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Circulating 25-Hydroxyvitamin D and Insulin Resistance in Patients with Coronary Heart Disease

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Abstract*: Background: Coronary heart disease (CHD) is a global epidemic, insulin resistance (IR) one of the predictors of coronary heart disease. Low concentration of vitamin 25-Hydroxyvitamin D(25(OH) D) associated with IR. The aim of our study was to explore the associations between 25(OH) Dand indexes of IRamong patients with CHD.Methods: This case–control study included 100 unrelated subjects. 60 patients with CHD and 40 healthy control group. Serum 25(OH) D levels were measured using a high-performance liquid chromatography (HPLC) according to. Results: Our results revealed that diabetic patient with CHD had significantly lower values of serum 25(OH) D compared to non-diabetic group as well as control group, there were significant negative correlations between serum 25 (OH) D levels and HOMA-IRas well as other studied parameters. Receiver operating characteristic analyses (ROC) revealed thatthe cut-off value of serum 25(OH) D levels was 31.1and the AUC was 0.996. The sensitivities and the specificities were 98.3%and 75%.Conclusion:25(OH) D levels are significant low in CHD patients as well as diabeticsubgroup compared to non-diabetic group. 25(OH) D levels are strongly correlated with insulin resistance thuslow25 (OH) D could be used asmarker of insulin resistance.*

Keywords: 25-Hydroxyvitamin D; CHD; insulin resistance

1. Introduction

Coronary heart disease (CHD), is the most common type of cardiovascular disease all over the world. The risk for cardiovascular disease (CVD) is two to eight-folds higher in patients with diabetes compared to non-diabetic individuals of similar age, sex, and ethnicity [1-3].Diabetes is associated with an increased risk of death after an acute myocardial infarction [4, 5]. Insulin resistance (IR) and impaired insulin secretion play a role in the pathogenesis of type 2 diabetes [6].

The prevalence CHD is increasing due to physical inactivity, nicotine abuse, bad nutrition practices [9] rapid globalization, urbanization, ageing of society, and an increase in chronic diseases [7,8]. Likewise, loss of traditional diet habits in new-industrial cultures [10].

Vitamin D deficiency is a risk factor for hypertension, type 1 diabetes, obesity, insulin resistance, defective insulin secretion [13-15], and various cancers [12]. Many environmental factors affect serum 25-hydroxyvitamin D levels e.g. vitamin D intake and ultraviolet exposure [11].

There were over 7.8 million cases of diabetes in Egypt in 2015(IDF, 2015). In our Egyptian population, cardiovascular complications of diabetes are the leading cause of morbidity and mortality. The goal of this study was to test the hypothesis that serum concentrations of 25(OH) D in patients with CHD are positively associated with defects in insulin action and insulin secretionamong patients with coronary heart disease.

2. Subjects and methods

2.1 Subjects

This case–control study included 100 unrelated subjects. 60 patients with CHD and 40 healthy control group, and they were matched to cases age,sex, BMI, ethnic origin (Caucasian), and sun exposurehabits.All patients were recruited from outpatient clinics of Endocrinology Unit of Internal Medicine Department of Zagazig University Hospitals. A retrospective analysis was conducted on patients with CAD, who underwent coronary angiography at cardiology department of Zagazig University Hospitals. The enrolled patients were divided into two groups: diabetic cardiac patients (n=25) and non-diabetic cardiac patients (n=35). The diagnosis of DM was based on fasting plasma glucose levels of ≥126 mg/dL (7.0 mmol/L) or 2-h postprandial plasma glucose levels of ≥200 mg/dL(11.1 mmol/L). The decision to perform coronary angiography was basedon symptoms consistent with the diagnosis, an abnormalelectrocardiogram (ECG), positive findings in standard exercisetests, or abnormal findings in radio nuclear studies.All patients were subjected to thorough history taking andfull clinical assessment including blood pressure, anthropometric variables includingbody mass index (BMI) calculated as weight in kg/height in (meters) 2 and waist circumference (cm)/hip circumference(cm) (WHR) were measured.

Exclusion criteria were: acute myocardial infarction within 48 hour, unstable CAD, variant angina, history of coronary artery bypass graft surgery, prior revascularization, left ventricular ejection fraction ≤ 30%.Subjects with acute or chronic inflammatory disorders, any acute infection, thrombocytopenia, malabsorption fat syndrome, acute and chronic liver disease, acute and chronic renal failure, nephrotic syndrome, urolithiasis, type 1 diabetes mellitus and primary hyperparathyroidism were excluded from the study.Also, we excluded all study participants receiving hormonal medications, vitamin D supplementation, or calcium for the last 6 months or scheduled to receive chemotherapy for malignancy and immunosuppressant therapy.Our study protocol, and written informed consent assigned by all participants. The ethical committees of Faculties of Medicine,Zagazig University approved.

2.2 Blood sampling

Blood samples were drawn from all subjects after an overnight fast and divided into 3 portions: 1 ml of whole blood was collected into evacuated tubes containing EDTA, for hematocrit, HbA1c. The second ml of whole blood was collected into evacuated tubes containing potassium oxalate and sodium fluoride (2:1) for fasting plasma glucose. Sera were separated immediately from remaining part of the sample and stored at −20 °C until analysisBiochemical and hormonal assays
Biochemical and hormonal assays

We determined fasting plasma glucose by the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol and triglycerides were assessed by routine enzymatic methods (Spinreact, Girona, Spain). HDL cholesterol was determined after precipitation of the apoB-containing lipoproteins. LDL cholesterol was calculated using the Friedewald formula [16]. Fasting serum insulin, was measured using high-sensitivity enzyme-linked immunosorbent assay kit provided by (DRG International, IRC, USA).. Serum 25(OH) D levels were measured using a high-performance liquid chromatography (HPLC) according to [17]. Homeostasis model assessments of IR were estimated including HOMA-IR and HOMA-b; an index of b-cell function [18]

2.3 Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 19; SPSS Inc., Chicago, IL, USA). Data were expressed using descriptive statistic (mean ± standard deviation) and were analyzed using t test, observed and expected frequencies were tested for significance using the Chi square test. Pearson correlation coefficient was used to assess the association between 25(OH) D, and other studied clinical as well as metabolic parameters. Receiver operating characteristic (ROC) analysis was performed to assess sensitivities, specificities, area under the curve (AUC), and the cutoff values of 25(OH) D for diagnosis of risk of CHD among studied subjects, linear regression analysis was done to detect the main predictors of 25(OH) D in patients with CHD. We considered P to be significant at <0.05.

3. Results

Among case individuals, 65% were male and 35% female, and in control individuals' 61% male and 39% female. The mean age of case group was 34.76±7.69 years and in controls 33.96±5.43years. The case and control individuals were thus balanced in terms of age and gender.

Clinical and biochemical characteristics of the studied groups:in patients with CHD, there were significant higher values of systolic blood pressure, diastolic blood pressure,waist circumference, body mass index, total cholesterol, triglycerides, LDL cholesterol ,fasting plasma glucose ,fasting serum insulin and HOMA-IRgroup compared to control group, however, there weresignificant lowers values ofHOMA β ,serum calcium and serum phosphorus(p <0.001).

Table 1: Clinical, anthropometric and laboratory characteristics of all studied subjects

	Control group (mean± SD) (n=40)	*CHD group (mean ± SD) (n=60)*	P
Systolic blood pressure (mm Hg)	113.25±6.65	130.1±12.06	<0.001*
Diastolic blood pressure (mm Hg)	711. ±4.41	84.41±7.31	<0.001*
Waist circumference(cm)	79.25±4.57	92.1±4.27	<0.001*
Body mass index (kg/m2)	22.6±1.17	31.6±1.192	<0.001*
Total cholesterol (mg/dL)	161.84±81.18	191.6±5.51	<0.001*
Triglycerides (mg/dL)	98.8±19.178	215.8±6.11	<0.001*
LDL cholesterol (mg/dL)	88.15±6.27	118.4±4.746	<0.001*
HDL cholesterol (mg/dL)	53.9±7.62	30.1±5.193	<0.001*
Fasting plasma glucose (mg/dL)	89.4±4.337	111.65±8.62	<0.001*
Fasting serum insulin (µU/mL)	6.2±0.301	13.32±7.59	<0.001*
HOMA-IR	1.37±0.135	3.69± 2.16	<0.001*
HOMA-β	106.7±161.61	59.4±2.36	<0.001*
Serum calcium (mg/dl)	9.29±0.539	7.77±0.31	<0.001*
Serum phosphorus (mg/dl)	3.12±0.46	2.02±0.21	<0.001*

HOMA-IR, homeostasis model assessments of insulin resistance; HOMA-b, an index of b-cell functions. * P < 0.05.

Clinical,anthropometric and laboratory parameters of CHD group, As shown in Table 2, diabetic cases group had significantly higher values of diastolic blood pressure, body mass index, fasting plasma glucose, LDL and triglycerides. .On the contrary, there was a significant lower value of serum calcium and serum phosphorusin diabetic group compared to non-diabetic group (p <0.001).

Table 2: Clinical, Anthropometric and laboratory characteristics of CHD patients

	Non-diabetic group (mean± SD) (n=40)	*Diabetic group (mean ± SD) (n=60)*	P
Systolic blood pressure (mm Hg)	128.71±13.52	132.1±9.57	0.223
Diastolic blood pressure (mm Hg)	71. 1±4.41	82.85±6.89	<0.05*
Waist circumference(cm)	92.68±3.64	91.04±4.96	0.154
Body mass index (kg/m2)	31.85±1.437	31.24±0.57	<0.05*
Total cholesterol (mg/dL)	188.46±4.64	196.14±2.88	0.591
Triglycerides (mg/dL)	212.6±4.741	220.48±4.718	<0.05*
LDL cholesterol (mg/dL)	115.8±3.96	122.2±2.802	<0.001*

HDL cholesterol (mg/dL)	30.14±1.396	29.8±0.8165	0.980
Fasting plasma glucose (mg/dL)	114.3±4.915	135.7±3.037	<0.001*
Fasting serum insulin (µU/mL)	14.49±9.424	13.76±2.35	0.625
HOMA-IR	4.09±2.66	4.62±0.87	0.225
HOMA-β	59.68±2.764	45.2±1.012	0.591
Serum calcium (mg/dl)	7.82±0.356	7.71±0.22	<0.001*
Serum phosphorus (mg/dl)	2.1±0.23	1.87±0.113	<0.001*

Comparison of serum 25(OH) Dlevels among the studied groups (Fig.1): Diabetic patient withCHD had significantly lower values of serum 25(OH) D(4.518±2.702 ng/ml) compared to non-diabetic group (11.26±6.12 ng/ml) **as** well ascontrol group (47.39±16.48 ng/ml) (p< 0.001) **(Fig .1).**

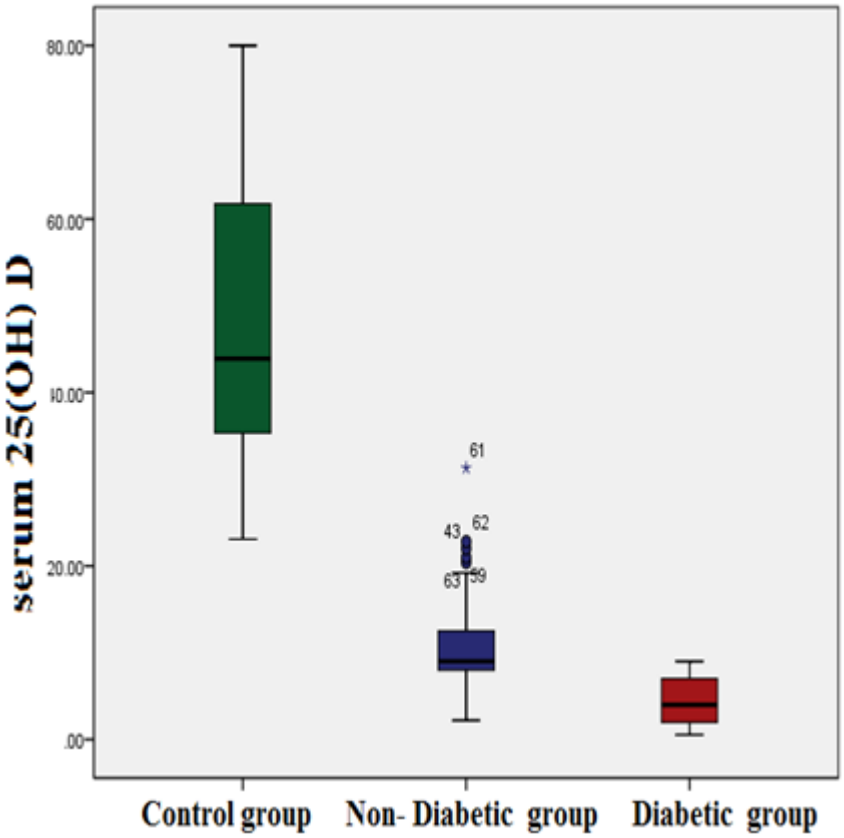

Figure 1: Comparison of serum 25(OH) Dlevels among the studied groups

Pearson correlations between serum 25(OH) Dand other parameters (Table 3): In patients with CHD, serum 25(OH) Dwas positively correlated with serum calcium and serum phosphorus. On the contrary, there were significant negative correlations between serum 25(OH) D level and HOMA-IR, diastolic blood pressure, systolic blood pressure, waist circumference, body mass index, totalcholesterol, triglycerides, LDLcholesterol, fasting plasma glucose and fasting serum insulin. Regarding insulin resistance indices; HOMA β there were non-significant correlation (p > 0.05).

Table 3: Pearson correlations between serum 25(OH)Dng/ml and other parameters of studied groups

Variable	*25(OH)D*	
	r	*p*
Systolic blood pressure (mm Hg)	-0.556	<0.001*
Diastolic blood pressure (mm Hg)	-0.681	<0.001*
Waist circumference(cm)	-0.777	<0.001*
Body mass index (kg/m2)	-0.852	<0.001*
Total cholesterol (mg/dL)	-0.332	<0.001*
Triglycerides (mg/dL)	-0.885	<0.001*
LDL cholesterol (mg/dL)	-0.827	<0.001*
HDL cholesterol (mg/dL)	0.112	0.265
Fasting plasma glucose (mg/dL)	-0.764	<0.001*
Fasting serum insulin (µU/mL)	-0.471	<0.001*
HOMA-IR	-0.518	<0.001*
HOMA-β	0.112	0.163
Serum calcium (mg/dl)	0.752	<0.001*
Serum phosphorus (mg/dl)	0.765	<0.001*

Multiple step wise regression analyses in patients withCHD;Our results showed thatserum 25(OH) D were independently correlated with LDL-cholesterol ,triglycerides and serum phosphorus (p< 0.05) .

Table 4: Multiple stepwise linear regression analyses in CHD patients to test the influence of the main independent variables against 25(OH) D (dependent variable).

Model		*Unstandardized Coefficients*		*Standardized Coefficients*	*t*	*p*	*95% C.I.*	
		B	*SE*	*Beta*			*Lower Bound*	*Upper Bound*
1	(Constant)	80.861	3.244		24.930	<0.001*	74.424	87.298
	Triglycerides	-0.341	0.018	-0.885	-18.818	<0.001*	-0.377	-0.305
2	(Constant)	55.202	10.767		5.127	<0.001*	33.832	76.571
	Triglycerides	-0.285	0.028	-0.740	-10.011	<0.001*	-0.342	-0.229
	Serum phosphorus	6.596	2.646	0.184	2.493	0.014	1.345	11.848
	(Constant)	24.299	18.481		1.315	0.192	-12.38	60.984
	Triglycerides	-0.285	0.028	-0.740	-10.011	0.000	-.342	-0.229
	Serum phosphorus	6.596	2.646	0.184	2.493	0.014	1.345	11.848
	LDL	0.483	0.237	.0337	2.041	0.044	0.013	0.954

25(OH)D; 25-Hydroxy vitamin D, LDL-C; low-density lipoprotein * P < 0.05

Accuracy of serum 25(OH) D in discriminating patients of insulin resistance among studied subjects by ROC Analyses: The cut-off value of serum 25(OH) D levels were determined by ROC; was31.1and the AUC was0.996(95% CI 0.987 -1.000, P<0.001) . The sensitivities and the specificities were 98.3% and 75% (**Fig. 2).**

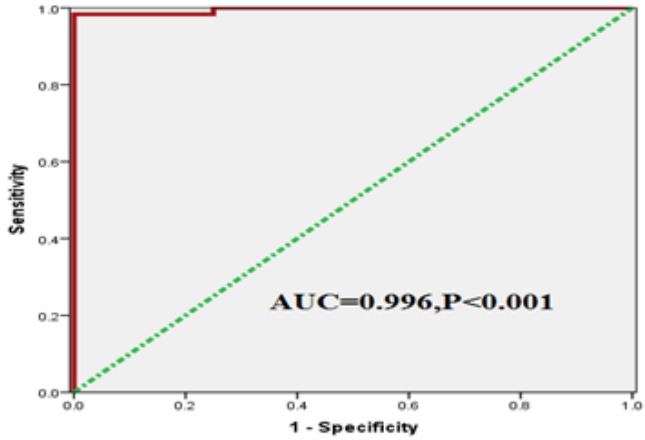

Figure 2: ROC curve: serum 25(OH) D in discriminating patients of insulin resistance among studied subjects

4. Discussion

Cardiovascular diseases (CVDs) account for about 38% of all deaths worldwide. The main cause of CVD is coronary heartdisease [19]. In Egypt, CVDs are now themain causes of death among Egyptians. In 1970, CVD had accounted for 12.4% of all deaths, whereas two decades later they were responsible for 42.5% of the mortality [20]. The prevalence, incidence, and mortality of all forms of CVD were strikingly higher for people with diabetes than those without diabetes [21,22].

The homeostasis model assessment of insulin resistance (HOMA-IR) is simple method to measure IR [23] and even in obese patients significantly correlates with the euglycemic clamp [24]. An identification of insulin resistant is highly important as the occurrence of type 2 diabetes coincides with the peak of IR [25].

The aim of the present study is to investigate serum concentration of 25-hydroxyvitamin D in patients with CHD. Furthermore, we aimed to clarify the possible associations of 25-hydroxyvitamin D, HOMA –IR, HOMA β, as well as other cardiovascular risk factors in cardiac patients with or without type 2 diabetes.

The main finding of the present study is that,patients with CHD had significantly lower values of serum 25(OH) D compared to control group. We stratified CHD group according to fasting plasma glucose into diabetic and non-diabetic groups. We found that diabetic patients with CHD had significantly lower values of serum 25(OH) D compared to non- diabetic group.

Our findings are in concordance with Siadat et al.,who found that low levels of 25 (OH) D are associated with significant coronary artery disease independent of cardiovascular risk factor [26].

Serum level of 25 (OH) D and 1α- hydroxylase enzyme act by binding to its intranuclear receptor in tissues in the form of autocrine and paracrine that causes angiotensin–renin system inhibition and induces or inhibits cell apoptosis and proliferation and development of cells as well. So, vitamin D deficiency can be effective on the occurrence of many disorders such as cardiovascular diseases and malignancies and Immune system diseases [27].

In line with our results Wang and colleagues revealed that vitamin D deficiency is associated with cardiovascular disease [28].

Kendricka et al., demonstrated that, Europeans who underwent coronary artery angiography with serum levels of 25(OH) D in the lowest quartile had the highest mortality rate by any reason and after adjustment with risk factors [29].

In agreement with our results,Leeet al**.** found that although vitamin D deficiency is prevalent in all patients with coronary artery disease history, it is more prevalent in patients with stable angina and also it is indicative of a worse prognosis (such as death, MI, cerebral stroke, or the need to revascularization) in them[27].

In agreement with our results,Lee and his colleagues [30] demonstrated thatlow 25-hydroxyvitamin D had adverse impact of on glucose homeostasis especially in patients with insulin resistance.

In order to evaluate the associations between serum 25(OH) D and other parameters**,** our results explored that was positively correlated with serum calcium and serum phosphorus. On the contrary, there were significant negative correlations between serum 25(OH) D level and HOMA-IRdiastolic blood pressure, systolic blood pressure, waist circumference, body mass index, total cholesterol, triglycerides, LDL cholesterol, fasting plasma glucose and fasting serum insulin.

Zhang et al., results indicate that low vitamin D status is also strongly associated with a high prevalence of diabetes in a Middle East population [31]. The possible mechanisms for this association may include the presence of vitamin D receptors in pancreatic beta cells to which circulating vitamin D binds [32]. Vitamin D has been well recognized for its role in regulating extracellular calcium flux, and insulin secretion is known as a calcium dependent process [33].

Similar to our results, several studies reported an impaired insulin release in association with vitamin D deficiency [34, 35].Also, Borissova et al. detected that vitamin D supplementation improved insulin secretion [36–40].

Our findings agreed with the results obtained by series of studies have shown that a positive correlation between low levels of serum 25-OHD3 and impaired insulin sensitivity [41, 42], T2DM, hypertension, hyperlipidemia and obesity is exist [43].

Against to our results, Davidson and collegues demonstrated that administration of vitamin D to individuals with low 25(OH)D concentrations and/or high risk of T2DM yielded little or no clinical benefit or changes in insulin action or secretion [44].similar finding observed by other studies [45-49].

Similarly to our results, in the article byMuscogiur and his colleagues observed that the prevalence of hypovitaminosis D was higher in diabetic patients than in control subjects [50].

In conclusion, serum 25(OH) D levels are significant low in CHD patients as well as diabetic subgroup compared to non-diabetic group. 25(OH) D levels are strongly correlated with insulin resistance thus low25 (OH) D could be used as marker of insulin resistance.We recommend further studies on a population to support these findings.

References

[1] Butler WJ, Ostrander LD Jr, Carman WJ, Lamphiear DE. Mortality from coronary heart disease in the Tecumseh Study: long-term effect of diabetes mellitus, glucose tolerance and other risk factors. Am J Epidemiol 1985;121:541-547.
[2] Klein R. Hyperglycemia and microvascular and macrovascular disease in diabetes. Diabetes Care 1995;18:258-268.
[3] Kleinman JC, Donahue RP, Harris MI, Finucane FF, Madans JH, Brock DB. Mortality among diabetics in a national sample. Am J Epidemiol 1988;128:389-401.
[4] Aronson D, Rayfield EJ, Chesebro JH. Mechanisms determining course and outcome of diabetic patients who have had acute myocardial infarction. Ann Intern Med 1997;126:296-306.
[5] Gu K, Cowie CC, Harris MI. Diabetes and decline in heart disease mortality in US adults. JAMA 1999;281:1291-1297.
[6] Lee JM, Okumura MJ, Davis MM, Herman WH, Gurney JG. Prevalence and determinants of insulin resistance among U.S. adolescents: a population-based study. Diabetes Care. 2006;29:2427–2432. doi: 10.2337/dc06-0709.
[7] Horton R. Offline: Chronic diseases—the social justice issue of our time. Lancet 2015;386:2378 10.1016/S0140-6736(15)01178-2
[8] Danaei G, Singh GM, Paciorek CJ, et al. The Global Cardiovascular Risk Transition: Associations of Four Metabolic Risk Factors with National Income, Urbanization, and Western Diet in 1980 and 2008.Circulation 2013;127:1493-502.
[9] Danaei G, Ding EL, Mozaffarian D, et al. The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS Med 2009;6:e1000058. 10.1371/journal.pmed.1000058.
[10] World Health Organisation. World Health Report. 2013. Available online: http://www.who.int/whr/2013/report/en/
[11] Gupta AK, Brashear MM, Johnson WD. Prediabetes and prehypertension in healthy adults are associated with low vitamin D levels. Diabetes Care 2011;34:658–60.
[12] Deleskog A, Hilding A, Brismar K, Hamsten A, Efendic S, Ostenson 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;55:1668–78.
[13] Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J ClinNutr 2004;79:820–5
[14] Kayaniyil S, Retnakaran R, Harris SB, Vieth R, Knight JA, Gerstein HC, Perkins BA, Zinman B, Hanley AJ. Prospective associations of vitamin D with beta-cell function and glycemia: the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort study. Diabetes 2011;60:2947–53.
[15] Mitri J, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic beta cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. Am J ClinNutr 2011;94:486–94.
[16] Frieldewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. ClinChem 18:499–502.
[17] Turpeinen U, Hohenthal U, Stenman UH (2003) Determination of 25-hydroxyvitamin D in serum by HPLC and immunoassay. ClinChem 49(9):1521–1524

[18] Matthews DR, Hosker JP, Rudenski AS Naylor BA, Treacher DF, Turner RC ,et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419.
[19] Wang Q. Molecular genetics of coronary artery disease.CurrOpinCardiol. 2005; 20(3): 182-188.
[20] Raja SG. Myocardial revascularization for the elderly: current options, role of off-pump coronary artery bypass grafting and outcomes. CurrCardiol Rev. 2012;8:26–36.
[21] Lam CS, Ho KT. Coronary artery ectasia: a ten-year experience in a tertiary hospital in Singapore. Ann Acad Med Singapore. 2004;33:419–422.
[22] Zografos TA, Korovesis S, Giazitzoglou E, et al. Clinical and angiographic characteristics of patients with coronary artery ectasia. Int J Cardiol. 2013;167:1536–1541.
[23] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–419. doi: 10.1007/BF00280883.
[24] Conwell LS, Trost SG, Brown WJ, Batch JA. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. Diabetes Care. 2004;27:314–319.
[25] Haymond MW. Measuring insulin resistance: a task worth doing: but how? Pediatr Diabetes. 2003;4:115–118. doi: 10.1034/j.1399-5448.2003.0002
[26] Zahra Dana Siadat, KeyvanKiani, MasoumehSadeghi, Amir Sina Shariat,[1] ZibaFarajzadegan, and Maryam Kheirmand Association of vitamin D deficiency and coronary artery disease with cardiovascular risk factorsJ Res Med Sci. 2012 Nov; 17(11): 1052–1055.
[27] Lee JH, Okefe JH, Bell D, Hensrud DD, Holick MF. Vitamin D deficienc an important, common, and easily treatable cardiovascular risk factor? J Am CollCardiol. 2008;52:1949–56.
[28] Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008;117:503–11.
[29] Kendricka J, Targherb G, Smitsa G, Chonchola M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination. Survey. Atherosclerosis. 2009;205:255–60.
[30] Lee JM, Okumura MJ, Davis MM, Herman WH, Gurney JG. Prevalence and determinants of insulin resistance among U.S. adolescents: a population-based study. Diabetes Care. 2006;29:2427–2432. doi: 10.2337/dc06-0709.
[31] Fang Fang Zhang, Suad Al Hooti, Sameer Al Zenki, HusamAlomirah, Kazi M Jamil, AravindaRao, Nasser Al Jahmah, Edward Saltzman, and Lynne M. Ausman. Vitamin D deficiency is associated with high prevalence of diabetes in Kuwaiti adults: results from a national survey. BMC Public Health. 2015; 16: 100
[32] Bland R, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE, et al. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. J Steroid BiochemMol Biol. 2004;89–90(1–5):121–5. doi: 10.1016/j.jsbmb.2004.03.115.
[33] Milner RD, Hales CN. The role of calcium and magnesium in insulin secretion from rabbit pancreas studied in vitro. Diabetologia. 1967;3(1):47–9. doi: 10.1007/BF01269910.
[34] Baynes KC, Boucher BJ, Feskens EJ, Kromhout D. Vitamin D, glucose tolerance and insulinaemia in elderly men. Diabetologia. 1997;40(3):344–7. doi: 10.1007/s001250050685.
[35] Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am JournaClin Nutrition. 2004;79(5):820–5. .
[36] Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients.Int J ClinPract. 2003;57(4):258–61.
[37] Inomata S, Kadowaki S, Yamatani T, Fukase M, Fujita T. Effect of 1 alpha (OH)-vitamin D3 on insulin secretion in diabetes mellitus. Bone and mineral. 1986;1(3):187–92.
[38] Orwoll E, Riddle M, Prince M. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. Am J ClinNutr. 1994;59(5):1083–7.
[39] Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial.Diabetologia. 2010;53(10):2112–9.
[40] Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2006;29(3):722–4. doi: 10.2337/diacare.29.03.06.dc05-2148.
[41] Chen Z, Caulfield MP, McPhaul MJ, Reitz RE, Taylor SW, Clarke NJ. Quantitative insulin analysis using liquid chromatography-tandem mass spectrometry in a high-throughput clinical laboratory.ClinChem 2013;59:1349–56.
[42] Taylor RL, Grebe SK, Singh RJ.Throughput analysis of 25-hydroxyvitamins D2 and D3 by LC-MS/MS using an automated on-line extraction.ClinChem 2005;51:A231–2.
[43] Pei D, Jones CN, Bhargava R, Chen YD, Reaven GM. Evaluation of octreotide to assess insulin-mediated glucose disposal by the insulin suppression test. Diabetologia 1994;37:843–5.
[44] Davidson MB, Duran P, Lee ML, Friedman TC. High-dose vitamin D supplementation in people with prediabetes and hypovitaminosis D. Diabetes Care 2013;36:260–6.
[45] Ljunghall S, Lind L, Lithell H, Skarfors E, Selinus I, Sorensen OH, Wide L. Treatment with one-alpha-hydroxycholecalciferol in middle-aged men with impaired glucose tolerance–a prospective randomized double-blind study. Acta Med Scand 1987;222:361–7.
[46] Lind L, Pollare T, Hvarfner A, Lithell H, Sorensen OH, Ljunghall S. Long-term treatment with active vitamin D (alphacalcidol) in middle-aged men with impaired glucose tolerance. Effects on insulin secretion and

sensitivity, glucose tolerance and blood pressure. Diabetes Res 1989;11:141–7.
[47] Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 2007;30:980–6.
[48] de Boer IH, Tinker LF, Connelly S, Curb JD, Howard BV, Kestenbaum B, Larson JC, Manson JE, Margolis KL, Siscovick DS, et al. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. Diabetes Care 2008;31:701–7.
[49] Tai K, Need AG, Horowitz M, Chapman IM. Glucose tolerance and vitamin D: effects of treating vitamin D deficiency. Nutrition 2008;24:950–6.
[50] Muscogiuri G1, Nuzzo V2, Gatti A3, Zuccoli A2, Savastano S4, Di Somma C5, Pivonello R4, Orio F6,7, ColaoA.Hypovitaminosis D: a novel risk factor for coronary heart disease in type 2 diabetes?. Endocrine. 2016 Feb;51(2):268-73.