International Journal of Science and Research (IJSR)
ISSN (Online) : 2319-7064
Index Copernicus Value (2013) : 6.14 | Impact Factor (2015) : 6.391

Metabolism of Leucine in Regulation of Insulin Secretion from Pancreatic Beta Cells (A Study in Khartoum State)

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Abstract: ***Branched-chain amino acids (BCAAs), including leucine, isoleucine and valine, are essential amino acids that cannot be manufactured in humans or other vertebrates and thus must be supplied in daily diet. BCAAs, in particular leucine, play a critical role in controlling protein synthesis by modulating translation initiation in various cells. Leucine is well known to acutely stimulate insulin secretion from pancreatic β cells by serving as both metabolic fuel and allosteric activator of glutamate dehydrogenase (GDH) (1) (2) (3). Recent reports indicate that leucine or its transaminated product α-ketoisocaproate (KIC) might impact on insulin secretion via a direct inhibition of β cell K_{ATP} currents (4). In the past decade, leucine had been demonstrated to activate the mammalian target of rapamycin (mTOR), a serine and threonine protein kinase that regulates protein synthesis and cell metabolism, in pancreatic β cells (5). To date, leucine has been proven to stimulate gene transcription and protein synthesis in pancreatic islets or other cell types by both mTOR-dependent and -independent pathways (6) (7) (8) (9). Leucine was reported to affect glucagon and insulin secretion in the pancreas (10). To our knowledge no research has been done to investigate leucine amino acid associated with insulin secretion in diabetic patients type two in Sudan. In this study, we intended to to find differences in the levels of leucine between Sudanese patients with diabetes mellitus type 2.and a control group and to measure the serum level of insulin in Sudanese patients with diabetes mellitus type 2. To correlate between the serum levels of leucine and the serum levels of insulin in Sudanese patients with diabetes mellitus type 2. To assess the relationship between the serum levels of leucine and the serum levels of insulin versus: HbA1c, Body mass index, duration of diabetes. To determine age, gender, life style association with diabetes mellitus type 2 in Sudan. Method: Descriptive analytic cross sectional and hospital based study. Samples were collected from different diabetes centers and hospitals in Khartoum state, Serum levels of leucine were measured using amino acid auto analyzer. Serum levels of insulin hormone were measured using ELIZA technique.HbA1c percentage were measured by ion exchange resin chromatography. Result: 87 Sudanese patients with type2 diabetes mellitus were enrolled in this study in contrast to 10 healthy volunteers (Age and sex matched) as control.53 male, 44 female. the age range from 20 to 80, our results showed significantly higher levels of leucine among the diabetic patients (mean=494.390) compared to a control group (mean=330.007), also significantly higher levels of insulin was observed among the diabetic patients (mean=15.912) compared to a control group (mean=7.72), our results showed significantly higher levels of HA1C (mean=8.9) in diabetic patients compared to a control group (mean=5.3) conclusion and recommendation: Significant difference in levels of insulin between diabetics and non-diabetics were observed. The altered levels of insulin in diabetic patients could be a suitable predictor of increasing leucine in their blood sample, is a condition in which there are excess levels of leucine circulating in the blood.***

Keywords: Insulin, Beta cells, Pancreas

1. Introduction

The prevalence of type 2 diabetes is soaring worldwide and is now recognized as one of the main threats to human health being associated with co morbidities, such as cardiovascular disease. The prevalence of DM in the Sudan, as in many other low-income countries, is increasing to epidemic proportions, leading to the emergence of a public health problem of major socio-economic impact. Before 1989 all knowledge about DM in the Sudanese population was based on a few hospital-based studies. Diabetes is a metabolic disease that is characterized by increased blood glucose, which may be due to the pancreatic β-cell dysfunction. This dysfunction leads to a lack of insulin production (type 1 diabetes, T1DM) or to development of insulin resistance (type 2 diabetes, T2DM). Insulin is the key hormone for metabolizing glucose; it facilitates glucose transport in- to cells, where glucose serves as an energy source.

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As aforementioned, high-protein diets are associated with impaired glucose tolerance, insulin resistance and an increased incidence of type 2 diabetes (11). Protein consists of amino acids (AAs). AAs were traditionally classified as essential or non-essential for humans and animals. Essential AAs cannot be synthesized from other compounds in the body at the level required for normal growth, so they must be obtained from food. Leucine, isoleucine and valine are named as branched-chain amino acids (BCAAs). BCAAs are the most abundant of the essential AAs. Leucine is the most abundant BCAA in many dietary proteins, it is found in cow milk, deferent types of cheese, yogurt, meat, chicken, sea food, white kidney beans, peanuts. Accounting for over 20% of total dietary protein obtained from the human diet. Of the AAs studied, the BCAAs have generated the most research interest, as they have emerged as potential biomarkers of metabolic disease. Circulating levels of BCAAs are elevated in individuals with obesity, impaired fasting glucose and type 2 diabetes (12). Furthermore, circulating

levels of BCAAs have the potential to predict development of type 2 diabetes (13) If cells do not get enough energy, there are other energy sources like lipids and proteins [4]. Deficiency of insulin contributes to increased gluconeogenesis, increased glycogenolysis and increased protein breakdown in skeletal muscle [5]. Therefore, the altered levels of amino acids can serve as potential biomarkers of diabetes. Type 2 diabetes is a condition characterized by abnormalities in carbohydrate, lipid and protein metabolism, with the most characteristic features being hyperglycemia and dyslipidemia. The underlying pathological aberrations comprise insulin resistance and bihormonal dysfunction of the pancreatic α- and β-cell Amino acids are important modulators of glucose metabolism, insulin secretion and insulin sensitivity. However, little is known about the changes in leucine amino acid metabolism in patients with diabetes.

2. Method and Materials

Study Approach: quantitative approach

Study Design: Descriptive analytic cross sectional and hospital based study.

Study Area: Samples were collected from different diabetes centers and hospitals in Khartoum state

Target Population and Sample Size: 88 Sudanese patients with type2 diabetes mellitus were enrolled in this study in contrast to 10 healthy volunteers (Age and sex matched) were involved as control.

Inclusion Criteria:

a- Test group :Sudanese patient with type 2 diabetes mellitus (male and female)
b- Control group: healthy volunteers were matched for age and sex.

Exclusion criteria: Patients with diabetic ketoacidosis, liver failure were excluded from the study.

Ethical consideration:

- Permission of this study was obtained from the local authorities in the area of the study.
- The aims and the benefits of the study were explained to the participants with assurance of confidentiality.
- Informed consents were obtained from all participants.
- Health education was provided to all participants.

Data collection and analysis:

Interview with the patients were done to obtain clinical data and to provide health education. Also questionnaire sheet were recorded by the patients.

Study Variables and Methods of measurement:

- Serum levels of leucine were measured using amino acid auto analyzer.
- Serum levels of insulin hormone were measured using ELIZA technique.
- HbA1c percentage was measured by ion exchange resin chromatography.

A total of 87 Sudanese patients with type2 diabetes mellitus were enrolled in this study in contrast to 10 healthy volunteers (Age and sex matched) were involved as control. The study population was divided into males (n =53) and females (n = 44)

Exclusion criteria included Patients with diabetic ketoacidosis, liver failure.

Venous blood samples were obtained in heparinised tubes after an overnight fast from each participant after signing a consent form. some of whole blood put in separate tube to test HBA1C by ion exchange resin chromatography, Plasma was separated within half an hour after collection by centrifugation at 3000 rpm for 5 minutes some of plasma separated for doing insulin test and kept at -20°C until analysis by ELIZA, the rest of plasma undergo Protein precipitated by 20% sulfosalicylic acid, centrifuged at 4°C for 15 min at 12000 rpm and the clear supernatant was kept at -80°C until analysis. Plasma leucine was determined by automated ion-exchange chromatography with ninhydrin, using an amino acid analyzer (Sykam S 334, Munich, Germany) following standard procedures. An amino acid standard solution was included in each run together with an internal control. Data Collection and Analysis: Data collected in the tabulated database sheet and analyzed by SPSS. The data included the age, gender, weight, height, bodymassindex, insulin, HBA1C, leucine findings

3. Results

A total of 87 with type 2 Sudanese diabetic patient where recruited in this study Males constituted 46 individuals (52.8%), and females 41 individuals (47.2%). The age range was from 20 years to 80 years. Results are shown in Table 1 and 2 and 3and 4and 5. We found significantly increased levels of leucine among the diabetic patients (mean=494.390) compared to a control group (mean=330.007), also significantly higher level of leucine seen among female diabetic patients (mean=136.610) compared to male diabetic patients (mean=126.53), also significantly higher levels of insulin was observed among the diabetic patients (mean=15.912) compared to a control group (mean=7.72), our results showed significantly higher levels of HA1C (mean=8.9) in diabetic patients compared to a control group (mean=5.3)

Table 1: Shows Frequency Distribution

Gender	*Frequency*	*Percentage*
Male	46	52.8 %
Female	41	47.2%
Total	87	100%

Figure 1: Shows Frequency Distribution

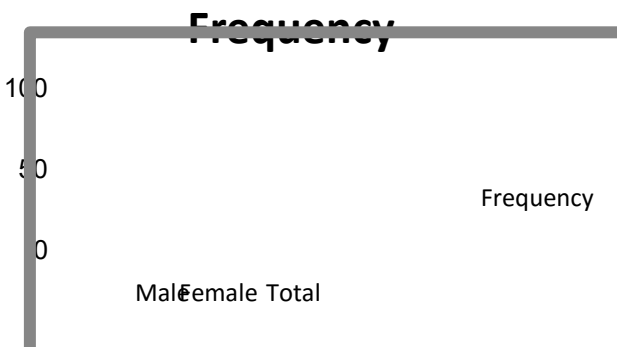
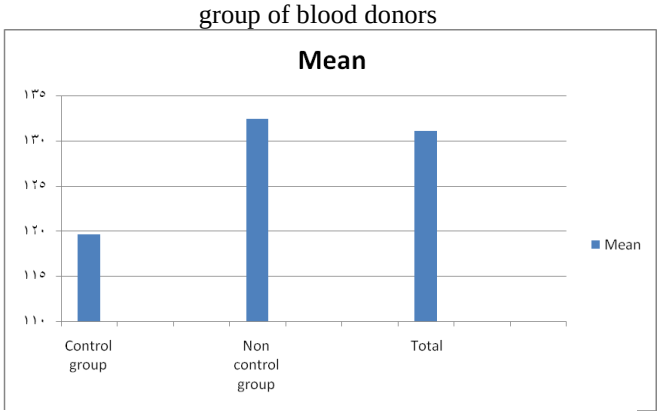


Table 2: Comparison of leucine level between patients with diabetes mellitus (type 2) and a control group of blood donors

Control, Noncontrol group		Leucine
Control group	Mean	119.655600
	N	10
	Std.Deviation	10.9985882
Non control group	Mean	132.422517
	N	87
	Std.Deviation	45.9503121
Total	Mean	131.106340
	N	97
	Std.Deviation	43.7956558

Figure 2: Shows Comparison of leucine level between patients with diabetes mellitus (type 2) and a control group of blood donors



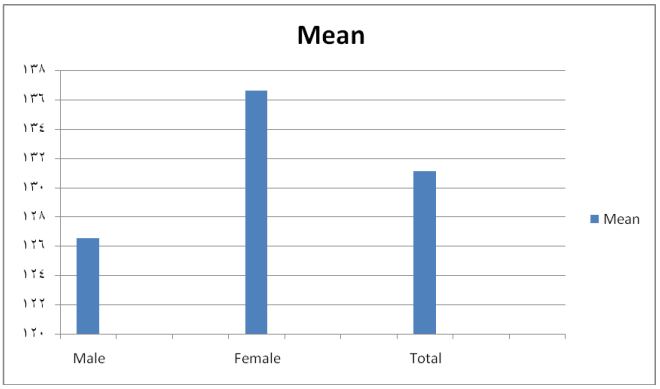
To date, the mechanism by which leucine up regulates GK and ATPβ still remains unknown. However, recent studies have suggested that leucine signaling pathway may have crosstalk with some transcriptors or nuclear receptors including PDX-1 (16), LXR (17) and PPARγ (18) (19) (20) in up regulation of GK and ATPβ.

Overall, the decrease in mitochondrial ATP synthesis rate is associated with the progression of pancreatic islet dysfunction and type 2 diabetes. To elevate cellular ATP synthesis rate by leucine-mediated up regulation of ATPβ or other metabolic enzymes may represent a potential intervention strategy for treatment of islet dysfunction and type 2 diabetes.

Table 3: Comparison of leucine level between male and female

Sex		Leucine
Male	Mean	126.536623
	N	53
	Std. Deviation	43.4435981
Female	Mean	136.610773
	N	44
	Std. Deviation	44.0802114
Total	Mean	131.106340
	N	97
	Std. Deviation	43.7956558

Figure 3: Shows Comparison of leucine level between male and female:



Only a few papers have directly addressed the question of sex dimorphism in protein metabolism in older persons. Surprisingly, two of these papers reported a higher muscle protein synthesis rate in older women as compared to BMI-matched and age-matched men (21) (26) despite the women having approximately 25% less fat-free mass, total muscle mass, and leg muscle volume than the men. It is unclear, however, when these differences begin to manifest. One recent study suggests that such a sexual dimorphism does not occur until later in life, as muscle protein synthesis was reported to be similar in middle-aged women and men (22) However, another paper reported higher protein turnover rates in women throughout adult life (21) adiposity can accelerate protein turnover (23) (24) (25) it is possible that the reported differences between men and women, when present, could be mainly driven by differences in relative body fat mass rather than sex. Future studies are warranted.

Table 4: Correlations between insulin in patients with diabetes mellitus (type2) and a control group of blood donors

Control, and Noncontrol group		Insulin
Control group	Mean	7.720000
	N	10
	Std. Deviation	1.8718974
Non control group	Mean	15.912644
	N	87
	Std. Deviation	2.5835258
Total	Mean	15.068041
	N	97
	Std.Deviation	3.5466400

Figure 4: Correlations between insulin in patients with diabetes mellitus (type2) and a control group of blood donors

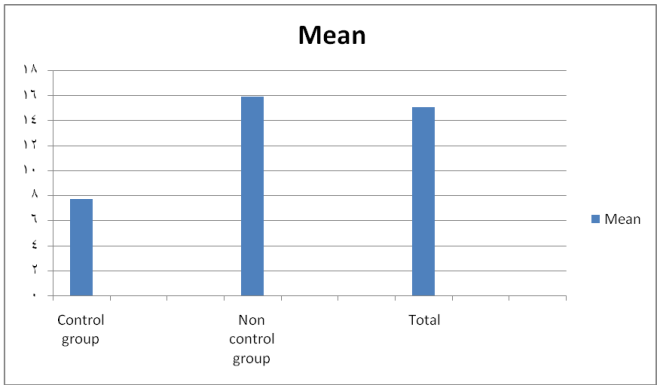
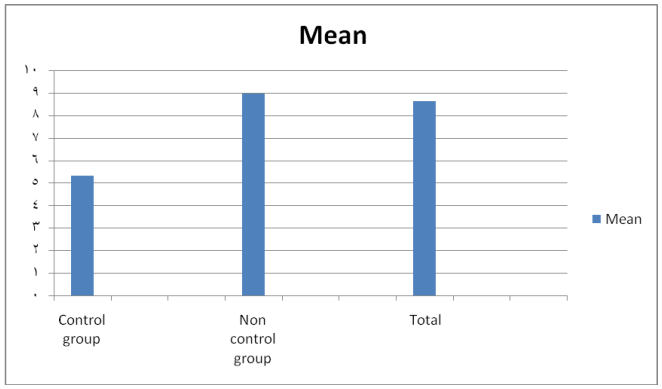


Figure 5: Shows Correlations betweenHbA1C in patients with diabetes mellitus (type2) and a control group of blood donors



Ingestion of proteins or amino acids together with carbohydrates leads to strong insulin secretion in humans and animal models (29) (30) (31) Leucine is one of the most potent insulin secret agog among the branched-chain amino acids that facilitates glucose-induced insulin release from pancreatic β-cells (32). The mechanisms by which leucine exerts its secret agog effects vary (33). Leucine can either serve as a fuel source for ATP production or be converted to α-ketoisocaproate, a metabolic intermediate that in turn inhibits K_{ATP} channel activity, leading to membrane depolarization and triggering insulin secretion (34), (35). Leucine also regulates insulin release by acting on glutamate dehydrogenase (32), a key enzyme that fuels amino acids into the tricarboxylic acid cycle (36). Additional routes of action include triggering calcium oscillations in pancreatic β-cells (33), (37) and regulating the expression of some key genes that are critical for insulin secretion in pancreatic islets (38).

Table 5: Correlations betweenHbA1C in patients with diabetes mellitus (type2) and a control group of blood donors

Control and Non control group		HbA1C
Control group	Mean	5.322222
	N	10
	Std.Deviation	.8227663
Non control group	Mean	8.960920
	N	87
	Std.Deviation	1.4388591
Total	Mean	8.619792
	N	96
	Std.Deviation	1.7515479

Glycated hemoglobin (hemoglobin A1c, HbA_{1c}, A1C, or Hb_{1c}; sometimes also referred to as being HbA1c or HGBA1C) is a form of hemoglobin that is measured primarily to identify the three month average plasma glucose concentration. The test is limited to a three month average because the lifespan of a red blood cell is three months. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. HbA_{1c} is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin. (39) (40). Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous three months before the measurement as this is the lifespan of red blood cells.

In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy. Monitoring HbA_{1c} in type 1 diabetic patients, for the purpose of assessing glycemic control and modifying therapy, may improve outcomes (41).

4. Conclusion and Perspective

Leucine plays important roles in regulation of insulin secretion and cell metabolism of pancreatic β cells via acute and chronic effects.

Allosteric regulation of GDH activity by leucine and/or other molecules has been demonstrated to be a potential intervention strategy for some insulin secretion disorders. In addition, further studies on the distinct mechanism (s) by which leucine regulates the expression of key metabolic genes in pancreatic β cells will shed new light on prevention and treatment of islet dysfunction and type 2 diabetes.

Throughout most points of the lifespan, men and women of similar health status and BMI display fairly similar protein turnover rates. However, some investigations have reported some minor sexual dimorphism in protein metabolism, which may be partly due to differences in fat-free mass and/or methodology. In periods of significant

changes in the hormonal milieu (puberty and menopause), sex differences may become more evident. Finally, anabolic stimuli such as feeding and exercise may help highlight any discrepancies in protein turnover between men and women. However, given the limited sample size of most of these studies it is still not possible to draw a solid conclusion. Future studies are warranted.

Acknowledgment

We sincerely acknowledge the participants diabetic patients who volunteered the blood samples for this study, in different diabetic centers in Khartoum state, deeply acknowledgement normal participants who volunteered blood samples for this study.

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