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The Effect of Glycemic Status on the Serum Amino Acid Profile of Diabetic Saudi Patients

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Abstract

Background: The disturbance in amino acids serum profile in Diabetes Mellitus has been studied previously; however, very obscured in the Saudi diabetic population. The aim of the current study is studying the relationship between the glycemic status and the amino acid profile in Saudi diabetic patients.

Methods: Representative sample of Saudi Diabetes Mellitus type-I patients were included in accordance with the national population distribution; and a panel of 17 amino acids of different categories (essential, semi-essential and metabolic indicator amino acids) were assessed in response to their glycemic status. Blood samples of normoglycemic and hyperglycemic patients were withdrawn and assayed for glucose, total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein. General biochemical serum profile was assessed (alkaline phosphatase (ALP), creatinine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT) and blood urea nitrogen (BUN)).

Results: Transient hyperglycemia significantly decreased three metabolic indicator amino acids (AAA, ALA and ORN); in addition to one essential amino acid (TYR) and one semi-essential amino acid (CYS2). The total level of amino acids, total level of essential amino acids, and total level of semi-essential amino acids did not change due to glycemic status. Only the total metabolic amino acids (AAA, ALA, CIT, HCY2 and ORN) significantly decreased in response to hyperglycemia.

Conclusion: Some amino acids (THR, CYS2, AAA, ALA and ORN) are sensitive to the glycemic status and can be used as surrogate marker for transient hyperglycemia.

Keywords: Diabetes mellitus; Diabetic complications; surrogate markers; Saudi Arabia

Introduction

Saudi Arabia is classified as level-4 burden Diabetes Mellitus (DM) country with annual new diagnosed patients in the year 2009 of exceeding half million capita. Diabetes mellitus is a debilitating progressive metabolic disease with expected worldwide burden of 300 million populations in the year 2020 [1]. Despite the significant decline in DM incidence in several industrialized countries, Saudi Arabia suffered from rise DM incidence in the past decade [2]. Saudi Arabia experienced significant economical flourish over the last two decades which might be coupled with improvement in health services. However, it was associated with decline in the healthy eating habits and increase in fast food consumption [3].

DM is a metabolic disease mainly attributed to elevated blood glucose level and lack of glucose utilization in the peripheral adipose and muscle tissues [4]. The debilitating trait of DM is attributed to several vascular, neurological, immunological, and biochemical alterations [5]. Serum glucose and c-peptide represents the major classical parameters to assess the hyperglycemic or diabetic status [6]. In addition, many other biomarkers have been used for detailed or specific assessment for DM such as glycogenated hemoglobin [7]. Many of these DM complications are biochemically related to amino acid metabolism and utilization [8].

Amino acids constitute the building units of all structural and functional proteins in the bio-system. Amino acids are essential to life in free or polymeric (peptides/polypeptide) form. Also, Amino acids play pivotal roles in physiological functions such as digestion, neurotransmission, pH homeostasis, lipid metabolism, enzymatic processes, and pain/inflammatory response control [9]. Many amino acids (non-essential) can be synthesized by the body from other amino acids. Essential amino acids must be obtained from the diet as they cannot be endogenously synthesized [10]. The eight essential amino acids in human biosysytems are: threonine (THR), valine (VAL), methionine (MET), isoleucine (ILE), tryptophan (TYR), phenylalanine (PHE), leucine (LEU) and lysine (LYS) [11]. Semi-essential amino acids are partially synthesized endogenously, however, does not satisfy the physiological needs. These include taurine (TAU), cysteine (CYS2), histidine (HIS) and arginine (ARG) [12]. Other amino acids are considered metabolic indicators for several physiological impairments such as, alpha-aminoadipic acid (AAA), beta-alanine (ALA), citrulline (CIT), homocysteine (HCY2), and ornithine (ORN). These amino acids provide information on conversion capability in the body or dietary adequacy of the essential amino acids [13].

Herein, in the current clinical study, we are checking the relationship between the glycemic status of Saudi DM patients and their amino acid plasma profile which might provide future insights into further metabolic traits of DM in Saudi population.

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Patients and Methods

Patients

A total of 65 adult DM patients (27 males and 38 females) entered the study after reading and accepting the institutional consensus. Patients have been allocated to normoglycemic group (n=29) or hyperglycemic group (n=36) based on random glucose concentration. All participants were clinically diagnosed with type-I DM according to the standard procedures within Saudi health care institutes (inclusion criteria). Type-I DM in Saudi institute is defined by fasting blood glucose more than 126 and/or random blood glucose more than 180 mg/dl. The study design was put in compliance with the terms of Helsinki declaration and was approved by the clinical ethics committee in Ministry of Health and all patients gave their informed consent in hospitals of the King Abdul-Aziz University. All analysis were performed in the laboratories of King Abdul-Aziz University hospital.

Clinical assessment

Body mass index (BMI) was calculated as body weight (in kg) divided by squared height (in meters). The procedure for the measurements of weight, height, waist circumference and hip circumference, systolic and diastolic blood pressure was according to the standard procedures within Saudi health care institutes. Any patient with combined sever illness or sever diabetic complications were excluded from the study (exclusion criteria).

Blood sampling

Venous blood samples (duplicate) were withdrawn from peripheral vein while the patient is supine position in heparinized collection tubes. Blood samples were mixed gently, centrifuged and the plasma layers were kept frozen at −20°C for further analysis. The second plasma sample was collected and kept in -80°C as backup.

Blood glucose determination

Blood glucose levels were automatically analyzed using glucose analyzer (Beckman Paragon, Fullerton, CA, USA). The assay principle depends on the rate of oxygen consumption by glucose oxidase enzyme into gluconic acid and hydrogen peroxide. Oxygen rate of consumption was determined by sensitive oxygen electrode. The instrument was calibrated prior to glucose determination using quality control samples provided with the solutions. The mean values obtained on the controls were within the values quoted by the manufacturer. The results were expressed by SI units (mmol/l). The intra and inter assay coefficient of variation were 3.7% and 1.9%, respectively.

Biochemical assessments

Biochemical assessments were assessed in isolated plasma using specific kits purchased from Dade Behring, Marburg, Germany. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed as previously described [14]. Creatinine was determined via picric acid chromophore interaction assay [15]. Creatinine kinase (CK), Alkaline phosphatase (ALP) and blood urea nitrogen (BUN) were determined in plasma using the manufacturer standard operating protocol of the kit.

Serum lipid analysis

Analysis of cholesterol and Triglyceride were perforemd on Cobas Mira S Clinical Analyzer, Roche Diagnostics. The assay for total cholesterol was done using the enzymatic method, which is an enzymatic colorimetric test with cholesterol esterase and cholesterol oxidase. Triglycerides were also assayed using an enzymatic colorimetric test with glycerol phosphate oxidase. A high density lipoprotein (HDL) cholesterol precipitating reagent, magnesium sulphate at concentration of 0.26 mol./l was used to remove low density lipoprotein (LDL) cholesterol, total cholesterol and very low density lipoprotein (VLDL). HDL cholesterol was, then, determined by an enzymatic colorimetric method. LDL cholesterol was estimated by using the formula: LDL cholesterol=total cholesterol − (HDL cholesterol+0.46×triglyceride). The intra and inter-assay coefficients of variation were 4.1% and 3.4%, respectively, for total cholesterol and 3.9% and 3.5% respectively, for triglyceride [16,17].

Amino acid analysis

The plasma was separated from heparinized blood samples by centrifugation at 4°C, and one aliquot were deproteinized with sulfosalicylic acid (10% w/v) as described, and the supernatant stored along with another aliquot of untreated plasma at -20°C. Individual amino acids were determined on the SSA supernatants by standard procedures using lithium buffer systems on a Beckman 121M Amino Acid Analyzer (Beckman Instruments. Inc., Palo Alto, CA). No specific measures were taken apart from minimizing the period between sampling and analysis, to assure the stability of glutamine, glutamate and aspartate. The values reported reflect this approach to sample handling [18]. Tryptophan was analyzed separately in subsequently trichloracetic acid precipitated plasma and dialysate by a fluorimetric method [19].

Statistical analysis

Data is expressed as scatter plot in addition to mean ± SD. Analysis of variance (ANOVA) with LSD's post hoc test was used for testing the significance between groups using SPSS® for windows, version 17.0.0. $P<0.05$ was taken as the cut off value for significance.

Results and Discussion

Glycemic, lipid profile and biochemical assessment in Saudi diabetic patients

In the current study, to evaluate the effect of glycemic status on amino acid profile in Saudi diabetic patients; fasting serum glucose level was taken as the primary criterion of group segregation into hyperglycemic and normoglycemic patient. Patient demographic criteria were assessed and there was no significant difference in mean body mass index (BMI) of different groups. Serum glucose of 140 mg/dl was considered as cut-off value for hyperglycemia. Mean blood glucose was found significantly higher in hyperglycemic group (216.8 ± 66.3 mg/dl) compared to normoglycemic group (109.7 ± 18.9 mg/dl) (Table 1). All patients undertaken for the study were on oral hyperglycemic treatment at the regular prescribed doses. Yet, the level of hyperglycemia in hyperglycemic groups reflects either treatment non-compliance or the need for dose adjustment.

With respect to lipid profile, no significant differences between normoglycemic and hyperglycemic patients were detected in total cholesterol, HDL-c, LDL-c or TG at $p<0.05$ (Table 1). Accordingly, the homogeneity in the metabolic profile could be assumed between both tested groups despite the transient elevated blood glucose levels. Only serum TG in was higher than normal value. That might be explained by the disturbance in lipolysis in DM rather than direct effect of glucose level. In addition, nutritional implication cannot be ignored as a determinant factor in increasing serum TG in Saudi diabetic patients.

	Normoglycemic group (n=29)	Hyperglycemic group (n=36)
Petient demographic	Male: n=13 Female: n=16 Age: 37.6 ± 4.7 years Height: 172.5 ± 21.7 cm Weight: 67.2 ± 3.1 Kg	Male: n=14 Female: n=22 Age: 33.6 ± 7.1 years Height: 168.4 ± 18.9 cm Weight: 64.6 ± 4.2 Kg
Glucose	109.7 ± 18.8	216.8 ± 66.3*
Cholesterol	168.5 ± 47.2	164.9 ± 30.2
HDL-c	54.2 ± 12.6	56.6 ± 9.4
LDL-c	94.2 ± 46.8	80.7 ± 31.6
TG	137.6 ± 47.6	154.5 ± 125.7
ALP	94.8 ± 24.18	100.6 ± 24.0
CK	0.88 ± 0.4	0.78 ± 0.6
ALT	21.1 ± 10.8	20.0 ± 8.9
AST	16.3 ± 6.4	16.2 ± 6.5
BUN	17.0 ± 12.1	13.9 ± 5.9

Table 1: Demographic information, glycemic, lipid profile and biochemical parameters in normoglycemic and hyperglycemic Saudi diabetic patients.

Serum triglycerides have been reported previously to be influenced by several metabolic disorders and hormone level fluctuation [20,21].

In terms of muscle (ALP and CK), kidney (BUN and CK), and liver (ALP, AST and ALT) biochemical markers, no significant changes in either of these parameters between hyperglycemic and normoglycemic Saudi diabetic patients at $p<0.05$ (Table 1). Yet, no patient with significant debilitation was allowed in this study; which appeared from the close and normal biochemical parameters of both tested groups.

Effect of glycemic status on the major categories of amino acids in plasma of Saudi diabetic patients

The total concentrations of major amino acid categories (essential, semi-essential and metabolic indicator amino acids) was assessed in plasma of hyperglycemic and normoglycemic diabetic Saudi patients. No significant difference between the total amino acid concentrations was detected between normoglycemic and hyperglycemic patients (Figure 1A). Also, the total essential and semi-essential was not significantly different between both tested groups (Figure 1B and 1C). This could be attributed to normal nutritional intake of both groups and absence of any debilitating disease [22]. However, the total level of metabolic amino acids was significantly lower in hyperglycemic groups (13.9 ± 2.9 mg%) compared to normoglycemic patients (5.7 ± 1.2 mg%) at $p<0.05$ (Figure 1D). Considering diabetes mellitus as a major metabolic disease, would explain the significant fluctuation between metabolic amino acids between both tested groups. The role of amino acids in the metabolic trait of DM has been suggested previously; however, very obscured in Saudi patients population [1].

Effect of glycemic status on the essential amino acids in plasma of Saudi diabetic patients

By analyzing the individual essential amino acids, only TYR showed significant decrease in plasma of hyperglycemic patients (4.3 ± 1.2 mg%) compared to normoglycemic group (1.2 ± 0.3 mg%) at $p<0.05$ (Figure 2E). The oxidized form of TYP has been reported to induce TYP depletion in DM [23]. Besides, the diabetes induced change in TYR level is associated with the neuronal complication of DM [24].

No significant differences in the other tested essential amino acids between hyperglycemic and normoglycemic diabetic Saudi patients (Figure 2). It is known that essential amino acids cannot be synthesized by the human regular physiological processes and must be taken from exterior dietary source. The similar level of all other essential amino acids can be explained in diabetic patient by the punctuate source of nutritional proteins representing the major external source of amino acids [10].

Effect of glycemic status on the semi-essential amino acids in plasma of Saudi diabetic patients

With respect to semi-essential amino acids, plasma concentrations of CYS2 was significantly decreased in hyperglycemic Saudi patients (2.9 ± 0.8 mg%) compared to normoglycemic group (1.2 ± 0.3 mg%) at $p<0.05$ (Figure 3-B). Other semi-essential amino acids did not show any significant change between hyperglycemic and normoglycemic groups (Figures 3A, 3C and 3D). CYS2 is essential for the protective role of glutathione in DM complications. The decreased level of CYS2 might implicate the early signs of DM-induced oxidative debilitation [25]. Plasma levels of CYS2 and HCY2 have been used as surrogate markers to assess renal glomerular filteration and kidney performance in diabetic patients [26].

Effect of glycemic status on the metabolic indicator amino acids in plasma of Saudi diabetic patients

Interestingly, three metabolic indicator amino acids (AAA, ALA and ORN) were significantly decreased in hyperglycemic patients compared to normoglycemic group at $p<0.05$. AAA plasma level dropped from 3.4 ± 0.8 mg% in normoglycemic patients to 1.4 ± 0.5

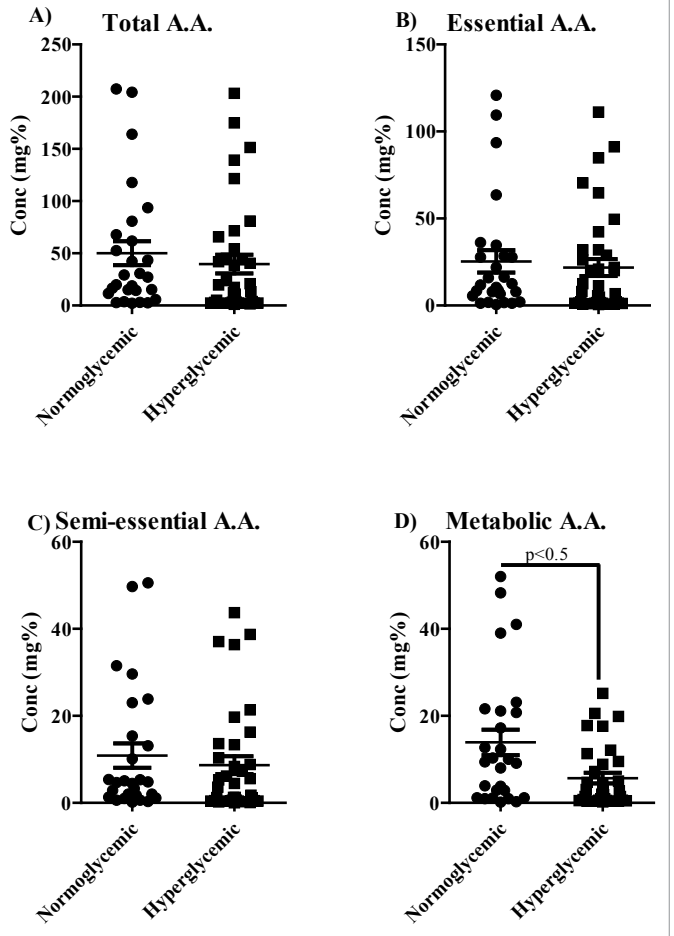

Figure 1: The relationship between the glycemic status and the plasma concentration of amino acids in DM type-I Saudi patients.

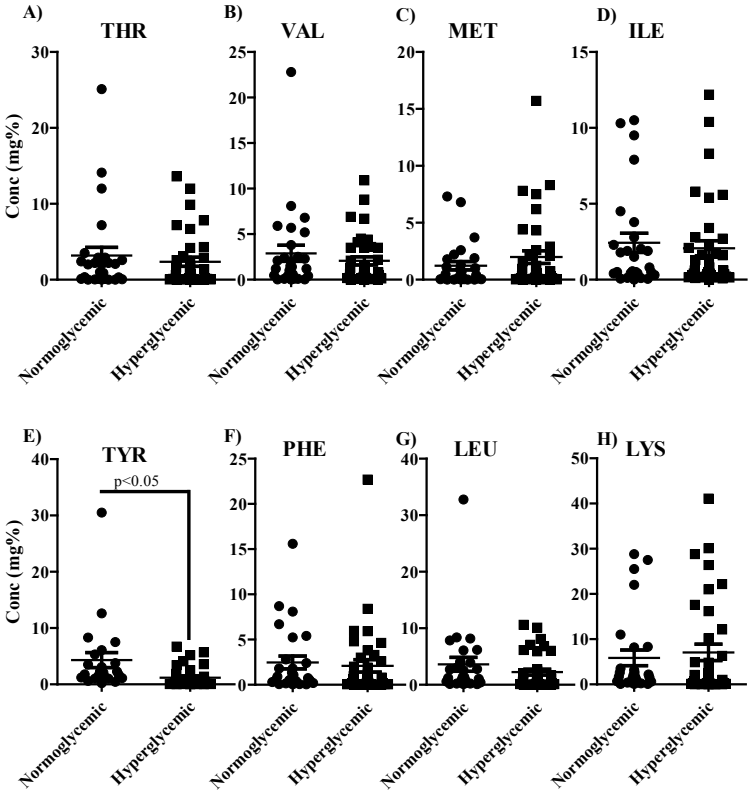

Figure 2: The relationship between the glycemic status and the plasma concentration profile of essential amino acids in DM type-I Saudi patients.

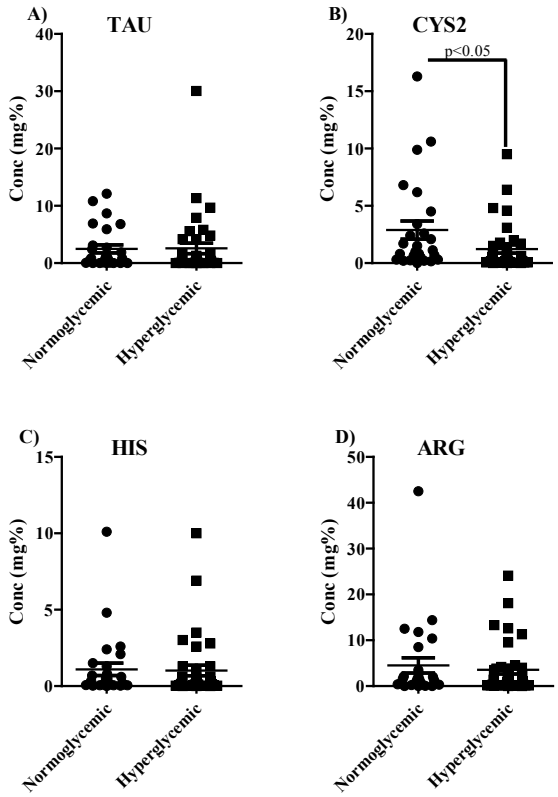

Figure 3: The relationship between the glycemic status and the plasma concentration profile of semi-essential amino acids in DM type-I Saudi patients.

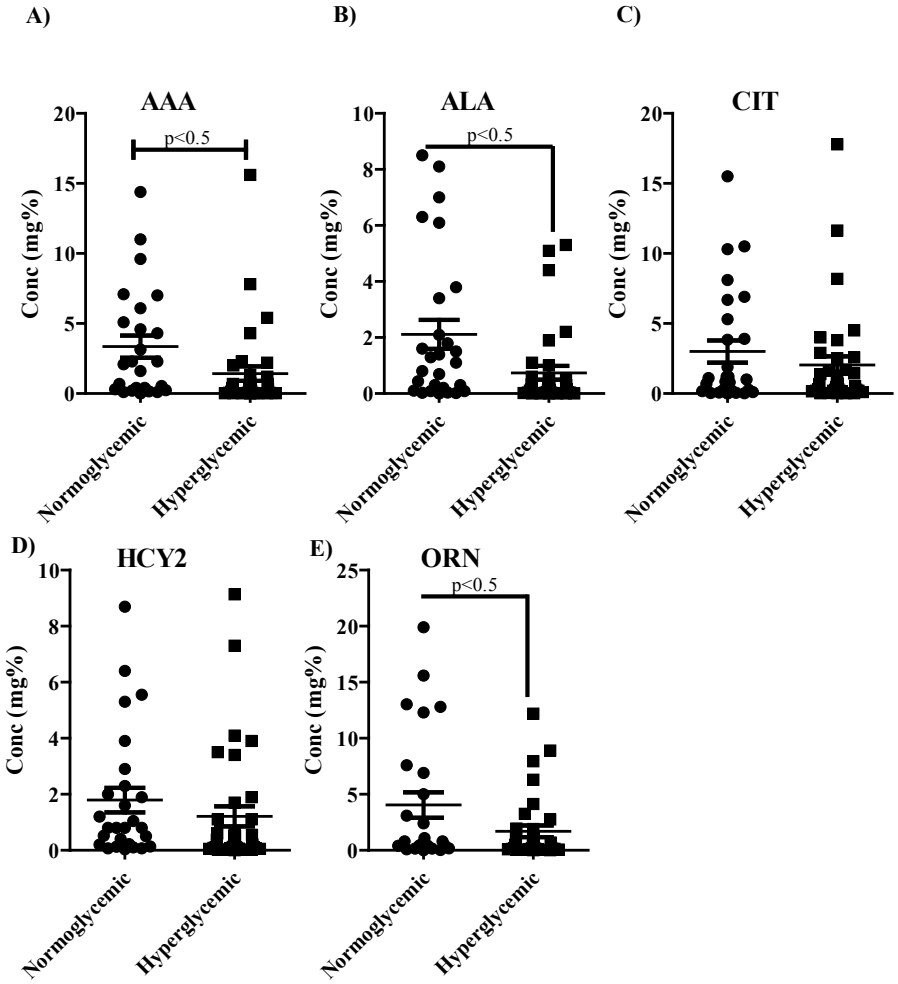

Figure 4: The relationship between the glycemic status and the plasma concentration profile of metabolic indicator amino acids in DM type-I Saudi patients.

mg% in hyperglycemic group (Figure. 4A). ALA and CIT dropped from 2.1 ± 0.5 and 4.1 ± 1.1 mg%, respectively in normoglycemic group to 0.7 ± 0.2 and 1.7 ± 0.5 mg%, respectively in hyperglycemic patients (Figures 4B and 4E). In contrary, the plasma levels of CIT and HYC2 did not change significantly due to hyperglecemia (Figures 4C and 4D). It was previously reported that 2-amino adipic acid (AAA) can be used as a marker of protein carbonyl oxidation in diabetes, renal failure and sepsis [27]. ORN as well has been strong influence of renal physiology and glomerular filtration rate [28]. Accordingly, AAA and ORN can be used as early surrogate marker for the major diabetic vascular renal complications. In addition, hyperglycemia was reported previously to interfere with the mitochondrial metabolism of CIT [29]. On the other hand, diabetic trait was reported to affect the release of ALA amino acid [30]. This would explain the decreased serum level of ALA after transient hyperglycemia.

Conclusion

We have shown the influence of transient hyperglycemia in Saudi diabetic patients on the serum level of several amino acids of different categories. It is suggested for the fore mentioned amino acids to be used as surrogate markers to expect some diabetic debilitating complications in Saudi patient population.

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