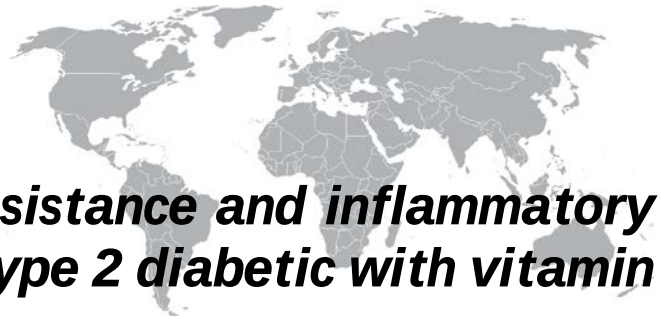
Eur J Gen Med 2016;13(4):91-96
ISSN:1304-3889 eISSN:1304-3897
DOI: 10.29333/ejgm/81757

Original Article



Association between insulin resistance and inflammatory cytokines among obese Saudi type 2 diabetic with vitamin D deficiency

Fadwa M. Al-Sharif[1]

ABSTRACT

Background: Vitamin D deficiency is now considered a public health problem around the world that is strongly associated with all-cause and cardiovascular mortality. However, vitamin D deficiency may play a role in mediating low-grade inflammation and insulin resistance among type 2 diabetic patients.

Objective: The aim of the present study was to investigate the possible relationship between inflammation, insulin resistance among obese Saudi type 2 diabetic with vitamin D deficiency.

Materials and Methods: One hundred obese Saudi patients with T2DM (60 males and 40 females). Their age was46.38 **±** 7.53 year, and a control group included one hundred healthy volunteers, who was gender and age matched.

Results: Obese T2DM patients showed significantly higher glucose, insulin, glycosylated hemoglobin (HBA1c), Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein(CRP) in addition to significantly lower values of the quantitative insulin-sensitivity check index (QUICKI) and 25(OH) vitamin D levels in comparison to controls. Serum levels of TNF-α , IL-6 and CRP showed an inverse relationship with QUICKI and a direct relationship with HOM-IR and HBA1c among obese Saudi type 2 diabetic with vitamin D deficiency.

Conclusion: Within the limit of there is an association between insulin resistance and inflammatory cytokines among obese Saudi type 2 diabetic with vitamin D deficiency.

Keywords: insulin resistance, type 2 diabetes, obesity, inflammatory cytokines, vitamin D deficiency

INTRODUCTION

Vitamin D deficiency is a worldwide epidemic health related problem (1,2) and potentially playing a role in the development of type 2 diabetes mellitus (T2DM) as In 2008, it was estimated that 1 billion individuals presented with vitamin D insufficiency or deficiency (3) and cardiovascular disease (CVD) (4), as well as with an increased overall mortality risk[5].The pathogenesis of T2DM remains unknown as there are many malfunctioning mechanisms that occur simultaneously which can contribute to the development of the disease (6); however increasing evidence suggests that vitamin D deficiency (as measured by serum 25-hydroxyvitamin-D3 concentration) may also contribute to the pathogenesis of T2DM (7-9). One follow-up study, through 20 years on 4,843 patients with T2DM, showed that vitamin D intake was associated with reduced prevalence of the T2DM (10).

Type 2 diabetes mellitus (T2DM) is a major global health problem. About six people approximately die every minute from the disease worldwide; a rate that will soon portray T2DM as one of the most prevalent health problems in the world (11). The number of people with diabetes is around 285 million and expected to reach 438 million by 2030 worldwide (12), ninety percent of which will have T2DM (13). T2DM is associated with increased morbidity and mortality due to its predisposing factor for cardiovascular disease and stroke (14,15). Cardiovascular disease is the leading cause of death in individuals with T2DM (16).

Clinical studies have demonstrated a positive correlation between circulating vitamin D (25-hydroxyvitamin D; 25(OH)D) levels and insulin sensitivity, they indicate that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes (17), either through a direct action via vitamin D receptor (VDR) activation or indirectly via calcemic hormones and also via inflammation (18,19). Chronic low-grade inflammation, frequently observed in obese individuals, is involved in the development of insulin resistance, which increases the risk of type 2 diabetes (20). Hotamisligil and colleagues were the first who stated the first link between obesity, inflammation and insulin action (21). The relations between 25(OH)D concentrations and inflammatory markers have been investigated in several studies, with most of these studies based on small samples or specific patient groups (22,23).

There is strong evidence that activation of inflammatory pathways interferes with normal metabolism and disrupts proper insulin signaling resulting in increased expression of pro-inflammatory cytokines (24). These cytokines can target cell membrane receptors, feeding into inflammatory response and exacerbating insulin resistance (25). Another important molecular mediators that link pro-inflammatory cytokine to inhibition of insulin signaling are suppressors of cytokine signaling (SOCS) 1 and 3, induced by IL-6, which lead to ubiquitinylation and degradation of insulin receptor substrate (IRS) proteins (26). There is increasing evidence from clinical and observational studies that a systemic, sub-clinical, low-intensity inflammatory reaction not only co-exists, but also precedes the development of T2DM (27,28). However, the inflammatory markers that have shown the strongest predictive capacity in the development of T2DM are C-reactive protein (CRP) and IL-6 (29, 30). Epidemiological studies have shown that TNF-a, CRP and IL-6 are positively correlated with BMI and percentage body fat (31, 32). Several recent human studies

[1] *King Abdulaziz University, Saudi Arabia*

Received: 07 Jan 2016, Accepted: 17 Mar 2016

Correspondence: *Fadwa M. Al-Sharif*
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, P.O. Box 80324, Jeddah, 21589, Saudi Arabia.

E-mail: dr_fadwa1@outlook.com

have associated vitamin D status with type 2 diabetes development (27).

The aim of the present study was to investigate the possible relationship between inflammation, insulin resistance among obese Saudi type 2 diabetic with vitamin D deficiency.

MATERIALS AND METHODS

Subjects

One hundred Saudi obese T2DM patients (60 males and 40 females) with body mass index (BMI) ranged from 31 to 35 Kg/m^2, were selected from the out-patient diabetic clinic of the King Abdalziz Teaching Hospital. They were checked for fasting/random glucose levels. Only participants have fasting blood sugar levels more than 5.6 mmol/L or random blood sugar level more than 7.8 mmol/L (impaired blood sugar) were included in this study and were further checked for type 2 diabetes mellitus as per recent American Diabetes Association criteria i.e. fasting blood sugar ≥7.0 mmol/L or post-prandial blood sugar ≥11.1 mmol/L [2-h plasma glucose 11.1 mmol/L during an oral glucose tolerance test] and glycosylated hemoglobin (HbA1c%) > 6.5% (33). Exclusion criteria included smokers, kidney insufficiency, congestive heart failure, pregnant female patients, hepatitis and respiratory failure. A detail clinical history and physical examinations were conducted which included the age, sex, symptoms suggestive of diabetes and family history of diabetes. Physical examinations included anthropometric measurements such as height, weight, body mass index (BMI) and waist circumference. Also, One hundred apparently healthy, medically free, and treatment naive individuals were recruited to serve as non-diabetic control. Informed written consent was obtained from each included subjects.

Laboratory Analysis

For the biochemical estimations, 5.0 ml fasting venous blood samples from the subjects were drawn after a minimum fasting period of 12 hours. Serum samples were stored at -80 ºC till further use.

A. Serum concentrations of 25-OH vitamin D: Measurement of 25(OH) vitamin D for all patients and controls were done by the commercial kit RIA (Elisa Kit; DiaSorin, Stillwater, MN, USA). Plasma 25(OH) vitamin D concentrations of less than 20 ng/ml were defined as 25(OH) vitamin D deficiency and less than 30 ng/ml as 25(OH)D deficiency and insufficiency [34,35].

B. Serum glucose, insulin and insulin resistance tests: Glucose was measured on the Hitachi 912 Chemistry Analyzer using the hexokinase reagent from Boehringer Mannheim (Indianapolis, IN 46256). For the oral glucose tolerance test; after an overnight fast, subjects were given 75 g of oral glucose dissolved in 250 ml of water and blood sugar was quantified after 2 hours. Human insulin was measured with an insulin kit (Roche Diagnostics, Indianapolis, IN, USA) using a cobas immunoassay analyzer (Roche Diagnostics). Insulin resistance was assessed by homeostasis model assessment (HOMA-IR). HOMA-IR = [fasting blood glucose (mmol/l) _ fasting insulin (mIU/ml)]/22.5 [36]. However, insulin sensitivity was assessed by the quantitative insulin-sensitivity check index (QUICKI) using the formula: QUICKI=1/[log(insulin) + log(glucose)] [37]. All serum samples were analyzed in duplicates.

C. Inflammatory cytokines measurements: Inflammatory cytokines included tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) levels were measured from frozen plasma samples stored at −80 °C. Enzyme-linked immunosorbent assays kits (ELISAs) were used to measure TNF-α and IL-6 (GE Healthcare Amersham, Biotrak Easy ELISA), which employs the quantitative sandwich enzyme immunoassay technique. However, C-reactive protein (CRP) level was quantified by enzymatic-colorimetric method using commercially available kits (Roche Diagnostics, Mannheim, Germany) at King Abdul-Aziz University Hospital.

Table 1: *Demographic and anthropometric characteristics of type 2 diabetic patients and control subjects*

	Mean +SD		Significance
	Diabetic group	Control group	
Age (year)	46.38 ± 7.53	45.64 ± 8.15	P >0.05
Gender (M/F)	60/40	55/45	P >0.05
BMI (kg/m^2)	30.23 ± 4.16	29.74 ± 4.51	P >0.05
Waist circumference (cm)	114.73 ± 8.11	111.25 ± 7.64	P >0.05
FBS (mg/dl)	176.12 ± 14.83*	89.65 ± 6.12	P <0.05
PPS (mg/dl)	232.45 ± 21.62*	116.18 ± 9.35	P <0.05

BMI= Body mass index; FBS = Fasting blood sugar; PPS = Postprandial blood sugar; (*) indicates a significant difference between the two groups, P < 0.05

Table 2: *Mean value and significance of biochemical parameters of type 2 diabetic patients and control subjects*

	Mean +SD		T-value	Significance
	Diabetic group	Control group		
25(OH)Vitamin D (ng/ml)	14.26 ± 3.51*	32.45 ± 5.83	7.61	P <0.05
CRP(mg/dl)	15.72 ± 2.84	10.36 ± 2.45	6.32	P <0.05
TNF- α (pg/mL)	5.87 ± 1.36	4.22 ± 1.21	5.73	P <0.05
IL-6 (pg/mL)	8.13 ± 2.42	5.74 ± 1.74	5.22	P <0.05
Insulin (mU/L)	15.56 ± 3.71*	8.13 ± 2.82	7.15	P <0.05
HBA1c (%)	9.31 ± 2.80	6.17 ± 1.36	6.43	P <0.05
HOMA-IR	5.72 ± 1.56*	2.85 ± 1.13	5.12	P <0.05
QUICKI	0.135 ± 0.063*	0.214 ± 0.087	6.36	P <0.05

HOMA-IR : Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, QUICKI : The quantitative insulin-sensitivity check index ; HBA1c = glycosylated hemoglobin; ICAM-1 – Inter-Cellular Adhesion Molecule ; VCAM-1 – Vascular Cell Adhesion Molecule; (*) indicates a significant difference between the two groups, P < 0.05

Table 3: *Pearson's correlation coefficients test value of the studied variables in the diabetic group*

	QUICKI (%)	HOMA-IR (%)	HBA1c (%)
TNF- α (pg/mL)	0.516**	0.637***	0.424*
IL-6 (pg/mL)	0.627**	0.508**	0.541**
CRP(mg/dl)	0.681 ***	0.452*	0.613 ***
HBA1c (%)	0.492*	0.635***	0.443*

TNF- α - tumor necrosis factor – alpha; IL-6 - Interleukin-6 ; CRP - C-reactive protein ; HOMA-IR : Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, QUICKI : The quantitative insulin-sensitivity check index ; HBA1c = glycosylated hemoglobin. Spearman's correlation was used *: P < 0.05 **: P < 0.01 ***: P < 0.001

Statistical Analysis

Independent t-test was used to compare mean differences between both groups. Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. The degree of correlation inflammation and insulin resistance among obese T2DM patients with vitamin D deficiency was detected by Pearson's product moment correlation coefficients (r).

RESULTS

One hundred obese Saudi T2DM patients were enrolled including 60 men and 40 women, had a mean age of 46.38 ± 7.53 year and one hundred healthy subjects, had a mean age of 45.64 ± 8.15 years, there was no significant differences in body mass index between both groups (Table 1).

Table 2 summarizes the comparison between T2DM patients and matched controls. Obese T2DM patients showed significantly higher glucose, insulin, glycosylated hemoglobin (HBA1c), Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein(CRP) in addition to significantly lower values of the quantitative insulin-sensitivity check index (QUICKI) and 25(OH) vitamin D levels in comparison to controls (Table 3).

Table 3 summarizes the relationship between parameters of glucose control and inflammatory cytokines among obese Saudi type 2 diabetic with vitamin D deficiency. Serum levels of TNF-

α, IL-6 and CRP showed an inverse relationship with QUICKI and a direct relationship with HOM-IR and HBA1c (Table 3).

DISCUSSION

Major evidence points towards a link between inflammation and type 2 diabetes, insulin resistance and diabetic complications. However, type 2 diabetic patients appear to be in a low grade inflammation status (38). Inflammation could be linked to type 2 diabetes pathogenesis and also to the development of common diabetic complications, mainly atherosclerosis (39). Type 2 diabetic patients presented higher plasma levels of cytokines such as TNF-α (40) and IL-6 (41). Higher concentrations of TNF-a and IL-6 in plasma have also been considered diabetes development predictors (42). TNF- α and IL-6 are long known to be involved in insulin resistance, obesity and type 2 diabetes (43, 44). Epidemiological studies show correlations between low serum 25(OH)D concentrations and increased insulin resistance, as well as impaired ß-cells function (45) and T2D risk (46-48). While, some studies have shown that vitamin D supplementation improves insulin sensitivity (49), and reduces inflammation biomarkers as CRP, TNF, IL6 and IL8 after vitamin D supplementation (50-53). Our data corroborate these findings and also demonstrate the association between high levels of systemic inflammatory cytokines and insulin resistance among obese Saudi type 2 diabetic with vitamin D deficiency.

Many studies reported inverse associations between concentrations of 25(OH)D with proinflammatory cytokines like CRP, IL-6, IL-23 or tumor necrosis factor (54-61). Vitamin D deficiency or insufficiency and cardio-metabolic disease may be linked by chronic inflammation (62, 63). Also, 25(OH)D was able to down-regulate the expression of TNF-a, IL-6, IL-1, and IL-8 (64), the underlying mechanism was that 1,25(OH)2D may improve insulin sensitivity and protect against beta cell cytokine-induced apoptosis by directly modulating the expression and activity of cytokines (65-67). One such pathway may be through down-regulation of NF-kB, which is a major transcription factor for TNF-a and other inflammatory mediators (68). Another pathway that may, at least in part, mediate the anti-apoptotic effect of 1,25(OH)2D on beta cell is through counteracting cytokine-induced Fas expression [69]. Several other immune-modulating effects of 1,25(OH)2D (e.g., blockade of dendritic cell differentiation, inhibition of lymphocyte proliferation, inhibition of foam cell formation and cholesterol uptake in macrophages, enhanced regulatory T-lymphocyte development) may provide additional pathways of protection against inflammation-induced type 2 diabetes risk (70, 71).

Some studies have shown that vitamin D may play a functional role on glucose tolerance through its effects on insulin secretion and insulin sensitivity (72, 73). In comparison to healthy controls, subjects with T2DM have significantly lower circulating concentration of 25 (OH)D (74). Vitamin D inadequacy has been associated with insulin resistance (IR) (75). Insulin resistance is associated with type 2 diabetes mellitus (76). Vitamin D deficiency is thought to influence insulin resistance and the pathogenesis of T2DM by affecting either insulin sensitivity, ß-cell function, or both (77, 78) Several cross-sectional studies have shown the existing inverse relationship between serum vitamin D (25[OH]D) and glycemic status measures, such as oral glucose tolerance tests, hemoglobin A1c (HbA1c), fasting plasma glucose, and insulin resistance (defined from the homeostatic model assessment [HOMA-IR]) as well as with conditions such as metabolic syndrome and obesity, in both diabetic and healthy subjects [79-81] , the underlying mechanism was that in order to develop glucose intolerance and T2DM, defects in pancreatic ß cell function and insulin sensitivity are often needed. There is evidence that vitamin D influence these mechanisms. However, vitamin D may also have beneficial effect on glucose metabolism improving systemic inflammation (82-84). The direct effect of vitamin D on pancreatic ß cells may be mediated by binding of its circulating active form 1,25(OH)D to the pancreatic ß cell vitamin D receptor (85). Alternatively activation of vitamin D may ocur within the ß cell by the 1α hydroxylase enzyme expressed in those cells (86). The indirect effects of vitamin D may be mediated via its role in regulating extracellular Ca and Ca flux through the ß cell. Insulin secretion is a Ca dependent process (87-89]).

CONCLUSION

Within the limit of there is an association between insulin resistance and inflammatory cytokines among obese Saudi type 2 diabetic with vitamin D deficiency.

REFERENCES

1. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc. 2013;88:720–755.
2. Thacher TD, Clarke BL. Vitamin D insufficiency. Mayo Clin Proc. 2011;86(1):50–60.
3. Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE. Blood 25- hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care. 2013;36:1422–1428.
4. Al Mheid I, Patel RS, Tangpricha V, Quyyumi AA. Vitamin D and cardiovascular disease: is the evidence solid? Eur Heart J 2013;34:3691–3698.
5. Tomson J, Emberson J, Hill M, Gordon A, Armitage J, Shipley M. Vitamin D and risk of death from vascular and non-vascular causes in the Whitehall study and meta-analyses of 12,000 deaths. Eur Heart J. 2013;34:1365–1374.
6. Leahy J. Pathogenesis of type 2 diabetes mellitus. Arch Med Res 2005;36:197-209.
7. Knekt P, Laaksonen M, Mattila C, Harkanen T, Marniemi J, Heliovaara M. Serum vitamin D and subsequent occurrence of type 2 diabetes. Epidemiology. 2008;19:666e71.
8. Thorand B, Zierer A, Huth C, Linseisen J, Meisinger C, Roden M. Effect of serum 25-hydroxyvitamin D on risk for type 2 diabetes may be partially mediated by subclinical inflammation: results from the MONICA/KORA Augsburg study. Diabetes Care. 2011;34(10):2320-2.
9. Dalgård C, Petersen MS, Weihe P, Grandjean P. Vitamin D status in relation to glucose metabolism and type 2 diabetes in septuagenarians. Diabetes Care. 2011;34(6):1284-8.
10. Zehra O, Tahseen A. vitamin D deficiency and type 2 diabetes. Postgrad Med J. 2010;86:18–25.
11. Rathmann W, Giani G. Global prevalence of diabetes: estimates for the year 2000 and projection for 2030. Diabetes Care. 2004;27(10): 2568–2569.
12. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care. 2011;34(6):1249–57.

13. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047-53.
14. Comeau P. New diabetes treatment and prevention strategies needed. CMAJ. 2007;176(10):1401–1402.
15. Moore AF, Florez JC. Genetic susceptibility to type 2 diabetes and implications for antidiabetic therapy. Annu Rev Med. 2008;59: 95–111.
16. Schering D, Kasten S. The link between diabetes and cardiovascular disease. J Pharm Prac. 2004;17:61-5.
17. Pittas AG, Sun Q, Manson JE Dawson-Hughes B, Hu FB: Plasma 25-hydroxyvitamin D concentration and risk of incident type 2 diabetes in women. Diabetes Care. 2010; 33:2021–2023.
18. Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin D in insulin resistance. J Biomed Biotechnol. 2012;2012:634195.
19. Thorand B, Zierer A, Huth C, Linseisen J, Meisinger C, Roden M, Peters A, Koenig W, Herder C :Effect of serum 25-hydroxivitamin D on risk for type 2 diabetes may be partially mediated by subclinical inflammation: results from the MONICA/RORA Augsburg study. Diabetes Care. 2011;34:2320–2322.
20. McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. Nutr J. 2008;28:7:4.
21. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor-necrosis-factor-alpha—direct role in obesity-linked insulin resistance. Science. 1993;259:87-91.
22. Haque UJ, Bathon JM, Giles JT. Association of vitamin D with cardiometabolic risk factors in rheumatoid arthritis. Arthritis Care Res (Hoboken). 2012;64(10):1497–504.
23. Jorgensen SP, Agnholt J, Glerup H. Clinical trial: vitamin D3 treatment in Crohn's disease -a randomized double-blind placebo-controlled study. Aliment Pharmacol Ther. 2010;32(3):377–83.
24. Gregor MF, Hotamisligil GS: Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415–445.
25. Solinas G, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, Grivennikov S, Wynshaw-Boris A, Scadeng M, Olefsky JM, Karin M. JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. Cell Metab. 2007;6:386–397.
26. Lebrun P, Van Obberghen E. SOCS proteins causing trouble in insulin action. Acta Physiol. 2008;192:29–36
27. Mezza T, Muscogiuri G, Sorice GP, Prioletta A, Salomone E, Pontecorvi A, Giaccari A. Vitamin D deficiency: a new risk factor for type 2 diabetes. Ann Nutr Metab. 2012;61:337–348.
28. Palomer X, Gonzalez-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab. 2008;10:185–197
29. Engström G, Stavenow L, Hedblad B, Lind P, Eriksson KF, Janzon L, Lindgarde F. Inflammation-sensitive plasma proteins, diabetes, and mortality of myocardial infarction and stroke: a population-based study. Diabetes. 2003;52:442–447.
30. Pickup JC: Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes mellitus. Diabetes Care. 2004; 27:813–823.
31. Pittas A, Joseph NA, Greenberg A. Adipocytokines and insulin resistance. J Clin Endocrinol. Metab. 2004;89:447–452.
32. Bermudez EA, Rifai N, Buring J, Manson JA, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. Arterioscler Thromb Vasc Biol. 2002;22:1668–1673.
33. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33(Suppl. 1):S62–9.
34. Holick MF. Vitamin D deficiency. New England Journal of Medicine. 2007;357:266–281.
35. Effraimidis G, Badenhoop K, Tijssen JG, Wiersinga WM. Vitamin D deficiency is not associated with early stages of thyroid autoimmunity. Eur J Endocrinol. 2012;167:43-8.
36. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from plasma FBS and insulin concentrations in man. Diabetologia. 1985;28:412–9.
37. Katz A, Nambi SS, Mather K, Baron DA, Follman DA, Sullivan F. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85:2402–2410.
38. Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. Diabetes Res Clin Pract. 2007;77(1):47-57.
39. Spranger J, Kroke A, Mohlig M. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study, Diabetes. 2003;52:812–817.
40. Moriwaki Y, Yamamoto T, Shibutani Y. Elevated levels of interleukin-18 and tumor necrosis factor-alpha in serum of patients with type 2 diabetes mellitus: relationship with diabetic nephropathy, Metabolism. 2003;2:605–608.
41. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X, Diabetologia. 1997;40:1286–1292.
42. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women, Diabetes. 2004;53:693–700.
43. Borst SE. The role of TNF-alpha in insulin resistance, Endocrine. 2004; 23:177–182.
44. Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes, Diabetes. 2002;51:3391–3399.
45. Khan H, Kunutsor S, Franco OH, Chowdhury R. Vitamin D, type 2 diabetes and other metabolic outcomes: a systematic review and meta-analysis of prospective studies. Proc Nutr Soc. 2013;72(1):89-97.
46. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care. 2004;27:2813–2818.
47. Lim S, Kim MJ, Choi SH, Shin CS, Park KS, Jang HC, Billings LK, Meigs JB. Association of vitamin D deficiency with incidence of type 2 diabetes in high-risk Asian subjects. Am J Clin Nutr. 2013;97:524–530.
48. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25- hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ. 2013;346:f1169.

49. 49- Talaei A, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol Metab Syndr. 2013;5:8.
50. Chen N,Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ. Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: a meta-analysis of randomized controlled trials. Nutrients. 2014;6:2206–2216.
51. Sharifi N, Amani R, Hajiani E, Cheraghian B. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. Endocrine. 2014;47:70–80.
52. Hopkins MH, Owen J, Ahearn T, Fedirko V, Flanders WD, Jones DP. Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: a randomized, controlled clinical trial. Cancer Prev Res (Phila). 2011;4:1645–1654.
53. Cavalcante IG, Silva AS, Costa MJ, Persuhn DC, Issa CI, de Luna Freire TL, Gonçalves MD. Effect of vitamin D3 supplementation and influence of BsmI polymorphism of the VDR gene of the inflammatory profile and oxidative stress in elderly women with vitamin D insufficiency: Vitamin D3 megadose reduces inflammatory markers. Exp Gerontol. 2015;66:10-6.
54. Haque UJ, Bathon JM, Giles JT. Association of vitamin D with cardiometabolic risk factors in rheumatoid arthritis. Arthritis Care Res (Hoboken). 2012;64(10):1497–504.
55. 55- Kim M, Na W Sohn C. Correlation between vitamin D and cardiovascular disease predictors in overweight and obese Koreans. J Clin Biochem Nutr. 2013;52(2):167–71.
56. Ngo DT, Sverdlov AL, McNeil JJ. Does vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations? Am J Med. 2010;123(4):335–41.
57. Patel S, Farragher T, Berry J. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis Rheum. 2007;56(7):2143–9.
58. Murr C, Pilz S, Grammer TB. Vitamin D deficiency parallels inflammation and immune activation, the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Clin Chem Lab Med. 2012;50(12):2205–12.
59. Amer M, Qayyum R: Relation betweenserum25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). Am J Cardiol. 2012;109(2):226–30.
60. Guillot X, Semerano L, Saidenberg-Kermanac'h N. Vitamin D and inflammation. Joint Bone Spine. 2010;77(6):552–7.
61. Dickie LJ, Church LD, Coulthard LR. Vitamin D3 down-regulates intracellular Toll-like receptor 9 expression and Toll-like receptor 9-induced IL-6 production in human monocytes. Rheumatology (Oxford) 2010;49(8):1466–71.
62. Reis JP, von Muhlen D, Miller III ER. Vitamin D status and cardiometabolic risk factors in the United States adolescent population. Pediatrics. 2009;124(3):e371–9.
63. Zittermann A, Schleithoff SS, Tenderich G. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? J Am Coll Cardiol. 2003;41(1):105–12.
64. Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C: Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. Diabetes Res Clin Pract. 2007 Jul;77(1):47-57.
65. Gysemans CA, Cardozo AK, Callewaert H, Giulietti A, Hulshagen L, Bouillon R. 1,25-Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice. Endocrinology. 2005;146:1956-1964.
66. Riachy R, Vandewalle B, Kerr Conte J, Moerman E, Sacchetti P, Lukowiak B. 1,25-Dihydroxyvitamin D3 protects RINm5F and human islet cells against cytokineinduced apoptosis: implication of the antiapoptotic protein A20. Endocrinology. 2002;143:4809-4819.
67. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol. 2005;97:93-101.
68. Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. Diabetes Res Clin Pract. 2007;77:47-57.
69. Cohen-Lahav M, Douvdevani A, Chaimovitz C, Shany S. The anti-inflammatory activity of 1,25-dihydroxyvitamin D3 in macrophages. J Steroid Biochem Mol Biol. 2007;103: 558-562.
70. Riachy R, Vandewalle B, Moerman E, Belaich S, Lukowiak B, Gmyr V. 1,25-Dihydroxyvitamin D3 protects human pancreatic islets against cytokine-induced apoptosis via downregulation of the Fas receptor. Apoptosis. 2006;11:151-159.
71. J. Oh, S. Weng, S.K. Felton, S. Bhandare, A. Riek, B. Butler. 1,25(OH)2 vitamin D inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. Circulation 120. 2009;687-698.
72. Mattila C, Knekt P, Mannisto S. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. Diabetes Care. 2007;30:2569–2570.
73. Palomer X, Gonzalez-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab. 2008;10:185–197.
74. Pietschmann P, Schernthaner G, Woloszczuk W: Serumosteocalcin levels in diabetes mellitus: analysis of the type of diabetes and microvascular complications. Diabetologia. 1988;31:892–895.
75. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient—a randomised, placebo-controlled trial. Br J Nutr. 2010;103:549–55.
76. Petersen KF, Shulman GI. Etiology of insulin resistance. Am J Med. 2006;119:S10–6.
77. Deleskog A, Hilding A, Brismar K, Hamsten A, Efendic S, Östenson CG. Low serum 25-hydroxyvitamin D level predicts progression to type 2 diabetes in individuals with prediabetes but not with normal glucose tolerance. Diabetologia. 2012;55(6):1668–1678.
78. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr. 2004;79(5):820–825.
79. Badawi A, Garcia-Bailo B, Arora P, Al-Thani MH, Sadoun E, Farid M, El-Sohemy A. The utility of vitamins in the prevention of type 2 diabetes mellitus and its complications: A public health perspective. In: Oguntibeju O, editor. Diabetes Mellitus - Insights and Perspectives. Rijeka: InTech Publ. 2013;1–16.
80. Brenner DR, Arora P, Garcia-Bailo B. Plasma vitamin D lev¬els and risk of metabolic syndrome in Canadians. Clin Invest Med. 2011;34(6):E377–E384.

81. Sharifi F, Mousavinasab N, Mellati AA. Defining a cutoff point for vitamin D deficiency based on insulin resistance in children. Diabetes Metab Syndr. 2013;7(4):210-3.
82. Van Etten E, Mathiu C. Immunoregulation by 1, 25 dihydroxy vitamin D3: basic concepts. J Steroid Biochem Mol Biol. 2005;97(1-2):93-101.
83. Christakos S, Barletta F, Huening M, Dhawan P, Liu Y, Porta A. Vitamin D target proteins: function and regulation J Cell Biochem. 2003;88(2):238-44.
84. Flores M. A role vitamin D in low intensity chronic inflammation and insulin resistance in type 2 diabetes mellitus. Nutr res Rev. 2005;18(2):175-82.
85. Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical localization of the 1,25(OH)2D3 receptor and calbindin D28k in human and rat pancreas. Am J Physiol. 1994;267(3Pt1):E356-60.
86. Bland R, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE. Expression of 25 hydroxy vitamin D3- 1 α-hydroxylase in pancretic islets. J Steroid Biochem Mol Biol. 2004;89-90(1):121-5.
87. Sooy K, Schermerhorn T, Noda M, Surana M, Rhoten WB, Meyer M. Calbindin-D (28k) controls Ca(+2) and insulin release. Evidence obtained from calbindin-d (28k) knock-out mice and beta cell lines. J Biol Chem. 1999;274(48):34343-9.
88. Beaulieu C, Kestekian R, Havrankova J, Gascon-Barre M. Calcium is essential in normalizing intolerance to glucose that accompanies vitamin D depletion in vitro. Diabetes. 1993;42(1):35-43.
89. Gedik O, Zileli MS. Effects of hypocalcemia and theophylline on glucose tolerance and insulin release in human beings. Diabetes. 1977;26(9):813-9.

◇◈◈◈◈◇