amount of vitamin D recommended for the general population based on age.

Table 5: Vitamin D dietary reference intake for the general population by the National Academy of Medicine (amount/day) [Adapted from (Ross, Taylor, Yaktine & Cook, 2011)]

Life Stage Group	EAR	RDA	UL
Children			
1–3 y	400 IU (10 µg)	600 IU (15 µg)	2,500 IU (63 µg)
4–8 y	400 IU (10 µg)	600 IU (15 µg)	3,000 IU (75 µg)
Males and Females			
9–70 y	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
> 70 y	400 IU (10 µg)	800 IU (20 µg)	4,000 IU (100 µg)
Pregnancy and Lactation			
14–50 y	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)

NOTE: EAR = Estimated Average Requirement; IU = International Unit; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Levels

8. Vitamin D Metabolism

The absorption of vitamin D occurs mainly in the small intestine. Once in the bloodstream, vitamin D is first converted in the liver to 25-hydroxyvitamin D [25(OH)D], which is then converted in the kidneys to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. $1,25(OH)_2D$ is the active form of the vitamin. DBP are used to transport 25(OH)D and $1,25(OH)_2D$ in the blood. Once released from DBP in the tissues, $1,25(OH)_2D$ triggers many metabolic reactions in the body through binding to “intracellular vitamin D receptor” (VDR) (Jones, 2012; Jones, Prosser, & Kaufmann, 2012). Figure 2 shows the pathway for vitamin D metabolism in the body.

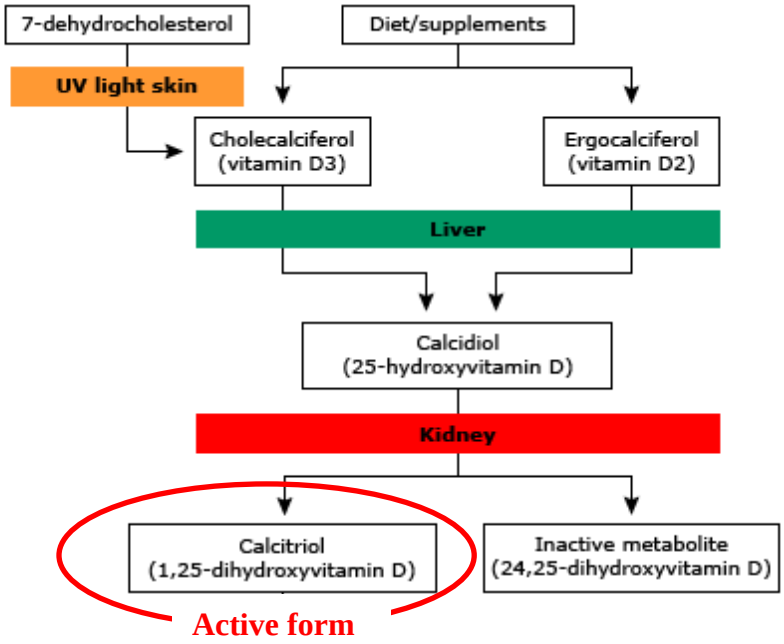

Figure 2: Vitamin D metabolism [Adapted from (Pazirandeh & Burns, 2017)]

9. Vitamin D Assessment

Recent analytical methods used to detect vitamin D levels are either chromatography based [with mass spectrometric detection or ultraviolet (UV)] or immunoassay based. Immunoassay based techniques are sensitive and require small volumes of the sample; in addition, they are "easily integrated into fully automated, random-access laboratory track systems, thus allowing rapid analysis times" (Couchman & Moniz, 2017). Nonetheless, a significant disadvantage of this technique is in its "selectivity due to cross reactivity with different vitamin D metabolites" like 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3. Moreover, another limitation of immunoassay based technique is that the analyte, 25(OH)D, must be first released from DBP before being measured, and the automated methods needed to accomplish this task are often proprietary (Couchman & Moniz, 2017). Enzyme-linked immunosorbent assay (ELISA) is an example of immune-based

techniques used to measure vitamin D; in this method the antigen used is bound to a solid phase consisting of polystyrene, polyvinyl and polypropylene tubes and microplates. The antigen and the antibody must be adsorbed appropriately to the microplates without any other interfering components (Engvall & Perlmann, 1971; Yalow & Berson, 1960). Chromatography-based techniques are also used for determining of vitamin D levels. First an extraction method is needed to disrupt protein binding, and then "25(OH)D_2 can be resolved chromatographically from 25(OH)D_3, and thus independently calibrated and quantified". At first, chromatography-based methods relied on "liquid chromatography (LC) with UV detection", but more recent methods emerged relying on LC with "tandem mass spectrometry (LC-MS/MS)". One of the advantages of the LC-MS/MS method is its selectivity "via chromatographic resolution of the individual metabolites". Using mass spectrometry with the chromatographic separation allowed the selective detection of vitamin D metabolites based on their mass to charge ratio values. Moreover, mass spectrometry allowed the insertion of "stable isotope-labeled internal standards" which enabled quantitative analyses of 25(OH)D levels. However, the LC-MS/MS technique is not free of limitations; "complete primary sample-to-result LC-MS/MS workflows are not yet automated to the extent of immunoassays", which means that the instrumentation parameters will differ from one laboratory to another and so would the results. On the other hand, the flexibility of the LC-MS/MS technique makes it the current gold standard technique for the assay of vitamin D (Couchman & Moniz, 2017).

10.Vitamin D Cutoffs

The definition and cutoff used to indicate vitamin D "deficiency" and "sufficiency" were subject to many controversies (Norman, 2008). 25(OH)D concentrations are used for

determining vitamin D status (Wimalawansa, 2018). Many studies claimed that the optimal 25(OH)D concentration to meet the needs of human tissues containing VDR is around 40 ng/mL (100 nmol/L). However, some studies revealed that the optimal concentration of vitamin D depends on the location and function of the tissue containing the VDR; the 25(OH)D requirements for synthesizing $1,25(OH)_2D$ in endocrine tissues are different than the requirements in the autocrine/paracrine tissues (Norman, 2008; Morris & Anderson, 2010; Spedding, Vanlint, Morris & Scragg, 2013; Anderson, Iida, Tyson, Turner & Morris, 2010). Table 6 shows the minimum 25(OH)D serum levels needed to prevent specific conditions.

Table 6: Minimum required serum concentration of 25(OH)D for different health conditions [Retrieved from (Spedding, Vanlint, Morris & Scragg, 2013)]

	Level of evidence NHMRC	**Minimum effective serum 25(OH)D concentration (nmol/L)**
Premature mortality	Level l	75
Falls prevention	Level l	95
Cancer prevention	Level ll	100
Respiratory infection prevention	Level ll	95
Diabetes prevention	Level ll	80
Depression treatment	Level ll	75
Dental disease	Level lll-2	>84
Cardiovascular disease	Level lll-2	80

NHMRC = National Health and Medical Research Council
Level I Systematic review of Level II studies
Level II Randomized controlled trial
Level III-1 Pseudo-randomized controlled trial
Level III-2 Comparative study with concurrent controls: non-randomized, experimental trial, cohort study, case-control study, or interrupted time series with a control group

According to the NAM, a minimal 25(OH)D concentration of 20 ng/mL (50 nmol/L) is considered adequate for bone health (Ross et al., 2011). In contrast, The Endocrine

Society in the USA recommended a 25(OH)D serum concentration in the range between 30 and 50 ng/mL (75 to 125 nmol/L) or 40 and 60ng/mL (100 to 150 nmol/L), for preventing vitamin D deficiency and maintaining bone health (Holick et al., 2011). The European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) recommends a minimum 25(OH)D serum level of 30 ng/mL (75 nmol/L) for the elderly population, at an elevated risk of falling and fractures (Pludowski, et al., 2018).

11. Treatment of Vitamin D Deficiency

In case of 25(OH)D concentration < 20 ng/mL or 50 nmol/L, a treatment with vitamin D is needed for a duration of 1 to 3 months. The dosage of the treatment is age and body weight specific (Pludowski, et al., 2018). Table 7 presents the age-based dose of vitamin D for the treatment of vitamin D deficiency as recommended by the Endocrine Society. Coexisting diseases should be taken into consideration when providing the treatment. For example, for cases of intestinal malabsorption, intramuscular doses or greater oral doses of up to 50 000 IU/2-3 times a week are needed. Sunlight or exposure to stimulated sunlight, from a specific light device that emits UVB radiation, are other alternative techniques to acquire vitamin D (Dabai, Pramyothin & Holick, 2012).

Table 7: Recommended dose of vitamin D for the treatment of vitamin D deficiency (Pludowski et al., 2013)

	Recommended dose of vitamin D
Neonates (< 1 month)	1000 IU/day (25 mg/day)
Infants (>1 month) and toddlers	2000–3000 IU/day (50–75 mg/ day)
Children and adolescents aged 1–18 years	3000–5000 IU/ day (75–125 mg/day)
Adults and elderly	7000–10,000 IU/ day (175–250 mg/day) or 50,000 IU/week (1250 mg/week)

12. Vitamin D Toxicity

Vitamin D toxicity is still a concern for medical doctors and the public healthcare system. This concern is behind the governments' resistance against vitamin D fortified foods such as milk and dairy. Serum 25(OH)D concentrations of up to 100ng/mL (250 nmol/L) are considered safe for children as well as adults, with the exception of cases of hypersensitivity to vitamin D; like "children and adults with idiopathic infantile hypercalcemia, Williams-Beuren syndrome, granulomatous disorders and some lymphomas" (Pludowski, et al., 2018). According to The Endocrine Society, vitamin D toxicity is extremely rare and a 25(OH)D serum level of 150 ng/mL (375 nmol/L) is needed before there could be evidence of toxicity (Holick et al., 2011; Holick, 2015).

Vitamin D toxicity is manifested by an increase in serum calcium and phosphate levels, which in turn leads to the deposition of calcium phosphate product in soft tissues like the kidneys, leading to nephrocalcinosis and atherosclerotic vascular calcification. Hypercalcemia also results in vasoconstriction and hypertension, in addition to a number

of other nonspecific symptoms like depression, confusion, constipation, polydipsia, polyuria and cardiac arrhythmias (Holick, 2015).

13.Prevalence of Low Vitamin D Status

Deficiency in vitamin D is a global issue that predominates in Asia as well as the Middle East. In these areas more than half of the population is deficient in vitamin D (<50 nmol/L or < 20ng/mL) (Wimalawansa, 2018). Figure 3 shows the prevalence of vitamin D deficiency among adults (≥ 18 years) worldwide. In Northern Europe and Southern Asia, vitamin D deficiency appears to be the highest with a range between 52% and 80% of the total population. Vitamin D deficiency also seems high in South America with a prevalence of 77% in Brazil. The prevalence is significantly lower in the United States where the values vary between 34 and 37% and in Australia and Canada with a prevalence of 31% and 20% respectively.

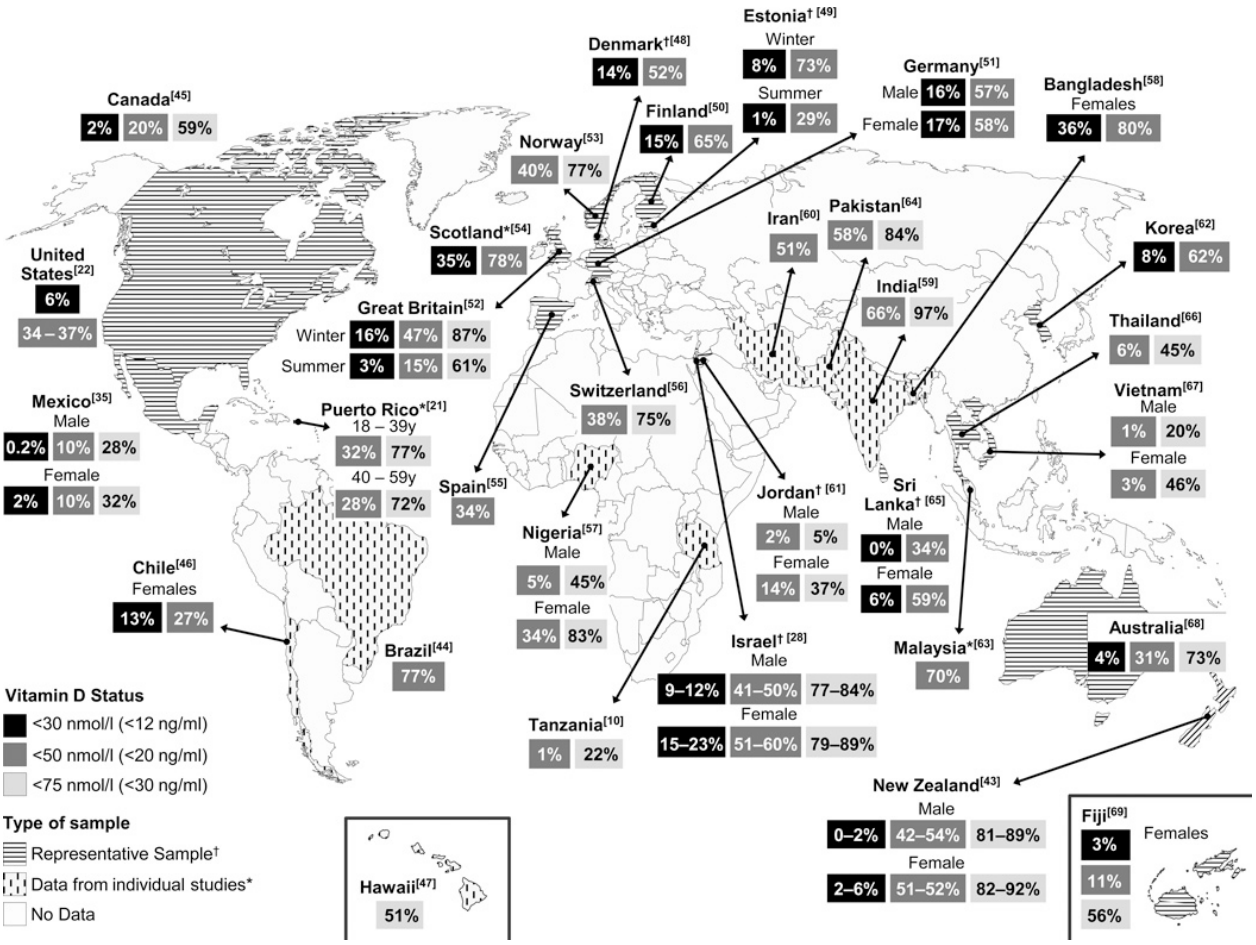

Figure 3: Prevalence of low vitamin D status among adults worldwide [Retrieved from (Palacios & Gonzalez, 2014)]

14. Vitamin D Functions

14.1. Skeletal Functions

The main function of $1,25(OH)_2D$ is to maintain calcium and phosphorus homeostasis in the blood. The normal serum calcium level in humans is maintained between 2.45 and 2.65 mmol/L. When the calcium level drops below this range, it triggers “a series of anti-

hypocalcemic events" to restore the calcium level within the normal physiologic range (Pludowski, et al., 2018).

The bones, intestines and kidneys are the main targets of $1,25(OH)_2D$ actions. On the level of the bones and skeletal tissues, $1,25(OH)_2D$ is responsible for controlling bone turnover with the help of the parathyroid hormone (PTH) (Pludowski, et al., 2018). In adults, severe vitamin D deficiency will lead to osteomalacia, while in children, it will manifest as rickets. Therefore, maintaining an adequate level of vitamin D is essential to prevent skeletal diseases like osteomalacia and rickets, as well as preventing falls and bone fractures (Wimalawansa, 2018). However, a randomized clinical trial among older women, aged 70 years and older, showed that very high doses of vitamin D supplements given at one time (500 000 IU of cholecalciferol/ year) resulted in a higher risk of falls and bone fractures after 3 to 5 years of treatment. Therefore, the protective effect of vitamin D is attainable when taken in adequate doses only (Sanders, et al., 2010).

14.2. Extraskeletal Functions

In the human body, almost all cells and tissues have VDR and also exhibit the 25(OH)D-1α-hydroxylase (CYP27B1) activity, which is the enzyme in charge of the conversion from 25(OH)D to $1,25(OH)_2D$ (Jones, 2012; Jones et al., 2012; Jones, Prosser, & Kaufmann, 2013; Norman, 2008). The synthesis of $1,25(OH)_2D$ and then its binding to VDR is responsible for upregulating around 2000 genes involved in several metabolic pathways, thus leading to the non-calcemic related benefits of vitamin D (Hossein-Nezhad, Spira, & Holick, 2013; Holick, 2003).

Evidence shows that 1,25$(OH)_2$D is involved in modulating cellular growth and differentiation as well as enhancing the immune system. High levels of VDR were reported in dendritic cells, macrophages as well as in T and B lymphocytes, which support the role of vitamin D in fighting bacteria and preventing chronic inflammatory and autoimmune diseases (Alves, Ishimura, Duarte, & Bueno, 2018). On the level of the cardiovascular system, there is an abundance of "vitamin D-related components" in the heart and blood vessels. Vitamin D deficiency leads to an increase in PTH, which in turn leads to an increase in blood pressure and myocardial contractility, thus affecting the cardiovascular system. A study among adults aged 18 years and older, in Saudi Arabia, showed that vitamin D deficiency (<20 ng/mL) was significantly associated with increased risk of coronary heart disease (odds ratio 6.5, $p< 0.001$) compared to vitamin D adequacy (≥20 ng/mL) (Aljefree, Lee, Alsaqqaf, & Ahmed, 2016). Moreover, adequate level of vitamin D in the body was also associated with reducing the risk of cancers (colorectal cancer, colorectal adenomas and breast cancer). Evidence showed that the optimal 25(OH)D concentration required to prevent cancer and improve the survival chance, is between 30 and 40 ng/mL (75-100 nmol/L) (Grant, 2016). Furthermore, the physiological level of vitamin D also plays a role in improving cases of neurodegenerative disorders like Alzheimer's disease, dementia and cognitive decline. A study showed that elderly people with vitamin D levels below 50 nmol/L (20 ng/mL) exhibited a higher risk of dementia and Alzheimer's disease compared to elderly people with 25(OH)D levels above 50 nmol/L (Littlejohns et al., 2014). Furthermore, an increasing body of evidence is suggesting a potential association between vitamin D deficiency and insulin insensitivity or abnormal glucose metabolism (Badawi et al.,

2014). Several studies have explored the association between low vitamin D status and type 2 diabetes mellitus (T2DM) and multiple mechanisms have been suggested to explain this association (Badawi et al., 2014; Huang et al., 2013).

15.IR and Vitamin D

15.1. Potential Mechanisms of Action

Vitamin D, with its anti-inflammatory properties, can improve the function of islet-cells, as well as insulin release and IR. Vitamin D deficiency may play a role in mediating low-grade inflammation resulting from an imbalanced innate immune system, related pro-inflammatory cytokines, and acute phase reactants" (Badawi et al., 2014). This mechanism was associated with many chronic disorders like obesity, metabolic syndrome and IR that enhance the risk of developing T2DM in vitamin D deficient individuals. In addition, insulin release is affected by gene polymorphism associated with the metabolism, transport and action of vitamin D, thus disrupting glucose homeostasis. Deficiency of vitamin D is related to hypocalcemia that lead to a decrease in glucose-stimulated insulin secretion in β-cell. Moreover, low vitamin D levels in the blood are associated with increasing parathyroid hormones (PTH) levels leading to decreased glucose uptake by the adipose cell, liver and muscle (Badawi et al., 2014). Furthermore, vitamin D was shown to have a direct effect on insulin function; $1,25(OH)_2D$ increases insulin's response to glucose by "stimulating the expression of insulin receptor in peripheral tissue" (Huang et al., 2013).

Additionally, IR is very common in overweight or obese patients (Hardy, Czech & Corvera, 2012) who, in general, are at higher risk of vitamin D deficiency due to the

following reasons. In adipose tissue, VDR is highly expressed and is highly responsive to the active form of vitamin D, 1,25$(OH)_2$D (Wimalawansa, 2018). There is a strong inverse association between high BMI, body fat and serum 25(OH)D (Wimalawansa, 2018). Many potential mechanisms have been suggested to explain this association. First, vitamin D is fat soluble, thus it is stored in adipose tissue where 25(OH)D is sequestered and converted to inactive metabolites (Pannu, Zhao & Soares, 2016). Therefore, obesity decreases bioavailable vitamin D level and increases vitamin D deficiency. Furthermore, vitamin D could be diluted in the excess fat mass present among obese individuals (Drincic, Armas, Van Diest & Heaney, 2012) or excreted in excess due to the inflammation related to obesity (Compher, Badellino & Boullata, 2008). As a result, when administering vitamin D supplements to obese people, a higher dose should be considered to make up for the excess adiposity (Wimalawansa, 2018).

15.2. Literature Review: Cross-sectional Studies, Cohort Studies and Randomized-Controlled Trials.

The association between vitamin D and IR received greater attention in the last decade (Al-Shoumer & Al-Essa, 2015). Several cross-sectional and cohort studies, as well as randomized controlled trials explored the link between these two variables.

Cross-sectional Studies

The three following cross-sectional studies were conducted in the three major countries in East Asia, including China, Japan and Korea, to assess the effect of vitamin D on IR in this region. The study conducted in China (n= 2708, mean age= 48.5 ± 12.6 years) showed that subjects with vitamin D deficiency [25(OH)D concentrations < 20 ng/ml (50

nmol/L] were 1.91 times more likely to develop IR (HOMA-IR>2.5) compared to subjects with adequate vitamin D levels [25(OH)D concentrations > 20 ng/ml (50 nmol/L] (Huang et al., 2013). Likewise, a study conducted among a sample of non-obese Korean participants without type II diabetes (n=1807, age range= 30-64 years) showed an inverse association between serum 25(OH)D level and IR not only among individuals with vitamin D deficiency (<20 ng/mL) but also among participants with vitamin D insufficiency (20 to 30ng/mL) (Ock et al., 2016). In line with the findings of the previous study, the level of IR increased as the levels of vitamin D decreased in a cross-sectional study among Japanese workers (n=494, age range=20-68 years) (Pham et al., 2012).

Similar results were observed in different ethnicities including American (Zhao et al., 2009) and Canadian adults (Badawi et al., 2014). An inverse linear association between plasma 25 [OH] D levels and HOMA-IR was observed among Canadian adults (n=1928, age range= 16-79 years, mean BMI=26.8 ± 0.3 kg/m^2), representing 96.3% of the Canadian people with and without diabetes, but excluding residents of "Crown lands, Aboriginal reserves, remote regions, health institutions, and full-time members of the Canadian Forces" (Badawi et al., 2014). Further, an inverse linear relationship between HOMA-IR and 25(OH)D levels was observed among US adults, without diagnosed diabetes (n=3206, age≥20 years) in a cross-sectional study (Zhao et al., 2009).

Conversely, the inverse association between vitamin D and IR was not observed among an older sample of European adults (n=446, age=35-70 years) with metabolic syndrome (Gulseth et al., 2010). Similar results were observed among a sample of non-diabetic participants in Brazil (n=53, age= 65.3 ± 10.3 years) meeting one or more of the following criteria "hypertension; body mass index (BMI) ≥ 25 kg/m^2; waist

circumference > 80 cm for women and > 94 cm for men; first-degree relatives with diabetes; women with large-for-gestational-age newborns or with gestational diabetes mellitus; fasting serum HDL-cholesterol < 35 mg/dl; and triglycerides > 250 mg/dl" (Giorelli, Matos, Saado, Soibelman & Dias, 2014). It is likely that this relationship could be altered in case of disease and older age, both factors could exacerbate IR.

While the previously mentioned studies explored the relationship between vitamin D and IR without addressing gender differences, other researchers examined this association across genders. For instance, in a cross-sectional study conducted among 1205 participants (609 males and 595 females) from Jerusalem (mean age=32.0 ± 1.3 years), the results showed that serum level of 25(OH)D was inversely associated with IR (HOMA-IR) among males but not females (Moore et al., 2014). This result may be due to the fact that IR is higher among males than females (Geer & Shen, 2009). Moreover, middle-aged men could be at a higher risk of developing type II diabetes compared to middle-aged women due to different deposition of body fat (Choi et al., 2009; Lipscombe & Hux, 2007; Moneta, 2011), therefore if low vitamin D levels were to have an effect on IR and the development of type II diabetes, this association would be more prominent in men than women (Moore et al., 2014). Similarly, in a cross-sectional sample of Arab Americans men and women (n=542, age range= 20-75 years), serum 25(OH)D concentration was negatively associated with IR among men but not women (Pinelli, Jaber, Brown & Herman, 2010). In contrast, Tao et. al., 2013 reported an independent inverse association between serum 25(OH)D concentration and IR (assessed using HOMA-IR) as well as β-cell function (assessed using HOMA-B) in females. This cross-sectional study was conducted among Chinese participants (n=1382, age 20-85 years);

therefore the difference in results could be due to the difference in ethnicity. As mentioned earlier, different ethnicities have genetic differences that affect their insulin sensitivity. Furthermore, in a sample of randomly selected non-obese non-diabetic student population (n=381, mean age=23.9 ± 3.9 years) in Lebanon, a significant inverse association between vitamin D and HOMA-IR was observed in the overall population. Nevertheless, when the analysis was stratified based on gender, this association was only significant among female and not male participants. This study was conducted among a small sample of young university students; it is possible that a larger sample size and a wider age range might have yielded significant correlations among the male participants (Gannage et al., 2009).

Conclusively, the difference in the association between the level of Vitamin D and IR among genders is still controversial and requires further assessment.

Cohort Studies

In a prospective cohort study, 489 non-diabetic individuals of different ethnicities (mean age=50 years ± 10 years), with a BMI ≥ 27 kg/m^2, were followed for 3 years in order to assess the relationship between baseline serum vitamin D level and beta cell function as well as IR. The results showed a significant association between baseline 25(OH)D concentration and HOMA-IR, but this relationship was diminished to non-significance after adjusting for the BMI. On the other hand, an independent significant positive association was found between baseline 25(OH)D concentration and beta cell function (Kayaniyil et al., 2011).

Another prospective cohort study was conducted on a much larger sample of non-diabetic Australian individuals (n=6537) who were followed up for 5 years in order to assess the effect of serum 25(OH)D concentration on insulin sensitivity. The results showed a significant independent positive association between vitamin D level and insulin sensitivity at 5 years. In this study the population was larger with a longer follow-up period than the previously mentioned study, and the population's BMI was heterogenous (normal weight, overweight and obese). These differences might be behind the significance of the association between these 2 factors (Gagnon et al., 2011).

Randomized Controlled Trials

A double-blind randomized controlled study was performed in Italy on a sample of 18 non-diabetic and vitamin D deficient (25[OH]D < 75 nmol/L or 30 ng/L) volunteers, aged between 18 and 70 years, who had a BMI ≥30 kg/m^2. The intervention lasted 3 months and consisted of placing all the participants on a hypocaloric diet while supplementing the intervention group with oral cholecalciferol at a dose of 25,000 IU/week and the control group with placebo. At the end of the 3 months, both groups lost body weight (vitamin D group: -7.5%, placebo group: -10%); however, the vitamin D group witnessed improved insulin sensitivity while no improvement was observed in the placebo group (Cefalo et al., 2018). Supplementation with vitamin D coupled with a hypocaloric diet for weight loss lead to improved insulin sensitivity in healthy obese individuals; thus this approach might represent a new potential therapeutic regimen for obese individuals with obesity-induced IR.

A double-blind randomized controlled study was performed on a population of non-Western immigrants in the Netherlands (n=130, age range= 20-65 years). The inclusion criteria included a BMI≥ 27 kg/m^2, impaired fasting or random plasma glucose (fasting plasma glucose= 5.6-6.9 mmol/L or random plasma glucose= 7.8-11.1 nmol/L) and vitamin D deficiency (serum 25[OH]D concentration <50 nmol/L). Similarly to the study mentioned above, the intervention involved two study groups; an intervention group receiving vitamin D supplementation (1200 IU/day) and a control group receiving a placebo. However, this study intervention lasted longer (16 weeks) than the intervention in the previously mentioned study (12 weeks). After 4 months of supplementation, the results showed that there was no significant effect of vitamin D supplementation on insulin sensitivity or beta cell function; nevertheless when patients who were diabetic at baseline were excluded, there was a significant improvement in beta cell function (insulinogenic index) in patients whose vitamin D level reached 60 nmol/L or more (Oosterwerff et al., 2014). Accordingly, this study suggests that correcting vitamin D status in overweight and obese adults might have a protective effect in reducing the incidence of diabetes.

The following study examined the effect of vitamin D supplementation on IR in children rather than adults. A triple-masked controlled trial was conducted on a population of 50 obese children aged between 10 to 16 years with metabolic syndrome. This intervention lasted for 3 months and involved two study groups; one group receiving vitamin D supplementation (300,000 IU one capsule/week) and one group receiving a placebo. The results showed that the vitamin D group exhibited a significant decrease in serum insulin and HOMA -IR compared to baseline and the placebo group (Kelishadi, Salek, Salek,

Hashemipour & Movahedian, 2014). This study showed that vitamin D supplementation has a beneficial effect on decreasing IR in obese children and not only in adults; however, it's important to highlight that the vitamin D status of the children was not measured at baseline to see whether they were deficient or sufficient in vitamin D.

The limited number of randomized control trials that specifically assess the effect of vitamin D on IR in non-diabetic, non-obese healthy adults hinders the availability of solid conclusive evidence regarding the association between these two variables in healthy populations.

A summary of the literature pertaining to vitamin D deficiency and IR can be found in tables 12 and 13 (Appendix A).

II. Manuscript

1. Introduction

Insulin resistance (IR) is a state where there is an increase in insulin secretion in the pancreas due to the fact that target cells like the muscles and adipose tissue no longer respond to normal amounts of insulin secretions (Salaroli et al., 2017). Therefore, insulin is unable to adequately take up glucose from the blood into the cells (Tam et al., 2012). An emerging body of evidence is suggesting that IR could play a fundamental role in the pathophysiology of many chronic conditions including Type II diabetes mellitus (T2DM), cardiovascular and metabolic abnormalities including the metabolic syndrome, polycystic ovarian syndrome (PCOS), nonalcoholic fatty liver disease, inflammation, cancer and Alzheimer's disease (Freeman & Pennings, 2018; Frozza, Lourenco, & Felice, 2018). Many of these conditions represent leading causes of death in adults in developed and developing countries, with alarming projections (Baena-Díez et al., 2016). Accordingly, an increasing number of studies is focusing on the understanding and prevention of IR. Weight reduction, diet as well as management of hyperglycemia can improve IR but total recovery is rare (ADA, 2010). From this perspective, there is a growing interest in assessing the relationship between IR and nutrients specifically vitamin D. This vitamin is gaining more attention in research due to its potential association with IR. Moreover, deficiency in vitamin D is a global issue that predominates in Asia as well as the Middle East where more than half of the population is deficient in vitamin D (Wimalawansa, 2018). With the high prevalence of both vitamin D deficiency and IR and the emergence of evidence relating vitamin D and IR, more

research is needed to explore this relationship especially that multiple research questions remain unanswered. This study aims to explore the association between vitamin D status and IR among employees in a private university in Lebanon.

2. Methodology

2.1. Study design

A cross-sectional study was conducted in Notre Dame University (NDU) in Lebanon. In September 2016, an email (Appendix B) was sent to all the employees in the 3 NDU campuses to invite them to participate in the study. The aim of the study was explained to the faculty members and staff, in their offices, by four trained nutritionists. Those who agreed to join the study completed a questionnaire through a 20-minute face-to-face interview. The participants were then requested to pass by the Nutrition Laboratory, after an overnight fast, to acquire anthropometric, biochemical and clinical measurements. In March 2017, the participants were re-visited by two trained nutritionists to fill a 24h dietary recall (24h multiple pass method recall) during a 10-minute face-to-face interview.

2.2. Ethical Considerations

The study was approved by the institutional review board at NDU. A signed informed consent (Appendix C), including the study's objectives and ensuring the participants' anonymity and confidentiality, was obtained from all the sample participants. The questionnaires and records were safely stored in a locked room and the computerized data files were protected by a password.

2.3. Study population

The participants were selected via convenient sampling, including NDU staff and faculty members in the main (Zouk Mosbeh), Shouf and North campuses who were older than 18 years of age (total population size =600) and excluding participants suffering from diabetes, cardiovascular disease or cancer.

The calculated sample size was estimated to be 316 participants, taking into consideration the prevalence of IR in Lebanon (44.6%) (Naja et al., 2012), the 95% confidence interval and the 5.5% margin of error.

2.4. Close-ended questionnaire

Independent variables:

Participants were requested to answer a close-ended background questionnaire (Appendix D) assessing socio-demographic information (i.e age, gender, education level, etc.), lifestyle habits (smoking and alcohol) and medical history. The physical activity level of the participants was assessed by using the short-form of the International Physical Activity Questionnaire (IPAQ-short form, Appendix E).

2.5. Food frequency questionnaire (Appendix F)

Independent variable:

A food frequency questionnaire was used to assess vitamin D intake of the participants. This food frequency questionnaire was developed by study investigators (El Hayek et al., 2014) and incorporated different categories of food items consumed in Lebanon.

2.6. 24h Recall (Appendix G)

Independent variables:

A 24h recall Multiple-Pass Method recall was administered to evaluate nutrient and calorie intake. It was conducted in a face-to-face interview by a trained dietitian using a 5-step approach. In Step 1, the participants reported all the food items they consumed in the last 24 hours. In Step 2 the interviewer asked a series of probing questions for food items that are frequently forgotten during the previous step, like alcoholic and non-alcoholic beverages, fruits, vegetables, sweets and salty snacks. In Step 3 the interviewer collected the time at which each item was consumed and the occasion for eating. In Step 4 the interviewer obtained the Detail Cycle including descriptions of each food consumed, in addition to the quantities of food and the location where the food was consumed. In Step 5 the interviewer asked a final review question called the Final Probe where the participant was given a last chance to recall any food item that had not been mentioned in the previous steps in the interview. Estimates of calorie and nutrient intake were obtained using the Nutritionist Pro diet analysis software.

2.7. Anthropometric, clinical and biochemical measurements

Dependent variable:

The homoeostasis model assessment (HOMA-IR) was used to assess IR. The HOMA-IR formula used is the following: HOMA-IR= {[fasting insulin (uU/mL)] × [fasting glucose (mmol/L)]}/22.5 and the cut off value used was 2.5 (Gutch et al., 2015).

Fasting blood samples were collected by a nurse and then stored at -20°C in the Nutrition laboratory at NDU main campus.

The dry chemistry analyzer Vitros 250 was used to assess blood glucose levels; while insulin from the same blood samples was measured by ELISA technique.

Independent variables:

Anthropometric, clinical and biochemical measurements of the study participants were taken in the Nutrition Laboratory, after an overnight fast. Anthropometric measurements included height, weight, body composition as well as waist circumference. The following procedure was followed to measure the height to the nearest 0.1 cm: heels together without any shoes and with the head touching the ruler. A mechanical weight beam scale was used to measure the weight to the nearest 0.1 kg with study participants wearing minimal clothing and no shoes. The following equation was used to calculate the BMI: Weight (kg)/ Height (m^2). A non-stretchable tailor measuring tape was used the measure the waist circumference to the nearest 1 cm during minimal respiration at the midpoint between the lower part of the rib cage and above the top of the iliac crest (CDC, 2015). The bioelectrical impedance analysis (BIA) machine InBody 720, available at the Nutrition Laboratory at NDU, was used to measure body composition. Serum 25(OH)D was measured using ELISA technique.

2.8. Statistical Analyses

The Statistical Package for Social Sciences (SPSS) version 23 for Windows was used for the statistical analyses. Statistical significance was determined by a p-value of less than

0.05. The characteristics of the sample were described using descriptive statistics and stratified by gender. Continuous variables were represented in terms of mean (± standard deviation) while categorical variables were represented in terms of n (valid percent %). Bivariate analyses, using chi-square or independent sample t-test, in addition to multiple logistic regression were used to assess the relationship between HOMA_IR categories (< 2.5 or ≥ 2.5) stratified by gender and other variables including vitamin D status.

Two cutoff values for vitamin D deficiency were assessed; the first was the National Academy of Medicine's (NAM) cutoff of 20 ng/mL, the second was the National Osteoporosis Foundation's (NOF) cutoff of 30 ng/mL. Normal and underweight BMI categories were merged together, since only 3 participants were found to be underweight and merging these 2 categories did not change the results.

A multiple logistic regression was used to adjust for confounding factors including all variables showing a p-value below 0.2 in the bivariate analyses; waist circumference, marital status, annual income, activity level, hypertriglyceridemia, CRP, body fat percentage, vitamin D status (≥ 20 ng/mL), medical morbidity and BMI. We tested for interaction between different variables and found a non-homogeneity of the OR ($p<0.05$) between marital status and gender, and between hypertriglyceridemia and gender; leading to a stratified analyses by gender.

3. Results

The study population consisted of 318 adult participants (48.1% male, 51.9% female) with an average age of 41.5 ± 11.1 years. The characteristics of the sample were summarized in table 8 and stratified based on gender. The majority of the participants

were married (62.9%) with low physical activity level (64.5%) and no medical morbidity (63.5%), they were also non-smokers (62.6%) and did not drink alcohol (74.2%). Male participants were older, with a higher BMI, higher blood glucose level, caloric intake and lower body fat percentage compared to female participants ($p<0.05$). Further, men had a lower educational level and showed a higher prevalence of smoking, drinking alcohol, overweight and obesity, IR, hypertriglyceridemia and high CRP compared to female participants ($p<0.05$).

Table 8: Socio-demographic, lifestyle, dietary, anthropometric and biochemical characteristics of the sample stratified by gender

	Total (n=318)		**Men (n=153)**		**Women (n=165)**		
Characteristics	**n or mean**	**% or SD**	**n or mean**	**% or SD**	**n or mean**	**% or SD**	**P-value**
Age (years)	41.5	11.1	44.3	11.8	39.0	9.8	<0.001
Marital status							
Single/Separated/Divorced	118	37.1	53[a]	34.6	65[a]	39.4	0.447
Married	200	62.9	100[a]	65.4	100[a]	60.6	
Education level							
High school	66	20.8	42[a]	27.5	24[a]	14.5	0.009
Bachelor degree	83	26.1	32[b]	20.9	51[b]	30.9	
Graduate	169	53.1	79[a,b]	51.6	90[a,b]	54.5	
Income ($)							
<2250	101	31.8	54[a]	35.3	47[a]	28.5	0.090
2250-4000	84	26.4	32[a]	20.9	52[a]	31.5	
>4000	133	41.8	67[a]	43.8	66[a]	40.0	
Alcohol drinking							
No	236	74.2	100[a]	65.4	136[a]	82.4	0.001
Yes	82	25.8	53[b]	34.6	29[b]	17.6	
Smoking							
No	199	62.6	83[a]	54.2	116[a]	70.3	0.005
Yes	119	37.4	70[b]	45.8	49[b]	29.7	
Physical activity level							
Low	205	64.5	94[a]	61.4	111[a]	67.3	0.333
Moderate/High	113	35.5	59[a]	38.6	54[a]	32.7	
Calories (Cal)	1942.1	811.5	2165.9	944.3	1744.9	611.6	<0.001
Vitamin D intake (µg)	2.3	3.3	2.6	4.2	2.1	2.1	0.158

Total dairy product intake (serving/day)	2.2	1.0	2.2	1.0	2.2	1.0	0.999
BMI[1]							
Underweight/Normal	123	38.7	26[a]	17.0	97[a]	58.8	<0.001
Overweight	119	37.4	78[b]	51.0	41[b]	24.8	
Obese	76	23.9	49[b]	32.0	27[b]	16.4	
Body fat percentage	30.7	7.8	27.5	6.9	33.6	7.5	<0.001
Waist circumference risky[2]							
No	165	51.9	82[a]	53.6	83[a]	50.3	0.635
Yes	153	48.1	71[a]	46.4	82[a]	49.7	
Medical morbidity							
No	202	63.5	91[a]	59.5	111[a]	67.3	0.185
Yes	116	36.5	62[a]	40.5	54[a]	32.7	
Vitamin D concentration (ng/ml)	28.1	14.1	27.9	15.4	28.2	12.9	0.848
Glucose (mmol/L)	5.0	0.9	5.3	1.2	4.8	0.5	<0.001
Insulin (uIU/ml)	12.1	8.0	12.3	6.8	11.8	8.9	0.604
HOMA_IR[3]							
Non-insulin resistant (<2.5)	175	61.2	71[a]	53.0	104[a]	68.4	0.011
Insulin resistant (≥2.5)	111	38.8	63[b]	47.0	48[b]	31.6	
Hypertriglyceridemia							
Normal TG[4] levels	222	69.8	82[a]	53.6	140[a]	84.8	<0.001
Hypertriglyceridemia	96	30.2	71[b]	46.4	25[b]	15.2	
Cholesterol[5]							
Desirable	200	62.9	100[a]	65.4	100[a]	60.6	0.447
High	118	37.1	53[a]	34.6	65[a]	39.4	
HDL[6]							
Normal	243	76.4	115[a]	75.2	128[a]	77.6	0.708
Low	75	23.6	38[a]	24.8	37[a]	22.4	

CRP[7]							
Low/Moderate	130	40.9	48[a]	31.4	82[a]	49.7	0.001
High	188	59.1	105[b]	68.6	83[b]	50.3	

[1]Body Mass Index: underweight <18.5 kg/m^2, normal 18.5-24.9 kg/m^2,overweight 25-29.9 kg/m^2, obese ≥ 30 kg/m^2
[2]Risky waist circumference: >102 cm in men, <88 cm in women
[3]Homeostatic Model Assessment of Insulin Resistance
[4]Triglycerides-Normal levels: <150mg/dL
[5]Desirable: <200mg/dL
[6]High density lipoprotein: normal levels ≥40mg/dL in men, ≥50 mg/dL in women
[7]C-reactive protein: low/moderate levels <3mg/L
Columns with superscripts without a common symbol differ, P<0.05

In table 9, the association between HOMA_IR and socio-demographic, lifestyle, dietary, anthropometric and biochemical factors was assessed and stratified by gender. The results showed a significant positive proportional association between HOMA_IR and BMI categories in both genders (p<0.001).Mean glucose level was also significantly higher among participants with IR for both men (p=0.003) and women (p<0.001). Similarly, body fat percentage was significantly higher and risky waist circumference was more prevalent among participants with IR for both men and women (p<0.001). As for marital status, a significant association with HOMA_IR was seen among women but not men; single/separated/divorced women tended to have more IR than married participants (p<0.001). Moreover, hypertriglyceridemia (p<0.001) and high CRP (p=0.008) were more prevalent with IR among female participants only.

Table 9: Association between HOMA_IR and socio-demographic, lifestyle, dietary, anthropometric and biochemical factors stratified by gender

	Men					**Women**				
	HOMA_IR[1] (<2.5)		**HOMA_IR (≥2.5)**			**HOMA_IR[1] (<2.5)**		**HOMA_IR (≥2.5)**		
Characteristics	**n or mean**	**% or SD**	**n or mean**	**% or SD**	**P-value**	**n or mean**	**% or SD**	**n or mean**	**% or SD**	**P-value**
Age (years)	43.9	12.8	43.4	10.5	0.807	39.0	9.0	39.0	11.1	0.978
Marital status										
Single/Separated/Divorced	24[a]	33.8	21[a]	33.3	0.954	32[a]	30.8	28[a]	58.3	0.001
Married	47[a]	66.2	42[a]	66.7		72[b]	69.2	20[b]	41.7	
Income ($)										
<2250	20[a]	28.2	24[a]	38.1	0.095	25[a]	24.0	16[a]	33.3	0.467
2250-4000	13[a]	18.3	17[a]	27.0		37[a]	35.6	14[a]	29.2	
>4000	38[a]	53.5	22[a]	34.9		42[a]	40.4	18[a]	37.5	
Physical activity level										
Low	39[a]	54.9	43[a]	68.3	0.114	67[a]	64.4	34[a]	70.8	0.437
Moderate/High	32[a]	45.1	20[a]	31.7		37[a]	35.6	14[a]	29.2	
Calories (Cal)	2203.9	1109.5	2123.1	720.6	0.623	1739.3	620.1	1756.9	599.1	0.870
Vitamin D intake (µg)	2.7	5.3	2.7	3.2	0.952	2.1	2.1	2.0	2.0	0.823
Total dairy product intake (serving/day)	2.1	1.1	2.3	0.9	0.344	2.2	0.9	2.1	1.1	0.534
BMI[2]										
Underweight/Normal	21[a]	29.6	4[a]	6.3	<0.001	71[a]	68.3	18[a]	37.5	<0.001
Overweight	37[b]	52.1	32[b]	50.8		25[a]	24.0	13[a]	27.1	
Obese	13[b]	18.3	27[b]	42.9		8[b]	7.7	17[b]	35.4	
Body fat percentage	25.0	6.5	29.4	6.2	<0.001	31.4	6.6	38.7	7.5	<0.001
Waist circumference										

risky[3]										
No	53[a]	74.6	21[a]	33.3	<0.001	67[a]	64.4	11[a]	22.9	<0.001
Yes	18[b]	25.4	42[b]	66.7		37[b]	35.6	37[b]	77.1	
Medical morbidity										
No	46[a]	64.8	37[a]	58.7	0.471	75[a]	72.1	29[a]	60.4	0.149
Yes	25[a]	35.2	26[a]	41.3		29[a]	27.9	19[a]	39.6	
Glucose (mmol/L)	5.0	0.5	5.6	1.6	0.003	4.7	0.4	5.1	0.6	<0.001
Hypertriglyceridemia										
Normal TG[4] **levels**	44[a]	62.0	31[a]	49.2	0.137	99[a]	95.2	30[a]	62.5	<0.001
Hypertriglyceridemia	27[a]	38.0	32[a]	50.8		5[b]	4.8	18[b]	37.5	
Vitamin D concentration (ng/mL)	29.7	15.2	25.2	13.7	0.073	28.4	12.7	27.0	13.7	0.519
CRP[6]										
Low/Moderate	26[a]	36.6	20[a]	31.7	0.553	61[a]	58.7	17[a]	35.4	0.008
High	45[a]	63.4	43[a]	68.3		43[b]	41.3	31[b]	64.6	

[1]Homeostatic Model Assessment of Insulin Resistance
[2]Body Mass Index: underweight <18.5 kg/m^2, normal 18.5-24.9 kg/m^2,overweight 25-29.9 kg/m^2, obese ≥ 30 kg/m^2
[3]Risky waist circumference: >102 cm in men, <88 cm in women
[4]Triglycerides-Normal levels: <150mg/dL
[5]NAM: 25 hydroxyvitamin D status as defined by the National Academy of Medicine (adequate≥20 ng/mL)
[6]C-reactive protein: low/moderate levels <3mg/L
Columns with superscripts without a common symbol differ, P<0.05

Among the study participants, 33.6% had serum 25 hydroxyvitamin D < 20ng/mL while 63.3% had serum 25 hydroxyvitamin D < 30ng/mL. Using logistic regression, to assess the relationship between healthy/unhealthy vitamin D status and IR, a significant inverse association was observed with serum 25 hydroxyvitamin D < 20ng/mL (p=0.028) in the total sample; however, this association was not significant when cutoff of 30 ng/mL was used. Further analyses were performed to explore

this relationship by gender. The results showed a significant inverse association among females (p=0.031) but not males at 25 hydroxyvitamin D < 20ng/mL (figure 4).

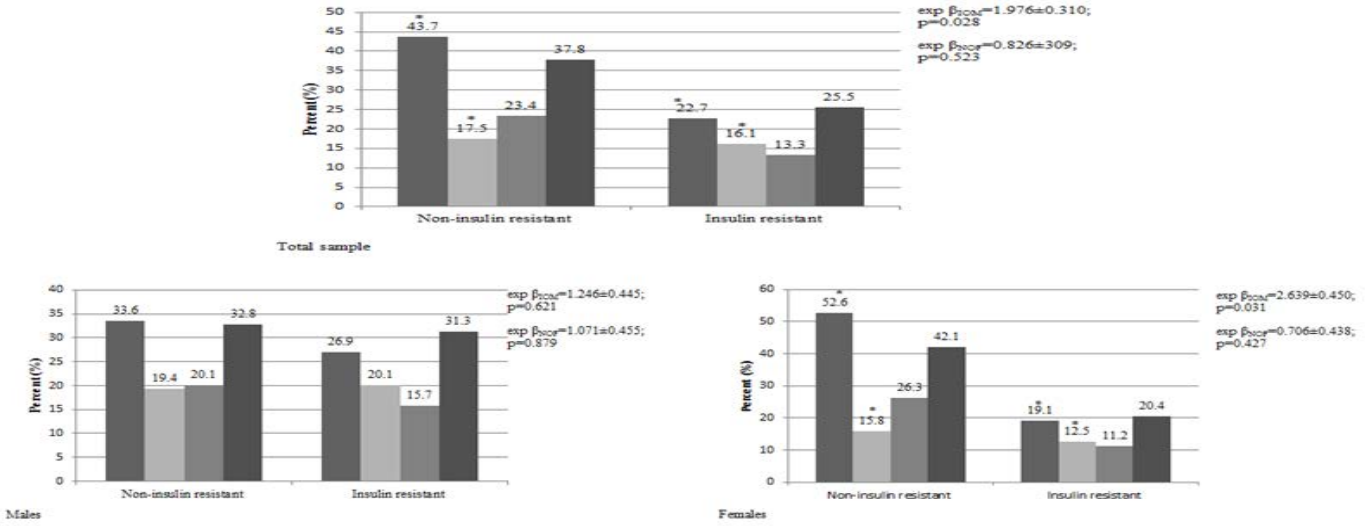

Figure 4: Association between HOMA_IR and vitamin D level in the total sample, males and females.

Notes: NAM: The National Academy of Medicine defined adequate vitamin D status as 25(OH)D ≥20 ng/mL; NOF: The National Osteoporosis Foundation defined sufficient vitamin D status as 25(OH)D ≥30 ng/mL; ■: 25(OH)D ≥ 20 ng/mL, ■: 25(OH)D < 20 ng/mL, ■: 25(OH)D ≥ 30 ng/mL, ■: 25(OH)D < 30 ng/mL.; *: significant association

Using multiple logistic regression, the association between vitamin D status, defined by 25(OH)D ≥20 ng/mL and 25(OH)D ≥ 30 ng/mL (data not shown) and HOMA_IR was no longer significant for both genders after adjusting for confounding factors. However, there was a significant independent association between HOMA_IR and waist circumference among males (p=0.008); and between HOMA_IR and marital status (p=0.006), hypertriglyceridemia (p=0.010) and body fat percentage (p=0.045) among females.

Table 10: Multiple logistic regression for HOMA_IR * and vitamin D status controlling for confounding factors among male participants

Characteristics	**Odds Ratio (OR)**	**95% CI**		**P value**
		Lower	**Upper**	
Waist circumference	4.139	1.455	11.775	0.008
Marital status	1.169	0.485	2.818	0.728
Annual income	0.855	0.539	1.358	0.507
Activity level	0.626	0.272	1.441	0.270
Hypertriglyceridemia	0.998	0.417	2.384	0.996
CRP[1]	0.633	0.260	1.542	0.314
Body fat percentage	1.024	0.936	1.121	0.607
25(OH)D ≥ 20 ng/mL[2]	1.148	0.496	2.657	0.747
Medical morbidity	1.042	0.449	2.422	0.923
BMI[3]	1.380	0.576	3.303	0.470

[1]C-reactive protein, [2]Adequate 25 hydroxyvitamin D status as defined by the National Academy of Medicine, 3 Body mass index
***Insulin resistance defined as HOMA_IR ≥ 2.5**
R^2=27.8%

Table 11: Multiple logistic regression for HOMA_IR * and vitamin D status controlling for confounding factors among female participants

Characteristics	Odds Ratio (OR)	95% CI		P value
		Lower	Upper	
Waist circumference	2.729	0.869	8.573	0.086
Marital status	0.289	0.118	0.705	0.006
Annual income	1.127	0.637	1.994	0.681
Activity level	0.674	0.262	1.737	0.415
Hypertriglyceridemia	6.585	1.561	27.775	0.010
CRP[1]	1.399	0.525	3.726	0.502
Body fat percentage	1.106	1.002	1.221	0.045
25(OH)D ≥ 20 ng/mL	0.763	0.298	1.954	0.572
Medical morbidity	1.051	0.384	2.879	0.923
BMI[3]	0.735	0.298	1.816	0.505

[1]C-reactive protein, [2]Adequate 25 hydroxyvitamin D status as defined by the National Academy of Medicine, 3 Body mass index
***Insulin resistance defined as HOMA_IR ≥ 2.5**
R^2=43.9%

4. Discussion

The current study showed that low vitamin D status was associated with IR in a sample of non-diabetic Lebanese adults. Yet, after stratifying based on gender this association was only observed among females but not males. When controlling for confounders using multiple logistic regression, this association was no longer significant in both genders. In males, the only factor associated with IR was elevated waist circumference, while in females; being single/separated/divorced, having high triglycerides level and body fat percentage were all associated with IR.

IR has become a major public health concern since it plays a fundamental role in the pathophysiology of many chronic diseases including Type II diabetes, cardiovascular diseases and metabolic abnormalities (Freeman & Pennings, 2018; Frozza, Lourenco, &

Felice, 2018). The prevalence of IR in our sample was 38.8%, which is concordant with the findings of Naja et al. (2012) in which the prevalence of IR was reported to be 44.6% among a national sample of 308 randomly selected adults in Lebanon with a mean age of 41.0 ±15.5 years. In line with our findings, other studies, that also used the HOMA-IR technique, reported similar prevalence rates of IR of 39.1% in Cameron County, Texas, USA among 1854 Hispanic adults >18 years of age (Qu et al., 2011) and of 46.5% among 2026 Venezuelan adults with a mean age of 39.7 ± 15.4 years in Maracaibo city (Bermudez & Salazar, 2016). In contrast, the lowest prevalence of IR of 15.5% was reported among 3354 Danish adults aged between 19-72 years (Friedrich et al., 2012). The variability in the prevalence of IR could be due to the different cutoff values used for HOMA_IR or to the difference in the characteristics of the study population including race, age, gender and obesity rates.

The prevalence of IR in the current study was higher in men (47.0%) compared to women (31.6%). This finding is concordant with the literature (Friedrich et al., 2012; Do, Lohsoonthorn, Jiamjarasrangsi, Lertmaharit & Williams, 2010); men are at higher risk of IR compared to women due to the higher amount of visceral and hepatic fat, in addition to the lack of the potential protective effect of estrogen (Geer & Shen, 2009).

In our study, bivariate analyses showed that IR was positively associated with BMI categories, body fat percentage and risky waist circumference in both genders. Those results are in line with the literature (Chung, Cho, Chung & Chung, 2012; Koike, Miyamoto & Oshida, 2009; Zadeh-Vakili, Tehrani & Hosseinpanah, 2011; Patel & Abate, 2013). Obesity is an established risk factor for IR, particularly abdominal obesity. The accumulation of visceral fat in obese individuals and the accumulation of fat in the

liver impairs insulin signaling and contributes to IR (Hardy, Czech & Corvera , 2012). However, when multivariate analyses were conducted, elevated waist circumference only remained significantly associated with IR in males. It is likely that waist circumference was associated with IR in men but not in women since male sex hormones favor the deposition of excess body fat around the abdominal area (Shi & Clegg, 2009). While in women, body fat percentage was associated with IR. It is well-established that female sex hormones favor fat deposition around the hips and thighs, accordingly it is likely that abdominal deposition of fat will not occur in females unless with excessive total body fatness (Palmer & Clegg, 2015).

Furthermore, insulin resistant men and women tended to have higher glucose levels than non-insulin resistant individuals. This increase in glucose level can be explained by the fact that IR is a state where there is an increase in insulin secretion in the pancreas due to the fact that target cells like the muscles and adipose tissue no longer respond to normal amounts of insulin secretions (Salaroli et al., 2017). Therefore, insulin is unable to adequately take up glucose from the blood into the cells (Tam et al., 2012); and thus leading to an increase in glucose blood level.

In females only, IR was associated with hypertriglyceridemia, CRP and marital status using bivariate analyses, but only hypertriglyceridemia and marital status remained significant in multivariate analyses. The positive association between IR, CRP and hypertriglyceridemia was reported by multiple studies, but in both genders (Moro et al., 2003; Gelaye et al., 2010). However, another study by Lichnovská, Gwozdziewiczová, & Hrebícek (2002) found a similar positive association between hypertriglyceridemia and IR among females only in a sample of 70 non-diabetic elderly individuals with an age

range of 43.9 to 71.3 years. The reason behind this gender difference is still unclear and further research is needed to explore this relationship. Yet, these differences could be explained by the potential effect of sex hormones on the inflammatory status of the individual (Alemzadeh & Kichler, 2014). As for the association between marital status and HOMA_IR, the results of this study were discordant to Bermudez et al. (2016), where they reported a significantly higher prevalence of IR among married compared to unmarried participants. The reason behind the higher prevalence of IR among single/separated/divorced women compared to married women in our study is still unclear since no differences were detected between the two groups on the level of their BMI, waist circumference, body fat percentage, caloric intake, fat intake, physical activity level and lipid panel.

Our bivariate findings suggested that low vitamin D status was inversely associated with IR in the total sample and in females only. These results are in line with another study conducted in Lebanon by Gannage et al. (2009) but on a younger population of 381 healthy university students with a mean age of 23.9 ± 3.9 years where vitamin D levels and HOMA_IR were inversely associated in the total sample and among women using spearman coefficient of correlation. In a study conducted by Pinelli et al. (2010) among a sample of 542 Arab American aged between 20 and 75 years, the results of spearman correlation coefficients showed an inverse association between vitamin D concentrations and HOMA_IR but only among males; no data was available on the total sample since all the data analyses was stratified by gender. In contrast, in a study conducted by Giorelli et al. (2014) among a sample of 53 non-diabetic participants with a mean age of 65.3 ± 10.3 years and meeting one or more of the following criteria “hypertension; body mass index

(BMI) ≥ 25 kg/m^2; waist circumference > 80 cm for women and > 94 cm for men; first-degree relatives with diabetes; women with large-for-gestational-age newborns or with gestational diabetes mellitus; fasting serum HDL-cholesterol < 35 mg/dl; and triglycerides > 250 mg/dl", the results of spearman correlation showed no association between vitamin D concentration and HOMA_IR in both genders. It is likely that this relationship could be altered in case of a metabolic abnormality, old age and racial differences. Regarding the findings of our study, the lack of significance of the association between serum 25(OH)D and HOMA_IR among men could be explained by ethnical differences, since the study that was performed in Lebanon yielded similar results as ours but on a different age group. Accordingly, future studies should further assess this association in Middle Eastern ethnicities and larger samples could be needed to confirm our findings.

When multiple regression was used to control for multiple confounding factors including BMI, the results showed that the association between vitamin D status and IR was no longer significant for both genders. These results are in concordance with a study conducted in Europe by Gulseth et al. (2010) among a sample of 446 older adults aged between 35 and 70 years with metabolic syndrome. In this study, the unadjusted analyses also revealed an inverse association between serum 25(OH)D and IR in the total sample, but when multiple linear regression was performed to adjust for the BMI and other confounding factors, the association was no longer significant. In contrast to the findings of our study, a significant inverse association between serum 25(OH)D concentration and IR was reported by Badawi et al. (2014) in a sample of 1928 Canadian adults with an age range of 16 to 79 years and mean BMI of 26.8 ± 0.3 kg/m^2. The individuals in the

previously mentioned sample were slightly overweight, while in the sample of the current study only 37.4% of the participants were overweight. The results of Badawi et al. were adjusted for multiple confounding factors (linear regression) including waist circumference; however they did not adjust for the BMI. Furthermore, another study, conducted in Japan by Pham et al. (2012) on a sample of 494 participants aged between 20 and 68 years yielded similar results as Badawi et al. but while adjusting for the BMI. However, in this study, the majority of the participants had a normal BMI and only 17% were overweight and 2.6% were obese; if more obese and overweight individuals were present in the sample, the results would have differed. Conversely, in a cross-sectional study conducted among 1205 participants from Jerusalem with a mean age of 32.0 ± 1.3 years, the results of multiple linear regression, adjusting for BMI, showed that serum level of 25(OH)D was inversely associated with IR among males but not females (Moore et al., 2014). The differences in the results, in the case of Moore et al., could be attributed to the fact that IR is higher among males than females (Geer & Shen, 2009). Moreover, middle-aged men could be at a higher risk of developing type II diabetes compared to middle-aged women due to different deposition of body fat (Choi et al., 2009; Lipscombe & Hux, 2007; Moneta, 2011), therefore if low vitamin D levels were to have an effect on IR and the development of type II diabetes, this association would be more prominent in middle-aged men than women (Moore et al., 2014). Furthermore, the differences in the findings could be due to the different racial backgrounds of the participants. Given the controversial results in the literature, further research and clinical trials are needed to eliminate ambiguity.

The present study has some limitations that need to be acknowledged. This study is cross-sectional in nature; therefore causal relationships cannot be inferred. Moreover, convenience sampling was used so the results cannot be generalized to the entire population. Additionally, the methods used to assess vitamin D levels (ELISA) and IR (HOMA_IR) are not the gold standard techniques, however those are the most commonly used techniques in research. The strengths of our study include direct measurement of the variables, adjustment for major potential confounding factors and as to our knowledge this is the first study to assess the association between vitamin D and IR in a middle-aged Lebanese sample.

5. Conclusion

Considering the controversial findings in the literature as well as the high prevalence of both, IR and vitamin D deficiency, further research is still needed to examine the association between these two conditions. Large-sized randomized-controlled trials are needed to draw definitive conclusions on the effect of vitamin D status on IR specifically in the Middle East, where the literature particularly aimed at assessing this topic is very limited. Moreover, these interventional studies must assess the effect of BMI as well as gender on this association. Since a different set of factors was associated with IR in every gender, accordingly future public health prevention efforts should be gender specific. A significant independent association was detected between HOMA_IR and waist circumference among males and between HOMA_IR and marital status, hypertriglyceridemia and body fat percentage among females; therefore, screening for IR should be targeted especially towards men with risky waist circumference and

single/separated/divorced women with high body fat percentage and hypertriglyceridemia.

APPENDIX A

Table 12: Summary of observational studies on vitamin D deficiency and insulin resistance

Reference (first author's last name), year of publication	**Sample characteristics (size, age range, gender distribution, nationality, other)**	**Study design & date of data collection**	**Exposure (independent variables) assessed & tool(s) of assessment**	**Outcomes (DV[s]) assessed & tool(s) of assessment**	**Main study findings**	**Limitations of the study**
Huang et al. (2013)	- n= 2708 (1326 males, 1382 females) - China - Mean age 48.5 ± 12.6 years - Exclusion criteria: altered vitamin D metabolism, alcohol or drug abuse, liver disease, consumption of lipid lowering medication, lack of a signed informed consent	- Cross-sectional - Between August and October 2008	- Serum 25(OH)D level by ACQUITY Ultra Performance Liquid Chromatography - Age, weight, height, smoking, alcohol consumption and sex via a general structured questionnaire - BMI calculated as weight in kilograms divided by the height in meters squared - Physical activity level using specified formulas	Blood samples were collected after 10h fasting. - FPG; PG (glucose oxidase method) - HbAlc, - Insulin (Centaur, Bayer Corporation, Bayer Leverkusen, Germany) - TC; TG; HDL; LDL; apoA; apoB; FFAs (AUTOLAB PM 4000, AMS Corporation, Rome, Italy) - LPL (enzyme-linked immunosorbent assay "ELISA") - HOMA-IR (Fasting glucose	- Positive association between serum 25(OH)D concentration and LPL. - Inverse association between LPL and IR as well as T2D. - Inverse association between serum 25(OH)D and IR as well as T2D.	- Cross-sectional in nature: causal relationships cannot be examined. - LPL activity was not measured.

				(mmol/L) × Fasting insulin (mU/L)/22.5)		
Ock et al. (2016)	- n= 1807 (628 males, 1179 females) - Korea - Age 30-64 years - Exclusion criteria: diagnosed with cancer in the last 2 years, currently under treatment or with a history of stroke, myocardial infarction or heart failure	- Cross-sectional - This study was part of the "Cardiovascular and Metabolic Disease Etiologic Research Center (CMERC) study The CMERC population is a multicenter prospective cohort, which consists of two community-based cohorts and one hospital-based cohort of people at high risk for cardiovascular diseases. In addition, participants were divided into normal and obese according to body mass index (BMI) and waist circumference". - In 2013-2014: May to October, November to April	- Serum 25(OH)D level (chemiluminescence immunoassay CLIA method: Liaison; DiaSorin, Dietzenbach, Germany). - Age, smoking, alcohol consumption, season of sampling and gender via a standardized questionnaire in a face-to-face interview - Weight and height: measurement by standardized techniques and equipment with "participants wearing light indoor clothing without shoes (cohort 1, DS-102 Jenix, Seoul, Korea for height; BB-150 CAS, Seongnam, Korea for weight; cohort 2, BSM 330, Biospace, Seoul, Korea)". - Blood pressure: measured "three times in the right arm using an electronic manometer (HEM-7080IC; Omron Healthcare Co. Ltd., Kyoto, Japan) after 5 minutes of rest in the sitting position, and the average of the final two measurements was used for the analysis".	Blood samples were collected after 8h of fasting: laboratory tests performed in (Seoul Clinical Laboratories, Seoul, Korea). - FPG (colorimetric method using an autoanalyzer ADVIA 1800 Auto Analyzer; Siemens Medical Solutions, Malvern, PA, USA). - The insulin serum level (radioimmunoassay SR 300; STRATEC, Birkenfeld, Germany) - Insulin resistance [HOMA-IR: fasting insulin (μIU/mL)×fasting glucose (mg/dL)/405].	- Inverse association between serum 25(OH)D and IR after adjusting for age, gender, waist circumference, alcohol consumption, smoking status, physical exercise, season, and cohort. - After dividing the subjects into normal and obese, this inverse association was observed for non-obese participants only. - Inverse association not only among individuals with vitamin D deficiency (<20 ng/mL) but also among participants with vitamin D insufficiency (20 to 30ng/mL).	- Blood samples not collected during the same season; results adjusted for season for more precision. - No available data on "Sunscreen use, sun exposure time, diet pattern, and calcium or vitamin D supplement use". - Cross-sectional in nature: causal relationships cannot be examined. - Some unmeasured confounding factors (calcium and parathyroid hormone levels) may have affected the results.

			- BMI: calculated as weight in kilograms divided by the height in meters squared - Waist circumference: measured "over the midpoint between the lower border of the ribs and the iliac crest in a standing position using a plastic tapeline". - Physical activity level using "the International Physical Activity Questionnaire" (IPAQ)			
Pham et al. (2012)	- n=494 (284 males, 210 females) municipal employees - Japan - Age 20-68 years - Exclusion criteria: long-term sick leave or maternity leave, history of diabetes, cancer, cardiovascular disease or under treatment for chronic hepatitis, without fasting status, missing data on serum insulin.	- Cross-sectional - July and November 2009	- Serum 25(OH)D by " a competitive protein binding assay" -Dietary and calcium intake: diet history questionnaire - Marital status, smoking and alcohol consumption, job position and occupational and non-occupational physical activities: questionnaire	- Serum insulin by chemiluminescence immunoassay - PG measured "enzymatically using Glucose CII-test Wako (Wako Pure Chemical Industries, Osaka, Japan), and the measuring device was an OLYMPUS AU640 (Olympus Corp., Tokyo, Japan)". -HOMA-IR: (fasting insulin (mU/ml) fasting glucose (mmol/l))/22.5	-Significant inverse association between serum 25(OH)D concentration and fasting insulin as well as HOMA-IR. -HOMA-IR was highest among subjects exhibiting both the lowest 25(OH)D concentrations as well as low calcium intake	- Cross-sectional in nature: causal relationships cannot be examined - Serum 25(OH)D deficiency might be an outcome rather than a predictor of insulin resistance -Insulin resistance was not assessed by the gold standard, the hyperinsulinemic euglycemic clamp - Bias caused by residual confounding is a possibility - Non-occupational physical activity was adjusted for as a dichotomous covariate - Use of a non-validated questionnaire for the assessment of non-

						occupational physical activity -Lack of info on the parathyroid hormone hinders our understanding of the possible effects of this hormone on the association between serum 25(OH)D and IR markers - The results might not be generalizable to other populations
Zhao et al. (2009)	- n= 3206 (1582 males and 1624 females) - United States - Age ≥20 years - Inclusion criteria: without diabetes or without physician-diagnosed diabetes	-Cross-sectional -Data from the "National Health and Nutrition Examination Survey" (NHANES) 2003–2006	-Age, gender, ethnicity, , smoking, alcohol consumption, physical activity, abdominal obesity, education, BMI and serum calcium concentrations - Serum 25(OH)D concentrations: using a radioimmunoassay procedure -Serum parathyroid hormone (PTH) concentrations: by the Elecsys 1010 analyzer using an electrochemiluminescent procedure	Blood samples were collected after 8h of fasting - Fasting and 2h glucose -Fasting insulin -Glycosylated hemoglobin -HOMA-IR: (fasting plasma insulin [mU/l] fasting plasma glucose [mmol/l])/22.5)	- Inverse association between serum 25(OH)D concentration and insulin resistance - Positive association of insulin resistance with PTH	- Cross-sectional in nature: causal relationships cannot be examined -No available data on sunlight exposure
Badawi et al. (2014)	- n= 1928 (927 males and 1001 females) - Canada - Age 16-79 years Exclusion criteria: residents of the "Crown lands, Aboriginal reserves, remote	- Cross-sectional - Data were used from the Canadian Health Measures Survey cycle 3.1 - March 2007–February 2009	-Age, gender, ethnicity -BMI calculated as weight in kilograms divided by the height in meters squared - Waist circumference "measured by using a measuring tape at the midpoint between the last floating rib and the top of the iliac crest in the midaxillary line"	-"Fasting insulin, glucose, C-reactive protein (CRP), total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL-C), as well as	- Inverse association between serum 25(OH)D concentration and insulin resistance in both men and women.	- Cross-sectional in nature: causal relationships cannot be examined

	regions, health institutions, and full-time members of the Canadian Forces",		- Systolic and diastolic blood pressures "measured by using BP-TRU automated oscillometric devices (BP-TRU Medical Devices, Ltd, Coquitlam, BC, Canada)" - Daily energy expenditure "calculated from self-reported leisure time physical activities during the past 3 months" - Serum 25(OH)D "measured through chemiluminescence assay by using the Liaison 25-hydroxyvitamin D Total assay (DiaSorin Inc., Stillwater, MN, USA)"	apolipoprotein A1 (ApoA1) and ApoB" -HOMA-IR: fasting plasma insulin [mU/l] fasting plasma glucose [mmol/l])/22.5		
Gannage et al. (2009)	- n= 381 (201 males and 180 females) - Lebanon - Age 23.9 ± 3.9 years Exclusion criteria: use of contraceptive pills or medication that may alter the lipid profile and, pregnancy	-Cross-sectional	-Age, gender, smoking, alcohol consumption, physical activity level -Height and weight measured using a manual scale -Waist circumference measured at the umbilicus -Systolic and diastolic blood pressure were "measured in seated subjects after a rest for at least 15 min using a mercury tensiometer" -BMI calculated as weight in Kg divided by height in meters squared -Serum 25OH)D was measured using the Dia Sorin RIA which is a chemiluminescent immunoassay (CLIA)	Blood samples collected after 12h fasting -Adiponectin measured using "a commercially available RIA kit (Linco Research, Inc., St Charles, MO, USA)". -Fasting insulin measured using "a commercial chemiluminescent assay (Immulite, DPC, Los Angeles, CA, USA)". -Glucose, TC, TG, and HDL were measured using an "automated COBAS Integra 400, Roche Diagnostics".	In the overall population: -Inverse association between serum 25(OH)D and BMI, SBP, WC, FPG, HOMA-IR and insulin levels. -Positive association between serum 25(OH)D and adiponectin and HDL. -25(OH)D is an independent predictor of SBP and FPG after adjusting for BMI and sex. In males: - Inverse association between serum 25(OH)D and WC, SBP and BMI; positive association with adiponectin. -Inverse association between serum 25(OH)D and LDL. -25(OH)D is an independent predictor of LDL and SBP after adjusting for BMI. In Females:	-Study performed among young well educated Lebanese students, thus with relative adequate vitamin D level -Small sample size -Cross-sectional in nature: causal relationships cannot be examined

				-LDL was calculated using the Friedwald equation. -HOMA-IR: fasting plasma insulin [mU/l] fasting plasma glucose [mmol/l])/22.5	-Inverse association between serum 25(OH)D and FPG as well as HOMA-IR.	
Gulseth et al. (2010)	-n= 446 -Europe-Caucasians -Age: 35-70 years -Inclusion criteria: Pan-European with the metabolic syndrome and a BMI between 20 and 40 kg/m^2	-Cross-sectional -Study among participants recruited in the LIPGENE study (NCT00429195) conducted in 8 European countries from 2005 to 2006	-Age, gender, physical activity level, smoking, alcohol consumption: using a questionnaire -Serum 25(OH)D through "high-performance liquid chromatography/mass Spectrometry"	-HOMA-IR and HOMA-β assessed using "HOMA indexes (HOMA2, version 2.2.2 http://www.dtu.ox.ac.uk/index.php?maindoc/homa) from fasting blood samples". -" Measures of insulin sensitivity (Si) were obtained using the MINMOD Millennium Program (version 6.02, Richard N. Bergman". -" The acute insulin response to glucose (AIR) was defined as the incremental area under the curve from 0 to 8 min. Disposition index was	-Inverse association between serum 25(OH)D and HOMA-IR, HOMA-β, fasting insulin, BMI and acute insulin response to glucose (unadjusted analyses). -No association between serum 25(OH)D and insulin action or secretion parameters after adjusting for BMI, age, geographic location and sex.	-Association assessed in only one ethnic group. -Few participants had severe vitamin D deficiency (<25 nmol/l or 10ng/ml) -Metabolic syndrome was an inclusion factor - Cross-sectional in nature: causal relationships cannot be examined

				calculated as AIRxSi".		
Giorelli, Matos, Saado, Soibelman & Dias (2014)	-n=53 (22 males and 31 females) -Brazil -age= 65.3 ± 10.3 years -Inclusion criteria: Non-diabetic, have at least one of the following conditions: "hypertension; body mass index (BMI) ≥ 25 kg/m2 ; waist circumference > 80 cm for women and > 94 cm for men; first-degree relatives with diabetes; women with large-for-gestational-age newborns or with gestational diabetes mellitus; fasting serum HDL-cholesterol < 35 mg/dl; and triglycerides > 250 mg/dl".	-Cross-sectional - December 2009 to July 2010	- Weight and height were measured with light clothes on -Waist circumference was measured "at the midpoint between the lower costal border of the last rib and the upper border of the iliac crest" -Hip circumference was measured "at the level of the greater trochanter" -Age, gender -BMI (kg/m^2) -Serum 25(OH)D_3 using a radioimmunoassay kit (DiaSorin, Stillwater, MN, USA)	Blood samples collected after 12h fasting and 5 minutes seated resting in a room without sun exposure -FPG assessed using an enzymatic method -Fasting inulin " by means of an immunometric method in a two-sided solid-phase chemiluminescent assay (Immulite 2000, Siemens TM, Los Angeles, USA)" - HOMA-IR: (glucose x insulin)/22.5 - Serum uric acid, creatinine, TG and TC - Microalbuminuria with the chemiluminescence method (24h urine samples) -GFR using the modification of diet in renal	-No association between serum 25(OH)D levels and HOMA-IR or other variables	- Cross-sectional in nature: causal relationships cannot be examined

				disease (MDRD) formula		
Moore et al. (2014)	-n=1205 (609 males and 595 females) -Jerusalem -age= 32 ±1.3 years -Exclusion criteria: offsprings with congenital disorders at birth	-Cross-sectional - The Jerusalem Perinatal Study was conducted between 1974 and 1976 among Jerusalem resident women who gave birth in this period and they were interviewed within two days postpartum. - Between 2007 and 2009 the Jerusalem Perinatal Follow-Up Study (JPS-1) was conducted to determine maternal and offspring genetic risk factors responsible for the correlation between maternal obesity and adulthood cardiometabolic risk factors in the offsprings.	- Between 2007 and 2009, interviews were conducted to collect socio-demographic and lifestyle data as well as anthropomorphic measurements -BMI (kg/m^2) -BP " measured as the average of three consecutive measurements performed after five minutes of sitting (Omron M7 automated sphygmomanometer)" -Serum 25(OH)D measured using "liquid chromatography tandem mass spectroscopy"	Blood samples were collected after 8h of fasting -Fasting plasma glucose, HDL and TG " measured on the VITROS 5,1FS Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ)" - Plasma insulin levels determined using "radioimmunoassay with the Human Insulin-Specific RIA Kit (Millipore, Billerica, MA)" -HOMA-IR	-Inverse association between serum 25(OH)D and HOMA-IR among males but not females.	-Cross-sectional in nature: causal relationships cannot be examined - The results might not be generalizable to other populations
Pinelli, Jaber, Brown & Herman (2010)	-n=531 (214 males, 317 females) -Arab-Americans -Age 20-75 years	-Cross-sectional -In 2009	-Age, gender -BMI - Ratio of Arab meals to total meals consumed in 1 week -Level of physical activity -Smoking -WC - Serum 25(OH)D was measured	- Fasting glucose, insulin, TG, HDL and A1C were measured -HOMA-IR	-75% of the study population was insufficient in vitamin D (5-20 ng/ml) -24% of the study population had vitamin D levels between 20 to 40 ng/ml (5-20 ng/ml)	-Factors affecting sun exposure were not assessed -Cross-sectional in nature: causal relationships cannot be examined

			using “the I Radioimmunoassay kit (DiaSorin, Stillwater, MN)”		-Serum 25(OH)D level was lower for glucose intolerant participants than normoglycemic participants; this difference was not present for women. - Inverse association between serum 25(OH)D and HOMA-IR, TG, FPG and A1C in men. -Positive association between serum 25(OH)D and HDL in women.	
Tao et. al. (2013)	-n=1382 females -Shanghai -Age= 20-85 years -Inclusion Criteria: without T2DM, normal blood count and normal liver and kidney function, did not take vitamin D and/or calcium supplements in the last 3 months	-Cross-sectional -February to March 2009	-Age, gender, smoking, alcohol consumption: through a questionnaire -Serum 25(OH)D determined using an “ECLIA Elecsys autoanalyzer (Roche Diagnostic GmbH, Mannheim, Germany)”	-Fasting blood samples: serum levels of calcium, glucose, albumin, insulin and phosphate -PTH level determined using an “ECLIA Elecsys autoanalyzer (Roche Diagnostic GmbH, Mannheim, Germany)” - HOMA-IR [fasting plasma insulin (mU/L)×fasting glucose (mmol/L)÷22.5] -HOMA-B [20×fasting plasma insulin (mU/L)÷(fasting glucose (mmol/L)–3.5)]	- Serum 25(OH)D level is independently and significantly inversely associated with insulin resistance (HOMA-IR) and β-cell function (HOMA-B) in a healthy Chinese female population	-Outdoor physical activity influencing both insulin sensitivity and vitamin D status might be responsible for the observed association. - Cross-sectional in nature: causal relationships cannot be examined. - Insulin resistance was not assessed by the gold standard, the hyperinsulinemic euglycemic clamp.

Kayaniyil et al. (2011)	-n=489 - Toronto and London, Ontario, Canada. -Age= 50 ± 10 years -Inclusion criteria: People at high risk for T2DM selected based on the presence of at least one of the following risk factors for diabetes: "obesity, hypertension, family history of diabetes, and/or a history of gestational diabetes or birth of a macrosomic infant"	- PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort study - Between May 2004 and December 2006 -The participants were followed for 3 years	-Serum 25(OH)D measured using "the DiaSorin 25 OH Vitamin D TOTAL competitive chemiluminescent immunoassay on the automated LIAISON Analyzer (Stillwater, MN)". -Gender, Age, Smoking, ethnicity, family history of diabetes determined through structured questionnaires. -Weight and height with participants in light clothing and without shoes -BMI (kg/m2) -WC measured as "minimal circumference between the umbilicus and xiphoid process" -Physical activity level assessed with "a version of the Modifiable Activity Questionnaire (MAQ)". -PTH measured using "an electrochemiluminescence immunoassay on the Roche Modular E170 Analyzer (Laval, Quebec, Canada)". - C-reactive protein measured in fasting samples using "Roche Modular's particle-enhanced immunoturbidimetric assay." -Blood pressure measured "twice in the right arm with the subject seated after a 5-min rest using an automated sphygmomanometer". -Vitamin D supplement use assessed with an open-ended question.	Fasting blood samples were collected. -FPG measured using "an enzymatic hexokinase method on the Roche Modular platform". -Serum insulin measured using "the Elecsys 1010 immunoassay analyzer (Roche Diagnostics, Basel, Switzerland) and the electrochemiluminescence immunoassay". -HOMA-IR: FPG x FPI/22.5 -Insulin sensitivity index of Matsuda and DeFronzo: IS_{OGTT}: $10{,}000/\sqrt{}$ (FPG x FPI) x (G x I) "where FPG refers to fasting plasma glucose, FPI refers to fasting plasma insulin, G refers to mean glucose during the OGTT, and I refers to mean insulin during the OGTT". - β-cell function: • Insulinogenic	- Significant association between baseline 25(OH)D concentration and HOMA-IR, but this relationship was diminished to non-significance after adjusting for the BMI. - Independent significant positive association between baseline 25(OH)D concentration and β-cell function.	-Serum 25(OH)D level was only collected at the beginning of the study, follow-up level of vitamin D would have been important in order to assess the effect of changes in vitamin D on the outcome variables. -No data on diet consumed. - Gold-standard measures of b-cell function and IR were not used. - Glucose data at 60 and 90 min of the OGTT was not available; could have increased the accuracy of the $AUC_{glucose}$ calculation. - Potential bias in the results since tte participants who remained for the follow-up clinic visits were mostly female, white and older than the participants who left the study. -Observational study: possible presence of confounding factors that may affect the correlation between serum 25(OH)D with the outcomes.

				index (IGI) divided by HOMA-IR IGI/IR • Insulin secretion sensitivity index-2 (ISSI-2): defined as the "ratio of the area under the insulin curve ($AUC_{insulin}$) to the area under the glucose curve ($AUC_{glucose}$), multiplied by IS_{OGTT}"		
Gagnon et al. (2011)	-n=5200(2340 males, 2860 females) -Australia -Age= average 51 years -Inclusion criteria: free of type 2 diabetes mellitus	-Cohort study - The data of this study originated from the Australian Diabetes, Obesity and Lifestyle (AusDiab) study (1999-2000). After 5 years (2004-2005), 5200 participants free of T2DM and with complete data returned to repeat the OGTT. - From April to September and October to March (2004-2005)	- Serum 25(OH)D measured using "the Liaison 25(OH)D TOTAL (DiaSorin Inc., Stillwater, MN), a direct competitive chemiluminescent immunoassay". - Total energy, dietary magnesium and calcium as well as alcohol consumption were determined using a self-administered validated food frequency questionnaire. - Age, gender, ethnicity, smoking, education level, physical activity, and family history of T2DM, were collected by trained interviewers using standardized	-FPG and 2-h plasma glucose measured at baseline using "a glucose oxidase method. -TC, TGand HDL measured by enzymatic methods using "an Olympus AU600 automated analyzer (Olympus Optical, Tokyo, Japan)". -Serum insulin measured using "a human insulin-specific radioimmunoassay (Linco Research,	-Independent positive association between serum 25(OH)D and reduced risk of T2DM after 5 years in Australian men and women. -No association between dietary calcium intake and the risk of diabetes.	- Serum 25(OH)D level was only collected at baseline. - Lack of data on the use of vitamin D and calcium supplements. - >90% of the participants were Europid, therefore the results cannot be generalized to other ethnic groups. - Observational study: possible presence of confounding factors that may affect the results. - 54% loss to follow-up: the lost participants might be

			questionnaires. -Weight, height, WC and BP determined using standard procedures.	St. Charles, MO)". - Insulin sensitivity estimated from "FPG and fasting insulin using HOMA. HOMA of insulin sensitivity (HOMA-S) was calculated with the HOMA-2 program".		different from the participants who were still included in the study on the level of age, physical activity, ethnicity, smoking status, blood pressure, BMI, WC, FPG, TG, serum 25(OH)D and calcium intake.

Abbreviations: "FFAs: free fatty acids; FPG: fasting plasma glucose; LPL: lipoprotein lipase; PAL: Physical activity level; PG: 2 h post-load glucose; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; TG: triglyceride; SBP: systolic blood pressure; GFR: glomerular filtration rate; T2DM: type 2 diabetes mellitus".

Table 13: Summary of randomized clinical trials on vitamin D deficiency and insulin resistance

Reference (first author's last name), year of publication	**Sample characteristics (size, age range, gender distribution, nationality, other)**	**Study design & date of data collection**	**Exposure (independent variables) assessed & tool(s) of assessment**	**Outcomes (DV[s]) assessed & tool(s) of assessment**	**Main study findings**	**Limitations of the study**
Cefalo et al. (2018)	-n=18 (4 males, 14 females) -Italy -Age: 35.3±11.0 years -Inclusion criteria: Non-diabetic, deficient in vitamin D	-Double-Blind, Randomized, Placebo-Controlled Trial. -Screening began in October 2013. -Participants were randomized to a hypocaloric diet (by	-Data on demographics, diseases, medications used and family history of diabetes were collected at baseline. -Data on weight, height, WC, hip circumferences were collected at baseline and after 3 months of intervention. -A hormonal assessment (PTH,	Fasting blood samples were collected. -TG, TC, HDL and LDL, oral glucose tolerance test (OGTT) and hyperinsulinemic euglycemic clamp	- Body weight decreased significantly in both groups with no between-group differences. -In the vitamin D group, serum 25(OH)D levels increased considerably and insulin sensitivity improved, while no changes were detected in the placebo group.	-Loss to follow-up due to the difficulty in complying to the diet.

	[serum25(OH)D< 30 ng/ml], BMI≥30kg/m^2	deducting 500 kcal from the participant's usual energy intake) and either oral cholecalciferol at 25,000 IU/week or placebo for 3 months.	serum 25[OH]D, thyroid function) as well as measurement of electrolytes (phosphorus and calcium) were performed at baseline and after 3 months of intervention. - Fat mass and lean mass were assessed by dual x-ray absorptiometry (Delphi-W densitometer; Hologic, Marlborough, Massachusetts) at baseline and after 3 months of intervention.	to asses IR were performed at baseline and after 3 months of intervention.		
Oosterwerff et al. (2014)	-n=130(52 males, 78 females) - Amsterdam-Netherlands -Age: 20–65 years -Inclusion criteria: non-Western immigrants, age between 20 and 65 years, and with BMI ≥27 kg/m^2 with impaired fasting glucose (FPG: 5.6 - 6.9 mmol/L or impaired random glucose: 7.8 - 11.1 mmol/L), vitamin D deficiency [serum 25(OH)D concentration < 50 nmol/L but not less than 10 nmol/L].	- Randomized placebo-controlled trial - 130 non-Western immigrants with prediabetes and vitamin D deficiency were randomly assigned after stratification by sex to receive either cholecalciferol (1200 IU/d) or a placebo for 16 weeks. Both groups received 500 mg Ca/day as calcium carbonate. -Recruitment period: August 2009 to May 2011	-Age, gender, ethnicity, smoking, alcohol consumption -Waist:Hip ratio -Serum 25(OH)D measured "in EDTA plasma samples stored at 2808C by using isotope dilution–online solid-phase extraction liquid chromatography–tandem mass spectrometry". -BMI -PTH measured in EDTA samples by using an immunoradiometric assay (Luminescence; Abbott) -Blood pressure -Anthropometric measurements	Fasting blood samples were collected. - Glycated hemoglobin (HbA1c) -75-g oral glucose-tolerance test (OGTT) after an overnight fast. -TC - Fasting insulin measured by using "an immunometric assay (Luminescence, Advia Centaur; Siemens Medical Solutions Diagnostics)". - Insulin-sensitivity index (ISI) - HOMA-IR	- After 4 months of supplementation, the results showed that there was no significant effect of vitamin D supplementation on insulin sensitivity or beta cell function; nevertheless when patients who were diabetic at baseline were excluded, there was a significant improvement in beta cell function (insulinogenic index) in patients whose vitamin D level reached 60 nmol/L or more.	- The treatment group was a little healthier at baseline despite randomization. - The dose of vitamin D might have been relatively low. -The 4 months duration of the treatment and follow-up might be considered short.
Kelishadi, Salek, Salek,	-n=50 - Isfahan, Iran.	- Triple-masked controlled trial	- Anthropometric measures -Systolic (SBP) and diastolic	Fasting blood samples were	- In the vitamin D group serum insulin and TG, as well as	- A larger sample size and longer

Hashemipour & Movahedian (2014)	-Age 10-16 years -Inclusion criteria: Age between 10-16 years, BMI ≥ three Z-scores and presence of Metabolic syndrome.	-2012 - In trial, one group received oral vitamin D (300,000 IU) supplement and the other group received placebo for 12 weeks.	(DBP) blood pressure measured by trained nurses according to standard protocols, using calibrated instruments. -Serum 25(OH)D analyzed using "the chemiluminescent immunnoassay (CLIA) method (25 OH VitD CLIA kit, Diasorin - Stillwater, MN,United States)".	collected. -FPG and lipid profile by "autoanalyzer with standard kits (Pars Azmoun - Tehran, Iran)". - Plasma insulin measured by "radioimmunoassay (RIA) (LINCO Research Inc)". -HOMA-IR: [HOMA-IR = (fasting insulin (mU/L) x fasting glucose (mmol/L)/22.5].	HOMA -IR and continuous metabolic syndrome score decreased significantly, both when compared with the baseline and with the placebo group. - No significant difference in LDL-C, HDL-C, fasting blood glucose, and blood pressure when compared with the baseline and with the placebo group.	follow-up period may have led to more favorable results.

Abbreviations: "FPG: fasting plasma glucose; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; TG: triglyceride; SBP: systolic blood pressure".

APPENDIX B

E-invite to participate in the study

Subject: Invitation to participate in a research study on the relationship between vitamin D and health

Dear NDU Community (Faculty members, Staff, Administrators)

This is an invitation to take part in a research study designed by a group of faculty members from the Faculty of Nursing & Health Sciences. The study entails a thorough health assessment including vitamin D status, blood pressure, blood lipids profile, blood glucose, blood CRP, waist circumference, body fat, blood pressure, alongside assessment of other variables that will be looked at.

Trained nutritionists/ dietitians will pass by your offices at some time of your convenience to conduct face-face interview which will last for about 30 minutes. In addition, a trained nurse will withdraw a blood sample for the biochemical assessment and measure blood pressure. Participants will be rewarded a free body composition (% body fat) assessment (worth $40), and a nutrition consultation. Scheduled visits will commence starting February 8, 2016.

If you agree to participate, please send a "yes" reply to this message. Shortly afterward you will be contacted by the study investigators to arrange for an interview at some time of your convenience.

Kindly note that this study has been reviewed by the University Research Committee and approved by the Assistant Vice-President for Research and Graduate Studies and Vice-President for Academic Affairs.

Should you have any questions or concerns, please feel free to contact at the telephone or email below.

Regards,
Signature of PI

APPENDIX C

Consent Form

Subject Code: ----------	Interviewer Name: ----------	Faculty: ----------
Date of birth: ----/ -----/ ----	Date of Interview: -----/ -----/ ----------	Time Required: ----------
(day/month/year)	(day/month/year)	

Faculty of Nursing & Health Sciences- Notre Dame University-Louaize (NDU)
Consent Form to Participate in a Research Study

THIS STUDY IS APPROVED BY THE IRB COMMITTEE (Ref #: IRBSP16_2_FNHS)

This is a consent form to participate in a research study. If you decide to participate, you will have to mark your consent below and return this form to the study investigators.

Who are we?

We are a group of researchers from the Faculty of Nursing & Health Sciences at NDU.

What is the purpose of the study?

We are interested to study the association between vitamin D status & several health outcomes (depressive symptoms, metabolic syndrome, & inflammatory markers) among Lebanese adults.

What does the study entail?

Trained nutritionists/ dietitians will pass by your offices at some time of convenience to conduct face-face interview for collection of dietary, lifestyle and other variables of relevance to the study. The interview will last for about 30 minutes. In addition, a trained nurse will take blood pressure measurements & collect a blood sample for measurement of vitamin D, blood sugar,

triglyceride and HDL levels, & markers of inflammation. In return, participants will be rewarded a free body composition assessment (worth $40), and a free nutrition consultation.

Is there any risk to participants in the study?

There is no risk in participating in this study. The information collected will be used only for the purpose described in this form.

What about anonymity, and/ or confidentiality?

You will not be asked to provide your name, or any other personal identifier. All data from this study will be maintained in a secure location, and access will be strictly limited to study investigators.

What are my rights as a study participant?

Taking part in this research is voluntary and declining to participate will not bear any negative consequences.

You have the right to withdraw anytime during the study.

Whom do I call if I have questions?

For questions about the study, contact the researchers at: 03-423443, or 09-218950 ext. 5048.

STATEMENT OF CONSENT:

I have read this form. I have had the opportunity to ask questions and have had them answered to my satisfaction. In addition, I have been assured that any future questions that I may have will also be answered by the research investigators.

☐ By checking this box, I indicate that I voluntarily agree to participate in this study.

☐ By checking this box, I indicate that I am not interested in participating in this study.

Date: _____

APPENDIX D

Background questionnaire

Subject Code: ---------- Interviewer Name: ---------- Faculty: ----------

Date of birth: ----/ -----/ -------- Date of Interview: -----/ -----/ ------- Time Required: -------

(day/month/ year) (day/month/year)

Background Questionnaire *(28 Q, 3 pages)*

Please check one box for each question where there are check boxes. If you do not wish to answer a question, please draw a line through it.

Medical history- I

1. **Have you been recently diagnosed by a doctor with any of the following <u>chronic</u> medical conditions?**

☐ No ☐ Yes (Check all applicable)

☐ Heart attack (نوبة قلبية) ; Heart failure (فشل القلب)	☐ Cancer (السرطان)
☐ Stroke (السكتة الدماغية)	☐ Neurological disease (multiple sclerosis…) (أمراض في الجهاز العصبي (التصلب اللويحي ...)
☐ Hypertension (إرتفاع ضغط الدم)	☐ Kidney disease (أمراض الكلى)
☐ Diabetes (السكري)	☐ Liver cirrhosis (تليف الكبد)
☐ Asthma (الربو)	☐ Thyroid gland disorders (اضطرابات الغدة الدرقية)
☐ Vitamin D deficiency	☐ Other: Specify: ----------

2. **If your answer is yes to question # 2, have you been taking any medication &/or supplement?**

☐ No

☐ Yes, Specify name of medication: _____

3. **Are you pregnant or breastfeeding?**

☐ No ☐ Yes

4. **Are you currently taking any oral contraceptive pills?**

☐ No
☐ Yes, Specify name: _____

5. **Have you previously taken oral contraceptive pills?**

☐ No
☐ Yes, Specify when: _____

6. **Do you have any physical disability (إعاقة جسدية)?**

☐ No
☐ Yes, Specify: _____

Sociodemographic, plus anthropometric measurements

7. **Gender**:

☐ Male ☐ Female

8. **Date of Birth:** ----------/ ---------/ ---------- (day/ month/ year)

9. **Body weight (kg)/Height (cm) (measured by researcher)** *(leave it empty)*

Body weight (kg) _____
Height (cm) _____

10. **Blood pressure measurement (mmHg):** *(leave it empty)* _____

11. **Waist circumference (cm):** *(leave it empty)* _____

12. **Body composition (total body fat %):** *(leave it empty)* _____

13. **Describe your permanent place of residence:**

☐ Urban ☐ Rural

14. **Marital status:**

☐ Single ☐ Separated
☐ Married ☐ Divorced

15. **Do you have children?**

☐ No
☐ Yes, How many? _____

16. Indicate your level of education

☐ High School (or equivalent)
☐ University graduate (Master's, Doctorate degree, or equivalent)
☐ University bachelor's degree (BA, BS)

.

Lifestyle questions

17. How many meals do you have per day?

☐ One
☐ Two
☐ Three
☐ Four or more

18. How often do you have your meals?

☐ Often ☐ Occasionally ☐ Rarely

19. How often do you have a breakfast?

☐ Daily ☐ Occasionally ☐ Rarely

20. During the past 3 months, have you been taking any vitamin D supplement?

☐ No ☐ Yes ☐ If yes, which supplement? *(Include dosage)* ----------

21. <u>If your answer is yes to Q#22</u>, then how often did you take the vitamin D supplement?

☐ Daily
☐ Every other day
☐ Less than 1x/ week

22. During the past 3 months, have you been taking <u>any other</u> vitamin or mineral supplement(s)?

☐ No ☐ Yes ☐ If yes, which supplement? *(Include dosage)* ----------

23. <u>If your answer is yes to Q#24</u>, then how often did you take the supplement(s)?

☐ Daily
☐ Every other day
☐ Less than 1x/ week

24. Have you been recently following a special diet (نظام غذائي خاص)?

☐ No
☐ Yes, Specify: _____

25. In the past 3 months, on average, how much time per day was you exposed to direct sunlight (between 10:00 am- 4:00 pm)? *(Think about averaging weekdays & weekend days)*

☐ 5 min or less
☐ 5 to 15 min
☐ 31 to 60 min
☐ More than 1 hour

☐ 16 to 30 min

26. How often do you use sunscreen?

☐ Rarely/ Never ☐ Sometimes ☐ Often

27. Do you smoke?

☐ Daily
☐ Occasional
☐ Former daily
☐ Former occasional
☐ Never smoked

28. Do you drink alcohol?

☐ Never/ Occasionally
☐ 1-2 drinks per week
☐ 1-2 drinks per day
☐ More than 2 drinks per day

APPENDIX E

International Physical Activity questionnaire

Subject Code: ----------	Interviewer Name: ----------	Faculty: ----------
Date of birth: ----/ -----/ ----	Date of Interview: -----/ -----/ ----	Time Required: ----------
(day/month/year)	(day/month/year)	

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

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

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

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

_____ days per week

☐ No vigorous physical activities → ***Skip to question 3***

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

_____ minutes per day

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

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

_____ days per week

□ No moderate physical activities → ***Skip to question 5***

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

_____ minutes per day

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

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

_____ days per week

□ No walking → ***Skip to question 7***

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

_____ minutes per day

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

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

_____ hours per day

APPENDIX F

Food Frequency Questionnaire

Subject Code: ---------- Interviewer Name: ---------- Faculty: ----------
Date of birth: ----/ -----/ ---- (day/month/year) Date of Interview: -----/ -----/ ---- (day/month/year) Time Required: ----------

Food Frequency Questionnaire: Vitamin D Intake

INSTRUCTIONS: Do your best to answer each question. State how often (if ever) you ate the following vitamin D-containing foods during the past 3 months, and then indicate the frequency, number of servings, & average portion size.

Food Item	**Never**	**Monthly**	**Weekly**	**Daily**	**Check Serving Size:** (mark one only)
EXAMPLE: Milk for drinking (including chocolate milk/ hot cocoa with milk)			10		● 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cup)
1.Milk for drinking (including chocolate milk/ hot cocoa with milk) Specify brand & type:					□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cup)
2. Milk on cereal, in soups, pasta, and desserts (ex. sahlab,muhallabieh, custard, riz bi halib, ...) Specify brand & type:					□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
3. Soy or rice milk, or orange juice with added calcium and vitamin D Specify brand & type:					□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cup)

4. Eggs and egg- based dishes (including yolk) (ex. Fried, hard boiled, omelette, quiche,…)					□ 1 large □ 1 medium □ 1 small
5. Fish: including salmon (canned, smoked, & fresh), oysters, or other fish Specify type:					□ 75 g (2.5 oz) □ 150 g (5 oz) □ 225 g (7.5 oz)
6. Margarine (ex. Crisco, Elle et Vire, Flora, etc.) Specify brand:					□ 5 ml (1 tsp) □ 15 ml (1 tbsp) □ 45 ml (3 tbsp)
7. Yogurt Specify brand & type:					□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 30 g (1 oz) □ 60 g (2 oz) □ 90 g (3 oz)
8. Cheeses (including cheddar, mozzarella, cheese singles, parmesan, gouda, brie, feta, blue, chevre, ...) Specify brand/ type:					□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 30 g (1 oz) □ 60 g (2 oz) □ 90 g (3 oz)
9. Ice cream Specify brand/ type:					□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Additional sources of vitamin D					
10. Fish liver oil (supplement)					□ 15 ml (1 tbsp) □ 30 ml (2 tbsp) □ 45 ml (3 tbsp)
11. Vitamin D or multivitamin supplement Specify brand:					□ 200 IU □ 400 IU □ 800 IU □ Other: ----------

APPENDIX G

24h Multiple Pass Method Recall

Time and occasion	Food or beverage consumed	Portion size	Method of preparation, Brand name	Where consumed?

Time and occasion	**Forgotten foods**	**Portion size**	**Method of preparation, Brand name**	**Where consumed?**

Additional comments (Step 5: Probing)

_____

_____

_____

_____

_____

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