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Study Of The Relationship Of The Branched Chain Amino Acids With Lipid Profile In Patients With Type 2 Diabetes Mellitus

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Abstract

Background: Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Current study was aimed to observe the total branched chain amino acids (BCAAs),valine, lucien,isoleucine, lipid profile levels and uric acid in type 2 diabetes mellitus patients. The objective of this study was to investigate the association between plasma branched chain amino acids with lipid profile in type 2 diabetes mellitus patients inThi-Qar, Iraq.

Methods: One hundred types 2 diabetes mellitus patients and an additional 75 non-diabetic controls were recruited. Age and body mass indexof the subjects were recorded. Plasmatotal branched chain amino acid, valine, leucineand isoleucine, serum glucose, HbA1c, total cholesterol, triglycerides, high density lipoprotein, very lowdensity lipoprotein and low density lipoprotein, uric acid levels were measured from the collected plasma and serum samples.

Results: Total branched chain amino acid, valine, leucine, isoleucine,the levels of serum glucose, HbA1c, total cholesterol, triglycerides, very low density lipoprotein and low density lipoprotein and uric acid showed significant increase in type 2 diabetes mellitus patients as compared to control group whereas the levels of high density lipoprotein showed a significant decrease in type 2 diabetes mellitus patients in comparison to control subjects ($P < 0.05$). In addition, current study demonstrated the characteristic diabetic dyslipidemia which is characterized by low HDL and high triglyceride. Plasma branched chain amino acid levels remained significantly associated with type 2 diabetes mellitus. Elevated serum BCAAs level are positively associated with dyslipidemia. In addition, glucose homeostasis could play a certain role in BCAAs-related dyslipidemia.

Keywords: Diabetes mellitus, Branchedchain amino acids, Lipid profile.

INTRODUCTION

Diabetes mellitus is a chronic disease caused by a combination of genetic, viral, environmental, and physiological factors. Diabetes is a set of disorders that affect the body's organs and have a common physiological condition: a high level of glucose (hyperglycemia) in the blood plasma (1). Inadequate insulin secretion and/or decreased tissue responses to insulin cause insulin deficiency at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action

frequently occur in the same patient, and it is often unclear which abnormality, if either alone, is the most common cause of hyperglycemia (2). Longterm damage, malfunction, and failure of multiple organs are all outcomes of diabetes mellitus. Diabetes mellitus is characterized by symptoms such as thirst, polyuria, blurred eyesight, and weight loss. Ketoacidosis or a non–ketotic hyperosmolar state can develop in its most severe stages, resulting in stupor, coma, and, in the absence of adequate treatment, death [3]. Type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and other causes are primary kinds of diabetes. T2DM is the most common type of diabetes, accounting for over 90% of all diagnosed cases (American Diabetes Association, 2008). Through their awful lengthy hyperglycemic course, the disease decreases cellular and humeral immune state and may promote latent opportunistic pathogens(4,5). According to estimates, there were 451 million people with diabetes globally in 2017 (ages 18–99 years) (6). T2DM reached epidemic proportions in Iraq in 2007, affecting around 2 million people, or 7.43 percent of the Iraqi population (7). BCAAs, which include the essential amino acids leucine (Leu), isoleucine (Ile), and valine (Val), not only provide substrate and govern protein synthesis(8), but also operate as signal molecules to regulate energy homeostasis involving glucose distribution and lipid metabolism(9,10). While it has been suggested that increased BCAAsin the circulation may have a role in the development of insulin resistance and T2DM by overloading of mitochondria with lipid substrates, resulting in mitochondrial stress and decreased insulin action (11,12). Recent genetic evidence supports the idea that insulin resistance causes greater circulating fasting BCAAs levels [13]. Unlike most amino acids, the catabolism of BCAAsdoes not occur in the liver. This is because the enzyme activity which catalyzes the first step of catabolism is low in the liver. For this reason, these amino acids quickly proliferate in the circulatory system following the protein intake [14].Type 2 diabetes is linked to groups of interconnected lipid and lipoprotein abnormalities in the blood [15]. An unusually high level of triglycerides (TG), a high proportion of low density lipoprotein cholesterol (LDL), low high density lipoprotein cholesterol (HDL), and high very low density lipoprotein (VLDL) are all typical lipoprotein abnormalities in type 2 diabetes [16]. The metabolic process for purines, the major components of nucleotides, produces uric acid (UA) (17). Obesity and insulin resistance, and hence type 2 diabetes, have been linked to hyperuricemia [18,19]. In a prospective follow-up research, however, it was discovered that elevated serum uric acid is linked to an increased risk of type 2 diabetes, regardless of obesity, dyslipidemia, or hypertension [20].

MATERIALS AND METHODS

This study is conducted at the Center of Diabetes and Endocrine Glands in Thi-Qar governorate, The study included 175 subjects; 75 normal healthy subjects (40 male, 35 female) as controls and 100 patients with Type 2 Diabetes (50 females and 50 males) with an age range of 35-70 years. The diagnosis of T2DM was performed on the basis of the recommended criteria by WHO (2006).

Venous blood samples were obtained after at least8h overnight fasting from the patient and controlgroups by venipuncture and collected in ethylenediaminetetraacetic acid (EDTA) and plain testtubes. The blood samples were centrifuged at at 3000rpm for approximately 10 minutes, and then plasma and serum wereseparated into plain test tubes,to collect plasma and serum and kept in the freezer (-20°C) until use unless used immediately to analyze biochemical parameters.

body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of body height (in meters), where the height and weight were measured (without heavy clothing and shoes), for each participant.

The quantitative determination of the fasting glucose concentration in Hemolysate Enzymatic reference method with hexokinase(21,22) by COBAS INTEGRA 400 plus system , Glycated hemoglobin (HbA1C) the quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in

whole blood on Roche clinical chemistry analyzers. The anticoagulated whole blood specimen is hemolyzed automatically onthe COBAS INTEGRA 400 plus analyzer with COBAS INTEGRA Hemolyzing Reagent Gen.2.(23,24,25)

Plasma Total Branched chain amino acid were measured using ELIZA kit (Mybiosource/USA) Plasma Valine, Lucien, Isoleucine were measured using Sample Preparation Kit for amino acid analysis (Germany). Lipid profiles including : the quantitative determination of total cholesterol, the quantitative determination of the triglycerides (26), the quantitative determination of the HDL-cholesterol (27,28), the quantitative determination of LDL-cholesteroland the quantitative determination of the uric acid concentration in serum by Enzymatic colorimetric test by COBAS INTEGRA 400 plus system. The VLDL-cholesterol level was calculated by using the formula (Triglyceride/5) = VLDL-cholesterol [29].

Statistical Analysis

The statistical analysis was done using SPSS v 23 the results were expressed as mean ± standard deviation (mean ± SD). It was used T test to compare study groups. Pearson's correlation was applied to determine the relationship among the present study parameters. P-values ($P<0.05$) were considered statistically significant.

RESULTS

A total of 175 subjects were included in the present study. There were 100 patients with type 2 DM and 75 healthy individuals considered as control group.

Table 1 shows the demographic features of all groups regarding age, gender, BMI , fasting blood glucose and HbA1c.

Figure1shows the positive correlation between FSG and BCAA in patients group with correlation coefficient (r= 0.31)and Figure2shows the positive correlation between HbA1c and BCAA in patients group with correlation coefficient (r= 0.13)

Table (1) Demographic featuresof diabetes patients and non-diabetes subjects.

Groups	NO.	Age mean± SD	Gen M/	BMI(Kg/m^2) mean± SD	FBG(mg/dl) mean± SD	HbA$_1$c (%)
Control	75	52.78±14.36	40 /35	24.56±3.89	90.26± 8.69	5.21±0.52
Patients	100	53.84±12.17	50/50	29.44±5.51	171.75 ±27.83	8.09±1.00
P.value				0.0001	0.0001	0.0001

Table 2show that there was a significant increase in Total BCAA(T BCAA) level in type 2 DM subjects compared with that of control (29.04±4.45 vs. 21.13±5.72 Ug/ml, $P < 0.05$) , valine (112.23±15.73 vs77.61±11.91 Umol/L($P <0.05$),), leucine(60.09±12.99vs46.54±7.85 Umol/L, ($P <0.05$))and isoleucine(38.21±7.72vs27.34±6.42 Umol/L, ($P <0.05$).

Table (2) Branched chain amino acid level of diabetes patients and non-diabetes subjects.

Groups	No	T BCAA(Ug/ml) mean±SD	Valine (Umol/L) mean±SD	Lucine (Umol/L) mean±SD	Isoleucine (Umol/L) mean±SD
Control	75	21.13±5.72	77.61±11.91	46.54±7.85	27.34±6.42
Patients	100	29.04±4.45	112.23±15.73	60.09±12.99	38.21±7.72
P-.value		0.028	0.040	0.0001	0.0001

Furthermore, as this study (Table 3) show that there was a significant elevation (P <0.05) was seen in serum triglyceride level in type 2 DM subjects (213.68±17.07mg/dl) compared with that of control were (103.61±31.82mg/dl) , a significant increase (P <0.05) was seen in serum total cholesterol in type 2 DM subjects (218.88±27.51mg/dl) compared with that of control were (161.42±22.56mg/dl), respectively. Also a significantly reduced (p < 0.05) was seen in serum HDL-C level in type 2 DM subjects (39.16±8.71 mg/dl) compared with that of controls (50.84±11.08 mg/dl), there was a significant increase (P <0.05) was seen in serum LDL-C level (136.98±32.99 vs. 89.86±23.71mg/dl), increases significantly (P < 0.05) in level of serum VLDL-C (42.72±9.41 Vs 20.72±6.36 mg/dl)and significantly increases(P < 0.05) in serum uric acid level (7.21±1.66 vs. 4.41±0.91) patients compared with controls.

Figure3,4,5,6,7shows the positive correlation between TC,LDL,TG,VLDL, uric acid and BCAA in patients group with correlation coefficient (r= 0.70,0.46,0.68,0.67,0.32) and **Figure 8** shows the negative correlation between HDLand BCAA in patients group with correlation coefficient(r= -0.14)

Table (3) Lipid profile and uric acid level of diabetes patients and non-diabetes subjects.

Groups	No	TC(mg/dl) mean±SD	TG(mg/dl) mean±SD	HDL(mg/dl) mean±SD	LDL(mg/dl) mean±SD	VLDL(mg/dl) mean±SD	Uric Acid(mg/dl) mean±SD
Control	75	161.42±22.56	103.61±31.82	50.84±11.08	89.86±23.71	20.72±6.36	4.41±0.91
Patients	100	218.88±27.51	213.68±17.07	39.16±8.71	136.98±32.99	42.72±9.41	7.21±1.66
P.value		0.005	0.001	0.031	0.038	0.001	0.006

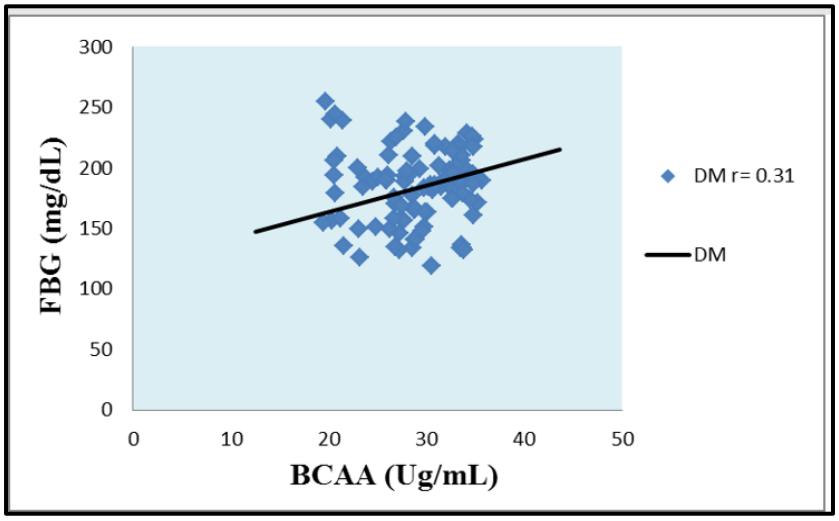

Figure (1) The correlation coefficient of BCAA with FSG for all subjects type 2 diabetes.

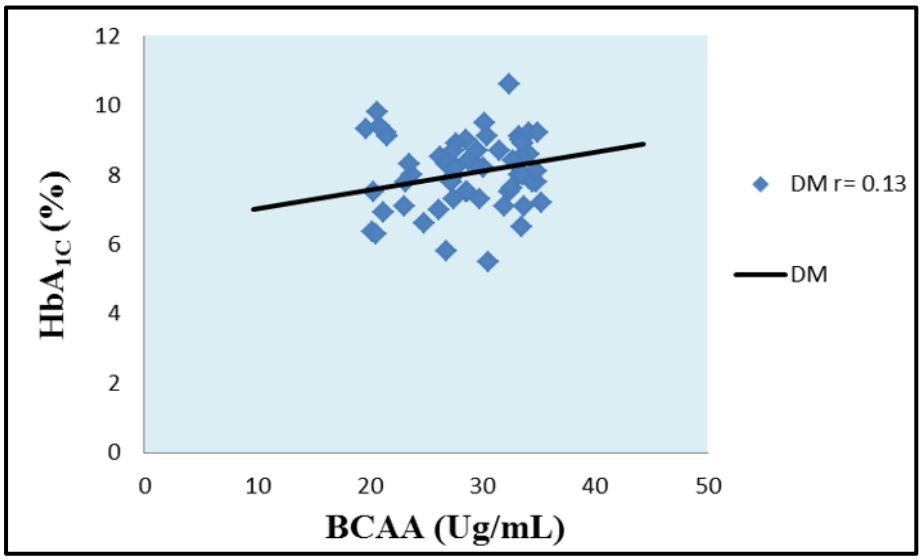

Figure (2) The correlation coefficient of BCAA with HbA_1c for all subjects type 2 diabetes.

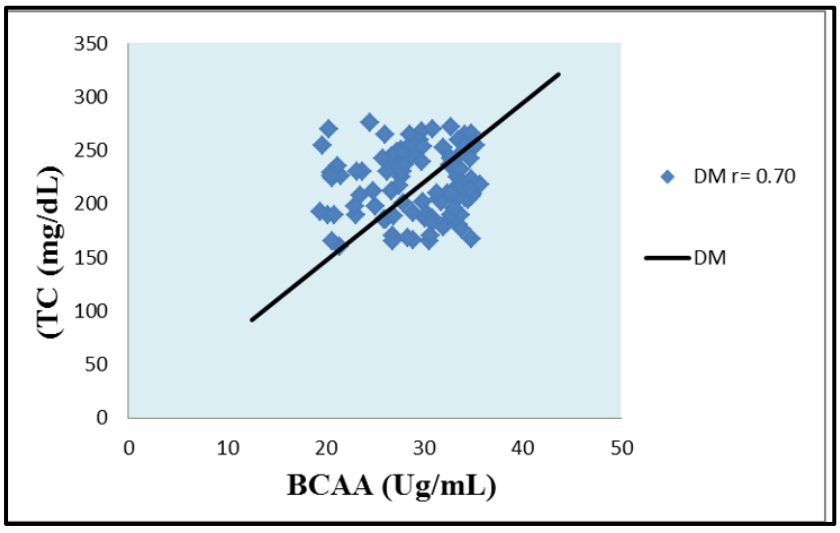

Figure (3) The correlation coefficient of BCAA with TC for all subjects type 2diabetes.

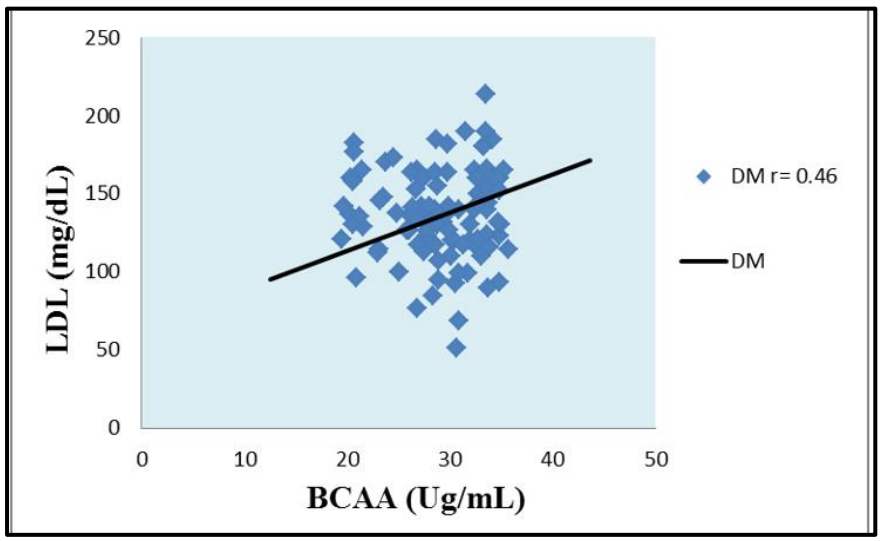

Figure (4) The correlation coefficient of BCAA with LDL for all subjects type 2 diabetes.

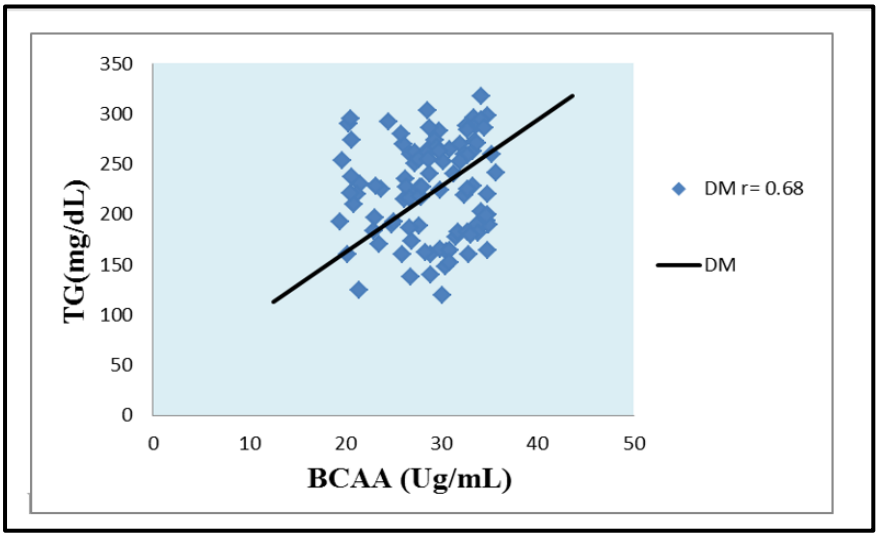

Figure (5) The correlation coefficient of BCAA with TG for all subjects type 2 diabetes.

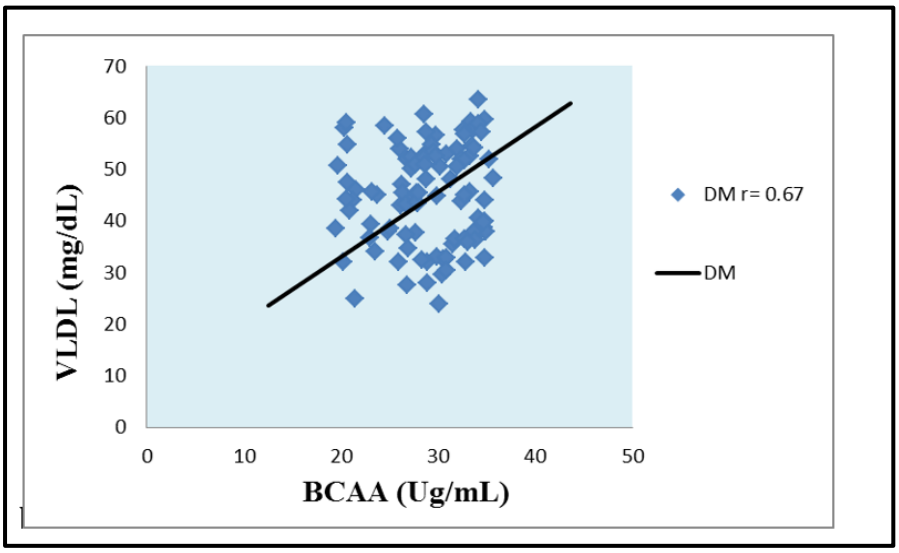

Figure (6) The correlation coefficient of BCAA with VLDL for all subjects type 2 diabetes.

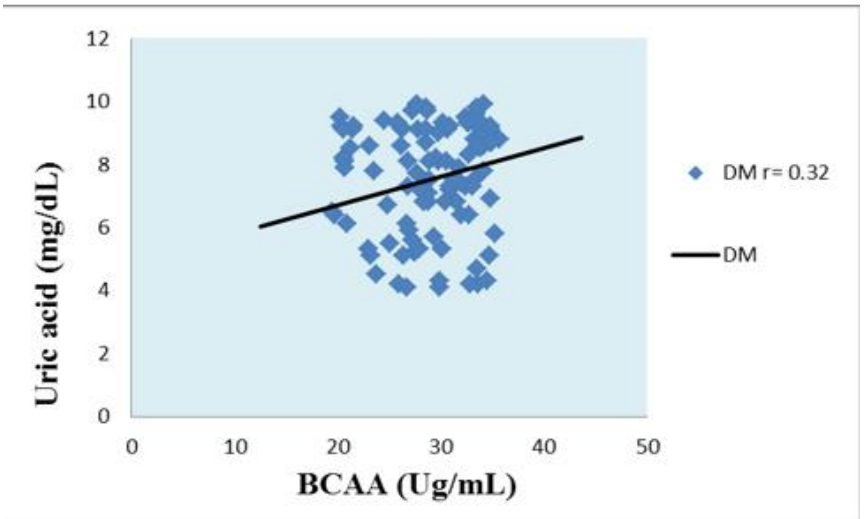

Figure (7) The correlation coefficient of BCAA with HDL for all subjects type 2 diabetes.

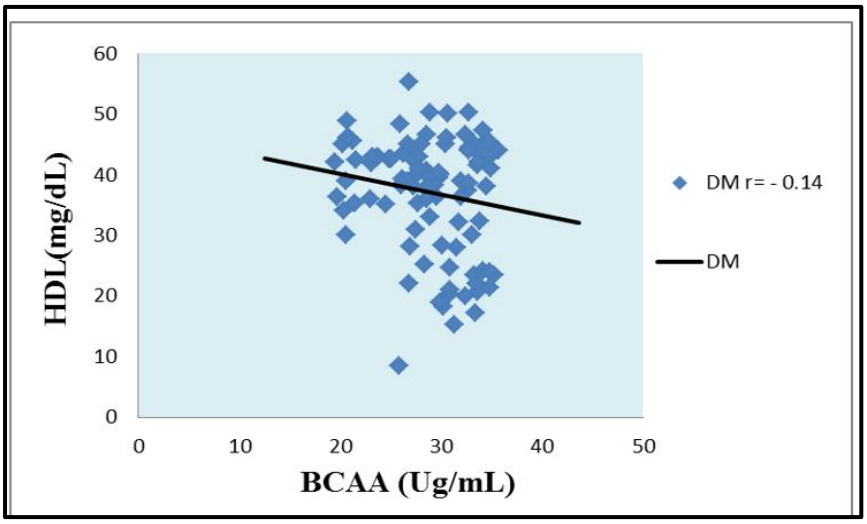

Figure (8) The correlation coefficient of BCAA with HDL for all subjects type 2 diabetes.

Discussion

T2DM is a global health problem due to its epidemic character and impact on a variety of health outcomes. T2DM raises the risk of atherosclerosis, dyslipidemia, and hypertension, among other things. (33).

One technique to monitor diabetes is to measure blood glucose levels. High blood glucose levels in diabetic patients could be attributed to a lack of or resistance to insulin, according to earlier studies (34). The authors of those investigations of a diabetic community found that the fasting blood glucose level is likewise increased, indicating inadequate DM control (35). Theincrease in HbA1c in this study's subjects corresponds to an increase in FBS in these patients. This finding agrees with the findings of (36) who discovered a favorable correlation between FBS and HbA1c. HbA1c 6.5 percent for diabetes diagnosis and 5.7-6.4 percent for the highest risk of diabetes progression, according to the American Diabetes Association (37). The HbA1c test, commonly known as the glycated or glycosylated hemoglobin level, determines the amount of glucose bound to hemoglobin and gives an estimate of the average blood glucose during the previous three months (38).When diabetic patients were compared to control people, the results showed a statistically significant rise in total BCAA. In a short-term dietary intervention, high protein or BCAA ingestion increased serum BCAAs concentration (39, 40), which appears to be able to partially explain insulin resistance induced by high protein intake, while another study suggest that defective insulin signaling can increase protein breakdown or turnover, resulting in BCAA buildup [41]. Furthermore, lower BCAAs oxidative enzyme activities or contents have been widely established in obese or diabetic mice as well as people (42, 43). In a T2DM rat model, Bajotto G (44) discovered decreased activity and content of hepatic BCKDC, a rate-limiting enzyme in BCAA catabolism. The same effect was seen by others (43,45).

When diabetic patients were compared to control people, the results showed a statistically significant rise in total cholesterol. This observation could be explained by a reduction in muscle activity or a reduction in cholesterol catabolism inhibition (46). It could, however, be ascribed to an increase in VLDL-C and LDL plasma concentrations (47), which could be related to hepatic VLDL synthesis or impaired clearance of VLDL-C and LDL from the circulation. Furthermore, diabetes patients' triglyceride levels were found to be

considerably higher than controls. This is due to insulin insufficiency, which causes hyperglycemia and fatty acid mobilization from adipose tissue. Excess fatty acids are collected in the liver and converted to triglyceride. Fatty acids from adipose tissue are mobilized for energy purposes (48). When diabetic patients were compared to controls, their VLDL cholesterol was considerably higher. This rise could be due to hyperinsulinemia, which raises triglycerides, LDL-C, and VLDL cholesterol. Insulin and growth hormone are known to stimulate VLDL cholesterol formation by boosting Apo-E and Apo-B 48 production and activating lipolysis in adipose tissues and triglycerides in the liver (49).Participants with T2DM had significantly higher LDL cholesterol levels than those without the disease. The latter could be explained by the fact that insulin increases the number of LDL receptors, therefore chronic insulin insufficiency, such as that found in T2DM, could be linked to a lower level of LDL receptor and a subsequent increase in LDL cholesterol levels (50).There is drop in serum HDL-Clevels which could be attributed to an increase in cholesterol ester transfer protein (CETP) activity, which moves cholesterol from HDL-C to VLDL-C, leaving HDL-C rich in triglycerides and less familiar to apolipoprotein-A, allowing it to be filtered more easily by the kidney. Furthermore, a drop in HDL-C levels could be related to an increase in hepatic lipase activity (51) or changes in liver function, both of which impede the formation of apolipoprotein-A1, the major protein for HDL-C (52).Hyperuricemia appears to be linked to the insulin-resistance syndrome and decreased glucose tolerance in Type 2 diabetes (53,54). Although uric acid is one of the most abundant antioxidants in the body (55), it can cause oxidative stress in a number of cells, including vascular smooth muscle cells (56), and hence contribute to cardiovascular disease progression (57,58).Reduced nitric oxide (NO) bioavailability in vascular smooth muscle and endothelial cells, as well as direct uric acid scavenging of NO, appear to be part of the pathogenic mechanism (59). Reduction in endothelial NO production by uric acid, has been also associated with endothelial dysfunction and insulin resistance (60,61).

Conclusion

In conclusion, our results illustrated that there was an elevated level in serum individual or total BCAAs in patients with type 2 diabetes and elevated serum BCAAs was positively associated with high HbA1c level, total cholesterol,LDL-C, TG and inversely associated with HDL-C. These provide a new potential explanation for serum BCAAs predictive ability of CVD events. However, further investigation needs to be done for predictive ability of BCAAs on dyslipidemia, CVD or other metabolic diseases.

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