بسم الله الرحمن الرحيم

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Association of some Amino Acids in Serum with Insulin with Secretion among Sudanese patients with type 2 Diabetes Mellitus - study in Khartoum state

العلاقة بين الاحماض الامينيه في مصل الدم وافراز الانسيولين لدي مرضي السكر من النوع الثاني في السودان – دراسة في ولايه الخرطوم

A Thesis submitted for the fulfillment of the awards of Ph.D degree in biochemistry

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الآية

"قالوا سبحانك لا علم لنا إلا ما علمتنا "

(البقرة 32)

Dedication

I dedicate this work to the soul of my father Mohammed Kheir, to my mother Amna, my husband Eljieli, my daughters Rama and Manal my sisters and brothers for their continuous patience and encouragement.

Acknowledgement

Thank and praise is due to Allah who is thankworthy and who has given me willpower and strength to accomplish this work.

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THESIS OUTCOME

Two papers were published:

1) **Metabolism of Leucine in regulation of insulin secretion from pancreatic beta cells**.

(Study in Khartoum State)

2) **Association of Alanine in Serum with Insulin secretion among Sudanese Patients with Type 2Diabetes Mellitus.**

(Study in Khartoum State)

ABSTRACT

A great number of people all over the world suffer from diabetes. Understanding the mechanisms by which amino acids regulate insulin secretion in vivo may reveal novel sites for targeting drugs for the therapy of type 2 diabetes in the future. A descriptive analytical cross-sectional and hospital based study was done. The objectives of this study were to determine in the levels of selected amino acids in patients with diabetes type 2 and to measure the serum levels of insulin in Sudanese patients with diabetes mellitus type 2. Samples were collected from different diabetes centers and hospitals in Khartoum State from October 2012 to January 2014. A total of 167 Sudanese patients with type2 diabetes mellitus were enrolled in this study with age ranged from 20 to 80 years and 47 healthy volunteers (age and sex matched) were involved as control. The study population was divided into males (n =116) and females (n = 98). Venous blood samples were obtained in heparinised tubes after an overnight fast from each participant. Whole blood was put in separate tubes for HBA1C test by ion exchange resin chromatography. Plasma was separated for running the insulin test using ELISA Plasma protein was 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 glutamate, alanine, luecine, arginine, were determined by automated ion-exchange chromatography with ninhydrin using an amino acid analyzer. Results showed significantly higher levels ofalanine(mean=494.39±242.19)(pvalue=0.000<0.05),leucine(mean=137.54±46.42),(pvalue=0.010<0.05),glutamate(mean=129.34±65.90),(pvalue=0.000<0.05),arginine (Mean=88.66±31.13) (P-value =0.000< 0.05)in diabetic patients. Significantly higher levels of alanine (mean=500.61±235.91) (p-value=0.018<0.05), insignificant increase in leucine (mean=136.31±44.75)(p-value=0.068>0.05),significant increase in glutamate(mean=132.41±63.01) (p-value=0.007<0.05),insignificant increase in arginine (mean=88.28±30.79) (p-value=0.082>0.05) were seen among female diabetic patients. Insulin level was significantly high among the diabetic patients (mean=15.96±2.52) (p-value =0.000<0.05), and was higher in females (mean=13.14±4.12)(p-value =0.000<0.05) than male,. HbA1C levels were significantly increased in diabetic patients (mean=8.97±1.53) (p-value =0.000<0.05), compared to control group, and was significantly high among female diabetic patients (mean=9.27±1.74) (p-value

=0.005<0.05) compared to male diabetic patients. Body Mass Index (BMI) was significantly higher in patients with diabetes mellitus type2 (mean=25.18±3.64)) (p-value =0.005<0.05) and was significantly high among female diabetic patients (mean=25.59±3.97) (p-value =0.005<0.05) compared to male diabetic patients. . In this study some of the known effects of the nutritional compounds on insulin secretion and β-cell metabolism were reviewed. Understanding the molecular mechanisms by which glucose, amino acids regulate insulin secretion may identify novel targets for future diabetes therapies. Although, there is a growing evidences suggesting the beneficial effects of nutrients such as amino acids for the treatment of diabetes. More research is needed to investigate and identify the potential effects of individual nutrient (specific amino acid) supplementation in human clinical trials. In addition, nutrient supplementation could be more effective in the early steps of β-cell dysfunction and, for this reason; the time of the nutritional intervention could be critical for the treatment of diabetes mellitus.

ملخص البحث

أعداد كبيرة من البشر علي نطاق العالم أصبحوا يعانون من الإصابة بمرض السكر.

إن فهم آلية تنظيم الأحماض الأمينية لإفراز الأنسولين داخل الجسم قد تستهدف إنتاج أدويه وعقارات لعلاج مرض السكر من النوع الثاني في المستقبل.

هذه الدراسة ترمي إلي تحديد وتقييم مستويات بعض الأحماض الأمينيه المرتبطة بإفراز الأنسولين لدي مرضي السكر النوع الثاني من السودانيين مقارنه بمجموعه ضابطه من الأشخاص الأصحاء وكذلك قياس مستوي الأنسولين لدي مرضي السكر من النوع الثاني ومقارنتها بنسبه الأنسولين عند الأصحاء.

هذه دراسة مقطعيه الرصد تم جمع العينات لها من مختلف المستشفيات ومراكز السكر في ولاية الخرطوم في الفترة من أكتوبر 2012 إلي يناير 2014,تم قياس معدل الأحماض الأمينيه باستخدام محلل الأحماض الأمينيه الأوتوماتيكي ,كما تم قياس معدل هرمون الأنسولين بجهاز الاليزا , وتم قياس معدل هيموقلبين أي ون سي بجهاز التبادل الأيوني .

شملت هذه الدراسة (167) مريض سكري النوع الثاني من السودانيين تتراوح أعمارهم بين (20)و(80) عام مقابل (47) متطوع من الأشخاص الأصحاء مع مراعاة التوافق في العمر والجنس وقسمت الدراسة إلي (116) شخص من الذكور و(98) من الإناث ,تم الحصول علي عينات دم وريدي في أنابيب هيبا رين بعد ليله صيام , اخذ بعض من الدم الكامل لقياس نسبه هيموقلبين أي ون سي بالتبادل الأيوني كما فصلت البلازما في جهاز الطرد المركزي خلال نصف ساعة للقيام باختبار الأنسولين , أما بالنسبة للأحماض الأمينيه فقد تم ترسيب البروتين بحمض السلفوسالسيلك بتركيز 20% تمهيدا لتحليله بواسطة جهاز تحليل الأحماض الأمينيه .

أظهرت النتائج زيادة مستوي الألانين (494.03) والليوسين (137.54) والقلوتاميت (129.30) والارقنين (88.66) بين مرضي السكر مقارنه بالعينات الضابطة, أيضا زيادة ملحوظة في مستوي الألانين (500.61) والقلوتاميت (132.42) لمرضي السكر الإناث مقارنه مع الذكور من مرضي السكر أما بالنسبة لليوسين (136.31) والأرقنين (88.28)كانت الزيادة غير ملحوظة.

لوحظ في هذه الدراسة ارتفاع نسبه الأنسولين لدي مرضي السكر (15.96) مقارنه بالعينات الضابطة كذلك وجد ارتفاع في نسبه الأنسولين لدي الإناث (16.67) مقارنه بالذكور من مرضي السكر , وجد ارتفاعا في نسبه هيموقلبين أي ون سي لدي مرضي السكر (8.9) مقارنه بالعينات الضابطة, لوحظ زيادة نسبه هيموقلبين أي ون سي لدي الإناث (9.2) مقارنه بالذكور من مرضي السكر ,وجد ارتفاع في معدل كتله الجسم لدي مرضي السكر(25.18) مقارنه بالعينات الضابطة , كما لوحظ ارتفاع معدل كتله الجسم لدي الإناث(25.59) مقارنه بالذكور من مرضي السكر.

في هذه الدراسة تم الاطلاع علي بعض الآثار المعروفة للمركبات الغذائية علي إفراز الأنسولين والتمثيل الغذائي في خلايا بيتا في البنكرياس. وفهم التقنيات الجزئية التي عن طريقها ينظم الجلوكوز والأحماض الأمينيه إفراز الأنسولين وسلامه الخلية, والتي قد تحدد أهدافا جديدة لعلاج مرضي السكر نسبه لتزايد الأدلة التي تشير إلي الآثار المفيدة للمواد الغذائية مثل الأحماض الأمينيه لعلاج مرض السكر . وفيما يتعلق بعلاج مرض السكر من النوع الثاني هنالك حاجه إلي مزيد من الأبحاث لدراسة وتحديد الآثار المحتملة للمغذيات الفردية (حامض أميني معين) كمكملات في التجارب السريريه علي البشر , بالأضافه إلي ذلك تم الاطلاع علي إن المكملات الغذائية يمكنها أن تكون أكثر فعاليه في الخطوات الأولي لعلاج الاختلال الوظيفي في خلايا بيتا و لكل ذلك فقد حان الوقت لاستخدام التدخل الغذائي ليكون حاسما لعلاج مرضي السكر .

LIST OF ABBREVIATIONS

ADMA	Asymmetric dimethyl arginine
ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BCAA	Branched chain amino acids
BCKA	branched-chain α-keto acids
BMI	Body mass index
CREB	cAMP response element binding
CVD	Cardiovascular disease
DNA	Deoxy nucleic acid
GABA	Glutamate-aminobutyrate
GAD	glutamate decarboxylase
GDH	Glutamate dehydrogenase
GLUT	Glucose transporter
GTP	Guanosine triphosphate
HbA1c	Hemoglobin A one C
HDL	High density lipoprotein
IDDM	Insulin dependent diabetes mellitus
IGT	Impaired glucose tolerance
IUGR	intrauterine growth restriction
mRNA	Messenger ribonucleic acid

MSG	monosodium glutamate
mTOR	mammalian target of rapamycin
NEFA	non esterified fatty acids
NIDDM	Non insulin dependent diabetes mellitus
NOS	Nitric oxide synthase
PC1	Pro hormone convertase
PCR	Polymerase chain reaction
T2DM	Type 2 diabetes mellitus
TCA	Tricarboxylic acid
TGN	Trans-Golgi network
TORC	Transducers of regulated CREB

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CHAPTER ONE

INTRODUCTION

CHAPTER ONE

Introduction

1-1-1 Diabetes mellitus:

Diabetes mellitus is a condition in which the body either does not produce enough, or does not properly respond to, insulin, a hormone produced in the pancreas. Insulin enables cells to absorb glucose in order to turn it into energy. In diabetes, the body either fails to properly respond to its own insulin, does not make enough insulin, or both, this causes glucose to accumulate in the blood, often lead to various complications. Many types of diabetes are recognized.

Diabetes mellitus type 1:

Results from the body's failure to produce insulin .Presently almost all persons with type 1 diabetes must take insulin injections.

Diabetes mellitus type 2: –

Diabetes mellitus type 2 or non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes – is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. (International Diabetes Federation. 2006.) Diabetes is often initially managed by increasing exercise and dietary modification. If the condition progresses, medications may be needed. Diabetes mellitus type 2 often affecting the obese.

Unlike type 1 diabetes, there is very little tendency toward ketoacidosis. (Kumar –et-al-2005) One effect that can occur is nonketonic hyperglycemia. Long-term complications from high blood sugar can include increased risk of heart attacks, strokes, amputation, and kidney failure. For extreme cases, circulation of limbs is affected, potentially requiring amputation. Loss of hearing, eyesight, and cognitive ability has also been linked to this condition.

Signs and symptoms:-

The classic symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), fatigue and weight loss (Fasanmade, et-al-2008). Type II diabetes has been associated with an increased risk of cognitive dysfunction and dementia through disease processes such as Alzheimer's disease and vascular dementia. Researchers have shown that reduced glucose tolerance has deleterious effects on memory in the elder (Cooke, et-al 2008).

Causes:-

Type 2 diabetes is due to a combination of lifestyle and genetic factors. (Convit, et-al. 2003) (Risérus, et-al 2009). Recently, intrauterine growth restriction (IUGR) or prenatal under nutrition (macro- and micronutrient) was identified as another probable factor (Ripsin, et-al, 2009).

1-1-2 Amino Acids:-

Specific amino acids are known to acutely and chronically regulate insulin secretion from pancreatic β-cells in vivo and in vitro. Mitochondrial metabolism is crucial for the coupling of amino acid and glucose recognition to exocytosis of insulin granules.. Mitochondria generate ATP, which is the main coupling messenger in insulin secretion, and other coupling factors, which serve as sensors for the control of the exocytotic process. Numerous studies have sought to identify the factors that mediate the key amplifying pathway over the Ca^{2+} signal in nutrient-stimulated insulin secretion. Predominantly, these factors are nucleotides (ATP, GTP, cAMP, and NADPH), although metabolites have also been proposed, such as long-chain acyl-CoA derivatives and glutamate. This scenario further highlights the importance of the key enzymes or transporters, e.g., glutamate dehydrogenase, the aspartate and alanine aminotransferases, and the malate-aspartate shuttle in the control of insulin secretion. In addition, after chronic exposure, amino acids may influence gene expression in the β-cell, which subsequently alters levels of insulin secretion. Therefore, amino acids may play a direct or indirect (via generation of putative messengers of mitochondrial origin) role in insulin secretion(Philip Newsholm, et al, 2006).

Glutamate:-

L-glutamate is the most highly debated amino acid with respect to stimulation of insulin secretion and the possible molecular mechanisms of its action on promotion of secretion

(Gao et-al 1999).

Alanine:-

L-alanine could stimulate insulin secretion under specific conditions of nutrient availability and that the mode of induction of insulin secretion may be a combination of increased ATP production and Na^+ co-transport (Curi, et-al -2005)

Leucine:-

The proposed mechanisms by which l-leucine stimulates insulin release from pancreatic β-cells include *1*) increased mitochondrial metabolism *2*) increased ATP production. In the presence of high glucose, leucine-induced insulin secretion is inhibited (Sener, et-al -2002)

Arginine.:

L-Arginine may alternatively be converted to l-glutamate and thus can influence insulin secretion (Broca, et-al -2003). However, no studies have yet explored l-arginine metabolism in detail in the β-cell; thus, the potential for l-glutamate generation remains to be determined.

1-2 Problems of the study :-

Due to aging, accelerated population growth, urbanization and high prevalence of obesity and an inactive lifestyle, the number of people with diabetes is increasing globally at a rapid speed. Important differences have been reported in the occurrence of DM and its complications between countries and between ethnic, cultural and even age groups within the same country. The prevalence of DM worldwide was estimated at 4% in 1995 and is expected to rise to 5.4% by the year 2025. Consequently, the number of adults with DM will rise from 139 million to 300 million by the year 2025(Murray et al,1996) The major part of this increase will occur in developing countries. There will be 70% increase, from 84 to 128 million, in developing countries, and a 42%increase from 51 to 72 million in the developed countries. According to WHO estimates in 2000 the burden of diabetes is massive globally, with 20-35% of the diabetic patients suffering from neuropathy,30-45% with retinopathy, 10-20% with nephropathy, and from 10 to 25%having cardiovascular disease . Thus, the effect of diabetes on mortality and morbidity, its rapidly growing prevalence, and the high economic and human cost give emphasis on diabetes as a major global public health problem

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.

Amino acids are important modulators of glucose metabolism, insulin secretion and insulin sensitivity. However, little is known about the changes in amino acid metabolism in patients with diabetes.

1-3 Objectives:

1-3-1General Objectives:

To determine the serum levels of glutamate, alanine, luecine, arginine as markers of insulin secretion in Sudanese with type 2 diabetes mellitus.

1-3-2 Specific objectives:

1- To measure the serum levels of glutamate, alanine, luecine, arginine, in Sudanese patient with diabetes mellitus type 2 compared to a control group.
2- To measure the serum levels of insulin in Sudanese patients with diabetes mellitus type 2 compared to a control group.
3- To measure the serum levels of HbA1c in Sudanese patients with diabetes mellitus type 2 compared to a control group.
4- To measure the serum levels of Body mass index in Sudanese patients with diabetes mellitus type 2 compared to a control group.
5- To correlate between the serum levels of glutamate, alanine, luecine, arginine, and the serum levels of insulin in Sudanese patients with diabetes mellitus type2
6- To correlate between the serum levels of glutamate, alanine, luecine, arginine, and the serum levels of HbA1c in Sudanese patients with diabetes mellitus type2
7- To correlate between the serum levels of glutamate, alanine, luecine, arginine, and the serum levels of Body mass index in Sudanese patients with diabetes mellitus type2.
8- To correlate between the serum levels of glutamate ,alanine ,luecine ,arginine versus:
   1- Age.
   2 - Gender.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

Literature Review

2-1 Diabetes mellitus:

Diabetes mellitus is a disease characterized by hyper glycemia; patients develop specific micro vascular and nonspecific macro vascular complications.

2-1-1 Clinical types of diabetes mellitus:

Human diabetes is defined into two forms:

Insulin dependent diabetes mellitus (IDDM, or Type 1), and non-insulin dependent diabetes mellitus (NIDDM, or type11).They differ in their basic mechanisms of development (WHO, 1980).

Recently a third type has been described by WHO study group and referred to as malnutrition-related diabetes mellitus (WHO, 1985).

2-1-2 Insulin dependent diabetes mellitus (IDDM,or type1):

This is also referred to as Juvenile onset, or ketosis-prone diabetes mellitus. It is an autoimmune disorder with abnormalities of both humeral and cellular immunity. It typically affects younger people. There is inherited predisposition and overt disease is triggered by an environmental influence such as a viral infection.

Pancreatic beta cells are destroyed over a prolonged period resulting in severe insulin deficiency which requires treatment by insulin injections.

IDDM is also associated with the development of ketoacidosis and is characterized by absolute deficiency of insulin. (it is often unmeasurable in the circulation when patients first present with ketoacidosis).

2-1-3 Non-insulin dependent diabetes mellitus (NIDDM, or type 11):

This referred to as maturity onset diabetes mellitus, and despite being typically a disorder of middle life has substantial inherited components to its pathogenesis. The development of disease in some subjects depends on environmental factors such as obesity, lack of exercise and smallness at birth; it is characterized by insulin resistance as well as insulin deficiency. The hyperglycemia in this patients can usually be controlled by dietary means alone or if not by addition of an oral hypoglycemic agents .These patients are less prone to develop ketosis .The disease may be caused by abnormality in the peripheral insulin receptors or partial insulin deficiency (Livson et al., 1997).

2-1-4 Tropical diabetes mellitus (malnutrition-related diabetes):

This type is distinct from the other two types common in developed countries. There are two distinguished sub classes:

a) Fibro calculus pancreatic diabetes mellitus:

This is associated with a high intake of cassava food and characterized by the absence of malabsorption caused by an exocrine pancreatic disease, and a history of recurrent abdominal pain from an early age. This type of diabetes mellitus is also characterized by the presence of pancreatic calculi on plain X-Ray and a history of formation of gallstones (Abu bakare et al., 1986).

b) Protein deficient pancreatic diabetes mellitus (previously known as J.type of ketosis resistant youth- onset diabetes mellitus):

This is characterized by a level of blood glucose greater than 200 mg\dl at any time. The onset of this type of diabetes mellitus can occur before thirty years of age.

Body mass index is less than 19kg\m^2 and no ketosis was observed on withdrawal of insulin. This type of diabetes mellitus prevails in patients of poor socioeconomic status. (Ahuj. 1985).

2-1-5 other types of diabetes include:

Secondary diabetes (secondary to disease of pancreas such as chronic pancreatitis, disease of other endocrine glands e.g., acromegaly or Cushing's syndrome, or drug treatment, e.g. with thiazide diuretics.

Other is insulin receptor abnormalities (such as mutations of insulin receptors or conditions with circulating antibodies to the receptor).

Gestational diabetes (diabetes that comes on during pregnancy and which often disappears once pregnancy is complete) and there were certain rare genetic syndromes

Diabetes may be associated with much genetic disorder. The contribution of these to the prevalence of diabetes over all is low, but the scientific importance of these dis orders lies in the insight they provide into the great variety of mechanisms is apparent in the range of biochemical pathology, from insulinopenia Wolfram disease or DIDMOAD syndrome to extreme insulin resistance in Menden hall syndrome, from auto immune IDDM is one of the forms of autoimmune poly glandular syndrome (schmidts syndrome)

To amore mysterios pancreatic beta cell destruction in Frederic ataxia.

The chromosomal loci for the genes responsible for the disorders, clearly the genes responsible are not on the same chromosome, this suggests that different genetic mechanisms underlie the diabetes in each disorder. A further interference is that diabetes can arise as a result of dysfunction in one or more genes at several possible chromosomal sites.

Patients with a strong family history of diabetes are referred to as having potential diabetes. Impaired glucose tolerance (IGT) is the term applied to these patients with glucose values during a glucose tolerance test intermediate between normal and diabetic .These patients suffer the macro vascular disease but are not affected by the specific micro vascular complications of diabetes.

They are also increased risk of impaired glucose tolerance worsening to diabetes (Livson et al., 1997).

2-1-6 Insulin dependent diabetes mellitus:

Etiology and predisposing factors:

IDDM is predominantly a Caucasian disorder most frequently encountered in Northern Europe, particularly Scandinavia it is less common in the southern hemisphere. There some

evidence to suggest that IDDM has increased in frequency since the 1950 .It has increased fourfold for example in Finland. The incidence of IDDM is highest between 10 and 14 years.

It affects males and females equally with a slightly earlier age of onset in girls (by 1-2 years). There is a seasonal variation, with a high incidence in winter than in summer. This seasonal variation is observed in both northern and southern hemisphere, i.e., winter presentation is more likely in both. This has been taken as

Evidence for a possible viral etiology or precipitant, other environmental agents that have been implicated as etiological factors from epidemiological studies include the nitrosamine compounds that are present in high concentration in certain smoked food preparations. The incidence of IDDM is increased in Icelandic boys under the age of 15 years who had been born nine month after a period of traditionally high intake of smoke mutton. Smoked mutton is rich in nitrous amine compounds.

These compounds are toxic to the pancreatic beta cells in experimental animals, such as mice and Chinese hamsters; there is no evidence for the compounds having an etiological influence in other countries.

Other nutrition factors have been proposed foreign proteins in the diet have been implicated in susceptible individuals. The incidence of IDDM is inversely related to the prevalence of breast -feeding (in some but not in all the studies that have examined the relationship). Cow's milk has, therefore, been proposed as an etiological factor. Antibodies to bovine serum albumin are observed more commonly in recently diagnosed diabetics than in controls. These antibodies cross react with a pancreatic beta cell peptide code P69 .This peptide is not normally presented on the beta cell surface, but if it were in certain circumstances(e.g., during inter current infection) the antibodies to bovine serum albumin would be capable of inducing cell damage .

Although this is an attractive idea, a relationship between cow's milk usage in infancy and the subsequent risk of developing has not been demonstrated consistently a clinical syndrome that includes diabetic ketoacidosis has been observed in subjects who ingested (deliberately or by accident) the rat poison vacor. This rodenticide has been developed for the control of warfarin-resistant rate populations. The diabetes, that follows is severely insulin deficient and extensive necrosis of pancreatic beta cells has been observed in those patients who died. Vacor has structural similarities to the experimental beta cell toxins streptozotocin, for example,

induces severe insulin deficiency when given in high dosage to adult rats. It causes beta cell necrosis in association with depletion of nicotinamide adenine dinucleotide (NAD) and ATP content. Administration of nicotinamide prevent the development of beta cell destruction, suggesting that NAD deficiency is of primary importance . Nicotinamide has a similar protective effect with vacor. (Livsonetal., 1997).

The pathology of the pancreas in IDDM is characterized by atrophy of the islets. When patients first present with IDDM, many of the islets are small with abundant fibrous trauma. The architecture of these islets is disrupted. In normal islets, the beta cells are located at the center and the other pancreatic islet cell types are around the periphery. In IDDM islets, the cell content is predominantly alpha and delta types, and a high proportion of the alpha cells is scattered throughout the exocrine tissue, outside the islets. The total number of beta cells in the pancreas is markedly decreased. The content of the other cells in pancreatic islets is normal. (livson et al., 1997).

2-1-7 Non insulin dependent diabetes mellitus:

Etiology and predisposing factors:

NIDDM is a disorder of middle and late life, the age of presentation varies between different populations. The pathology of the pancreas in NIDDM is quite different from IDDM. The extensive beta cell destruction is not observed. Total beta cell mass is diminished but this is highly variable. On average, the beta cell mass is 60% of that observed in the non-diabetic pancreas. The lymphocytic infiltration of IDDM is also absent. A myeloid tissue, which is present in the non-diabetic pancreas with increasing age, is observed to a greater extent in NIDDM, but the role of the myeloid tissue in the pathogenesis of NIDDM is uncertain at present. Both insulin resistance and insulin deficiency are observed in patients with NIDDM.

Insulin deficiency:

Insulin deficiency is observed in all patients, with established NIDDM. Even when circulating insulin concentrations appear to be normal or even elevated above those observed in the non –diabetic state, the levels are lower than would be observed in anon-diabetic in whom the blood glucose was elevated to the diabetic values. In order to establish whether insulin deficiency or resistance is the primary defect in NIDDM, several groups have

investigated subjects predisposed to NIDDM, but before the disorder appears, such at risk populations include first degree relatives of patients with NIDDM, un affected co twins of diabetics, women with gestational diabetes in a previous pregnancy and young un affected members of populations in which the prevalence of diabetes is particularly high. These studies have demonstrated that circulating insulin levels in response to glucose are frequently elevated early in the development of NIDDM. This hyper insulinaemia is however, by no means universal. As diabetes progresses and hyperglycemia develops, insulin deficiency is universal.

Defective processing of proinsulin to insulin has been proposed as a possible primary defect. Proinsulin converted to insulin within the beta cell granule under the influence of two enzymes PC_2 and PC_3. Rare inherited defects of proinsulin processing have been described that result in hugely elevated proinsulin concentrations and diabetes in affected individuals.

Insulin resistance:

Insulin resistance, like insulin deficiency, is universal in established NIDDM. The term refers to the fact that there is a subnormal response to endogenous or exogenous insulin. This resistance to insulin actions extends too many of insulin actions on glucose metabolism. There is resistance to the stimulation of glucose uptake by muscle and liver, and resistance also to the action of insulin to suppress hepatic glucose production.

The muscle defect is primarily in the non-oxidative pathway of glucose metabolism, i.e., in glycogen synthesis. The resistance extends to adipose tissue metabolism, with a reduced ability to suppress lipolysis. This results in an increase in circulating non-esterified fatty acid concentrations, which reduces further the sensitivity to insulin. The cause of insulin resistance is unknown in the majority of subjects. In rare patients, there is a structural abnormality of the insulin receptor or of one of the proteins involved in insulin action intracellularly for the remainder insulin resistance reflects an abnormality early in the intracellular pathways of insulin action occasional patients have circulating antibodies to the insulin receptor that diminish insulin action. For the majority, however, the causes remain unknown.

Insulin resistance is associated with impaired glucose tolerance and affecting large blood vessel and the association with hypertension.

Insulin resistance is also associated with dyslipidemia (raised triglyceride and decreased high density lipoprotein cholesterol this dyslipidemia is observed in insulin resistance subjects even in the absence of diabetes, although glucose tolerance is frequently impaired, by world

health organization criteria. This combination of impaired glucose tolerance, insulin resistance, hypertension and dyslipidemia is referred to as X syndrome (or keavens syndrome). Although a formal link between the lipid disturbance and the vascular disease in NIDDM and in syndrome X has not been established, a causal relationship seems likely.

Pathogenesis of the metabolic disturbances observed in NIDDM:

Insulin resistance in adipose tissue has a primary role in the metabolic disturbances of NIDDM. Adipose tissue lipolysis is normally very sensitive to insulin.

Resistance to insulin's anti-lipolytic action results in elevated non esterified faty acids (NEFA) concentrations

The greater the increase in NEFA, the greater the degree of hyper glycaemia. The increase in plasma NEFA decreases muscle glucose uptake further, through the operation of the glucose fatty acid cycle. Increased hepatic NEFA oxidation leads to increased gluconeogenesis and, therefore, a rise in hepatic glucose output. The resultant increase in fasting glucose concentrations compounds the problem in two ways. First, it leads to a further decreased in insulin secretary capacity this deleterious effect, or glucotoxicity, on insulin secretion is well recognized although poorly understood.

Second glucotoxicity exists also for insulin action, in that arise in fasting glucose levels inhibits subsequent insulin mediated glucose uptake; the changes are reverse all with insulin sensitivity improving its glucose level can be induced to fall.

The increase in fasting NEFA concentrations has additional effects on lipoprotein metabolism VLDL triglyceride secretion by the liver is increased in NIDDM. This is driven by the high NEFA substrate supply (in the presence of adequate amount of insulin for this process).Inconsequence, circulating triglyceride concentration are elevated. The elevation is exaggerated by deficient activity of lipoprotein lipase. This enzyme is present on the endothelial cell surface where it hydrolysis triglyceride rich lipoproteins such as VLDL and chylomicrons levels of high density lipoprotein cholesterol (HDL cholesterol) very inversely with these of triglyceride rich lipoproteins, particularly VLDL. This combination of high triglyceride and low HDL cholesterol together with insulin resistance, has particular importance in the development of macro vascular disease in diabetic. (Livsonetal ., 1997).

2-1-8 Diabetes mellitus in Sudan:

Diabetes mellitus seems take an under estimated health problem in developing countries due to lack of large surveys the prevalence rate in most of these countries is 2-5% (Ekoe, 1985)

Arabs appear to have a high prevalence of NIDDM, Buccus and coworkers (1982) using specific criteria, found a prevalence of 9.6% among male Saudi Arabians in the age group 45-64.

This may at least be partly related to obesity and recent urbanization.

In Africa, diabetes mellitus has become an important cause of morbidity and mortality as a result of the continuing tread towards the urbanization. NIDDM has become more common due to an increase in obesity while IDDM carries a high mortality from ketoacidosis. The prevalence of each type of diabetes mellitus varies considerably across the continent for example a high proportion of patients have type 11 diabetes mellitus in west and south Africa where obesity is less and many patients are lean(Michael, 1990).

In Sudan diabetes mellitus is a common medical problem with considerable morbidity (Abo Asha and Mokhtar, 1976) from the study done by the ministry of Health Registry in 1976 for hospital admission all over the country, diabetic patients seeking treatment constitute about 1.0 to 2.5% of the total population.

Diabetes mellitus is a common cause of severe morbidity recessatating hospital admission was due to diabetes mellitus (Abu Asha and Mokhtar, 1976).

Recently increase in the incidence of diabetes mellitus has been observed especially among urbanized population indicating that diabetes mellitus is emerging as important health problem (Abdel Halim, 1979).

The majority of diabetes are in poor glycemic control this has been attributed to poor compliance to drug, diet and the problem associated with insulin injections (El Mahdi et al .,1989). The morbidity and mortality in diabetes were mostly related to muscular complication and to infection.

Diabetes mellitus stands out as a very important factor in the development of heart disease in the Sudan (Ahmed et al., 1989).

In contrast to other tropical countries, no tropical diabetes mellitus (malnutrition – related diabetes) was demonstrated in the Sudan (El Mahdi et al., 1989).

2-1-9 Biochemistry of diabetes mellitus:

Plasma glucose:

Post prandial hyperglycemia is the major diagnostic criteria for diabetes mellitus. There are abnormalities in both glucose production and peripheral glucose disposal in diabetes mellitus (Koter man et al., 1981). The defect in insulin secretion or resistance to the action of insulin result in the dysregulation of hepatic production of glucose or decreased peripheral utilization of glucose leading to the hyperglycemia the maintenance of a steady –state plasma glucose levels depends on a closed feed lack loop relationship between the circulating glucose levels and the pancreatic islet hormones. Hyperglycemia play a week risk factor in the development of Atherosclerosis (West, 1978).

Diagnostic criteria:

The diagnostic criteria for diabetes were laid down by the world health organization in 1985. They are outlined in table (1-1) :

Table (2-1) world health organization (1985) diagnostic criteria for diabetes (75 gm glucose in an oral glucose tolerance test:

Whole blood glucose			Plasma glucose	
Diabetes	Venous	Capillary	Venous	Capillary
Fasting	≥6.7	≥6.7	≥ 7.8	≥ 7.8
2hour	≥10.0	≥11.1	≥ 11.1	≥ 12.2
Impaired glucose tolerance				

Fasting	≤ 6.7	≤ 6.7	≤ 7.8	≤ 7.8
2hour	6.7-9.9	7.8-11.0	7.8-11.0	8.9-12.1

Note:

Diabetes may be diagnosed without a glucose tolerance test if classical symptoms are present and random plasma venous glucose is ≥ 11.1 mmol\liter (or equivalent) or if the patient is asymptomatic and random glucose levels exceed this limit on two or more occasions.

2-2 Insulin

2-2-1 History

Discovery

In 1869 Paul Langerhans, a medical student in Berlin, was studying the structure of the pancreas under a microscope when he identified some previously unnoticed tissue clumps scattered throughout the bulk of the pancreas. The function of the "little heaps of cells", later known as the islets of Langerhans, was unknown, but Edouard Laguesse later suggested they might produce secretions that play a regulatory role in digestion. Paul Langerhans' son, Archibald, also helped to understand this regulatory role. The term "insulin" originates from *insula*, the Latin word for islet/island.

In 1889, the Polish-German physician Oscar Minkowski, in collaboration with Joseph von Mering, removed the pancreas from a healthy dog to test its assumed role in digestion. Several days after the dog's pancreas was removed, Minkowski's animal keeper noticed a swarm of flies feeding on the dog's urine. On testing the urine, they found there was sugar in the dog's urine, establishing for the first time a relationship between the pancreas and diabetes. In 1901, another major step was taken by Eugene Opie, when he clearly established the link between the islets of Langerhans and diabetes: "Diabetes mellitus is caused by destruction of the islets of Langerhans and occurs only when these bodies are in part or wholly destroyed." Before his work, the link between the pancreas and diabetes was clear, but not the specific role of the islets.

2-2-2 Synthesis, physiological effects, and degradation

Synthesis

Insulin is produced in the pancreas and released when any of several stimuli are detected. These stimuli include ingested protein and glucose in the blood produced from digested food[1]Carbohydrates can be polymers of simple sugars or the simple sugars themselves.

If the carbohydrates include glucose, then that glucose will be absorbed into the bloodstream and blood glucose level will begin to rise. In target cells, insulin initiates a signal transduction, which has the effect of increasing glucose uptake and storage. Finally, insulin is degraded, terminating the response.

Insulin undergoes extensive posttranslational modification along the production pathway. Production and secretion are largely independent; prepared insulin is stored awaiting secretion. Both C-peptide and mature insulin are biologically active. Cell components and proteins in this image are not to scale.

In mammals, insulin is synthesized in the pancreas within the β-cells of the islets of Langerhans. One million to three million islets of Langerhans (pancreatic islets) form the endocrine part of the pancreas, which is primarily an exocrine gland. The endocrine portion accounts for only 2% of the total mass of the pancreas. Within the islets of Langerhans, beta cells constitute 65–80% of all the cells.

Insulin consists of two polypeptide chains, the A- and B- chains, linked together by disulfide bonds. It is however first synthesized as a single polypeptide called preproinsulin in pancreatic β-cells. Preproinsulin contains a 24-residue signal peptide which directs the nascent polypeptide chain to the rough endoplasmic reticulum (RER). The signal peptide is cleaved as the polypeptide is translocated into lumen of the RER, forming proinsulin.(Jennifer C Lovejoy (1999).) In the RER the proinsulin folds into the correct conformation and 3 disulfide bonds are formed. About 5–10 min after its assembly in the endoplasmic reticulum, proinsulin is transported to the trans-Golgi network (TGN) where immature granules are formed. Transport to the TGN may take about 30 min.

Proinsulin undergoes maturation into active insulin through the action of cellular end peptidases known as prohormone convertase (PC1 and PC2), as well as the exoprotease carboxy peptidase(E.Darshan S. Kelley (2008).)

The end peptidases cleave at 2 positions, releasing a fragment called the C-peptide, and leaving 2 peptide chains, the B- and A- chains, linked by 2 disulfide bonds. The cleavage sites are each located after a pair of basic residues (lysine-64 and arginine-65, and arginine-31 and -

32), and after cleavage these 2 pairs of basic residues are removed by the carboxy peptidase.(DariushMozaffarian et al. (2004).) The C-peptide is the central portion of proinsulin, and the primary sequence of proinsulin goes in the order "B-C-A" (the B and A chains were identified on the basis of mass and the C-peptide was discovered later).

The resulting mature insulin is packaged inside mature granules waiting for metabolic signals (such as leucine, arginine, glucose and mannose) and vagal nerve stimulation to be exocytosis from the cell into the circulation (Paul Wilkinson;et al (2004).)

The endogenous production of insulin is regulated in several steps along the synthesis pathway:

- At transcription from the insulin gene
- In mRNA stability
- At the mRNA translation
- In the posttranslational modifications

Insulin and its related proteins have been shown to be produced inside the brain, and reduced levels of these proteins are linked to Alzheimer's disease.(Ebbesson SO et al. (1999).(Ebbesson SO et al. (2005).)

Beta cells in the islets of Langerhans release insulin in two phases. The first phase release is rapidly triggered in response to increased blood glucose levels. The second phase is a sustained, slow release of newly formed vesicles triggered independently of sugar. The description of first phase release is as follows:

- Glucose enters the β-cells through the glucose transporter, GLUT2.
- Glucose goes into glycolysis and the respiratory cycle, where multiple, high-energy ATP molecules are produced by oxidation, leading to a rise in the ATP: ADP ratio within the cell.
- An increased intracellular ATP: ADP ratio closes the ATP-sensitive SUR1/Kir6.2potassium channel (see sulfonylurea receptor). This prevents potassium ions (K^+) from leaving the cell by facilitated diffusion, leading to a build up of potassium ions.

- As a result, the inside of the cell becomes more positive with respect to the outside, leading to the depolarization of the cell surface membrane.
- On depolarization, voltage-gated calcium ion (Ca^{2+}) channels open which allows calcium ions to move into the cells by facilitated diffusion.
- An increased intracellular calcium ion concentration causes the activation of phospholipase C, which cleaves the membrane phospholipids phosphatidyl inositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate and diacylglycerol.
- Inositol 1, 4, 5-trisphosphate (IP3) binds to receptor proteins in the plasma membrane of the endoplasmic reticulum (ER). This allows the release of Ca^{2+} ions from the ER via IP3-gated channels, and further raises the intracellular concentration of calcium ions.
- Significantly increased amounts of calcium ions in the cells cause the release of previously synthesized insulin, which has been stored in secret or vesicles.

This is the primary mechanism for release of insulin. Other substances known to stimulate insulin release include the amino acids arginine and leucine, parasympathetic release of acetylcholine (via phospholipase C), sulfonylurea, cholecystokinin (CCK, via phospholipase C) anSchinner, S.; et al (2005).) the gastro intestinally-derived incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP).

Release of insulin is strongly inhibited by the stress hormonenorepinephrine (nor adrenaline), which leads to increased blood glucose levels during stress. It appears that release of catecholamine's by the sympathetic nervous system has conflicting influences on insulin release by beta cells, because insulin release is inhibited by α_2-adrenergic receptors (Kazunori Koyama et al. (1997).)(And stimulated by β_2-adrenergic receptors.(Michael Roden et al. (1996). The net effect of nor epinephrine from sympathetic nerves and epinephrine from adrenal glands on insulin release is inhibition due to dominance of the α-adrenergic receptors.-

When the glucose level comes down to the usual physiologic value, insulin release from the β-cells slows or stops. If blood glucose levels drop lower than this, especially to dangerously low levels, release of hyperglycemic hormones (most prominently glucagon from islet of Langerhans alpha cells) forces release of glucose into the blood from cellular stores, primarily

liver cell stores of glycogen. By increasing blood glucose, the hyperglycemic hormones prevent or correct life-threatening hypoglycemia.

Evidence of impaired first-phase insulin release can be seen in the glucose tolerance test, demonstrated by a substantially elevated blood glucose level at 30 minutes, a marked drop by 60 minutes, and a steady climb back to baseline levels over the following hourly time points.

2-3-1 Effect of insulin on glucose uptake and metabolism:

Insulin binds to its receptor (1), which starts many protein activation cascades (2). These include translocation of Glut-4 transporter to the plasma membrane and influx of glucose (3), glycogen synthesis (4), glycolysis (5) and triglyceride (6).

The actions of insulin on the global human metabolism level include:

- Control of cellular intake of certain substances, most prominently glucose in muscle and adipose tissue (about two-thirds of body cells)
- Increase of DNA replication and protein synthesis via control of amino acid uptake
- Modification of the activity of numerous enzymes.

The actions of insulin (indirect and direct) on cells include:

- Increased glycogen synthesis – insulin forces storage of glucose in liver (and muscle) cells in the form of glycogen; lowered levels of insulin cause liver cells to convert glycogen to glucose and excrete it into the blood. This is the clinical action of insulin, which is directly useful in reducing high blood glucose levels as in diabetes.
- Increased lipid synthesis – insulin forces fat cells to take in blood lipids, which are converted to triglycerides; lack of insulin causes the reverse.
- Increased esterification of fatty acids – forces adipose tissue to make fats (i.e., triglycerides) from fatty acid esters; lack of insulin causes the reverse.
- Decreased proteolysis – decreasing the breakdown of protein
- Decreased lipolysis – forces reduction in conversion of fat cell lipid stores into blood fatty acids; lack of insulin causes the reverse.

- Decreased gluconeogenesis – decreases production of glucose from non sugar substrates, primarily in the liver (the vast majority of endogenous insulin arriving at the liver never leaves the liver); lack of insulin causes glucose production from assorted substrates in the liver and elsewhere.
- Decreased autophagy - decreased level of degradation of damaged organelles. Postprandial levels inhibit autophagy completely.(Bergaminiet al (2007) .)
- Increased amino acid uptake – forces cells to absorb circulating amino acids; lack of insulin inhibits absorption.
- Increased potassium uptake – forces cells to absorb serum potassium; lack of insulin inhibits absorption. Insulin's increase in cellular potassium uptake lowers potassium levels in blood. This possibly occurs via insulin-induced translocation of the Na+/K+-ATPase to the surface of skeletal muscle cells.(Benziane et al (2008))(Clausen T (2008).
- Arterial muscle tone – forces arterial wall muscle to relax, increasing blood flow, especially in micro arteries; lack of insulin reduces flow by allowing these muscles to contract.
- Increase in the secretion of hydrochloric acid by parietal cells in the stomach
- Decreased renal sodium excretion.(Gupta AK et al (1997).).

Insulin also influences other body functions, such as vascular compliance and cognition. Once insulin enters the human brain, it enhances learning and memory and benefits verbal memory in particular (Benedict et al (2004) .) Enhancing brain insulin signaling by means of intranasal insulin administration also enhances the acute thermoregulatory and gluco regulatory response to food intake, suggesting that central nervous insulin contributes to the control of whole-body energy homeostasis in humans.(Benedict et al (2010).)

2-3-2 Hypoglycemia

Although other cells can use other fuels (most prominently fatty acids), neurons depend on glucose as a source of energy in the non starving human. They do not require insulin to absorb glucose, unlike muscle and adipose tissue, and they have very small internal stores of glycogen. Glycogen stored in liver cells (unlike glycogen stored in muscle cells) can be converted to glucose, and released into the blood, when glucose from digestion is low or absent, and the glycerol backbone in triglycerides can also be used to produce blood glucose.

Sufficient lack of glucose and scarcity of these sources of glucose can dramatically make itself manifest in the impaired functioning of the central nervous system: dizziness, speech problems, and even loss of consciousness. Low blood glucose level is known as hypoglycemia or, in cases producing unconsciousness, "hypoglycemic coma" (sometimes termed "insulin shock" from the most common causative agent). Endogenous causes of insulin excess (such as an Insulinoma) are very rare, and the overwhelming majority of insulin excess-induced hypoglycemia cases are iatrogenic and usually accidental. A few cases of murder, attempted murder, or suicide using insulin overdoses have been reported, but most insulin shocks appear to be due to errors in dosage of insulin (e.g., 20 units instead of 2) or other unanticipated factors (did not eat as much as anticipated, or exercised more than expected, or unpredicted kinetics of the subcutaneously injected insulin itself).

Possible causes of hypoglycemia include:

- External insulin (usually injected subcutaneously)
- Oral hypoglycemic agents (e.g., any of the sulfonylureas, or similar drugs, which increase insulin release from β-cells in response to a particular blood glucose level)
- Ingestion of low-carbohydrate sugar substitutes in people without diabetes or with type 2 diabetes. Animal studies show these can trigger insulin release, albeit in much smaller quantities than sugar, according to a report in *Discover* magazine, August 2004, p 18. (This can never be a cause of hypoglycemia in patients with type 1 diabetes, since there is no endogenous insulin production to stimulate.)

2-4-1 Diseases and syndromes

There are several conditions in which insulin disturbance is pathologic:

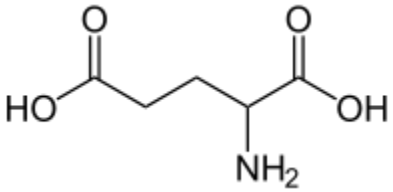
- Diabetes mellitus – general term referring to all states characterized by hyperglycemia
  - Type 1 – autoimmune-mediated destruction of insulin-producing β-cells in the pancreas, resulting in absolute insulin deficiency
  - Type 2 – multifactor syndrome with combined influence of genetic susceptibility and influence of environmental factors, the best known being obesity, age, and physical

inactivity, resulting in insulin resistance in cells requiring insulin for glucose absorption. This form of diabetes is strongly inherited.

- Other types of impaired glucose tolerance (see the Diabetes)

- Insulinoma - a tumor of pancreatic β-cells producing excess insulin or reactive hypoglycemia.
- Metabolic syndrome – a poorly understood condition first called Syndrome X by Gerald Reaven, Reaven's Syndrome after Reaven, CHAOS in Australia (from the signs that seem to travel together). It is currently not clear whether these signs have a single, treatable cause, or are the result of body changes leading to type 2 diabetes. It is characterized by elevated blood pressure, dyslipidemia (disturbances in blood cholesterol forms and other blood lipids), and increased waist circumference (at least in populations in much of the developed world). The basic underlying cause may be the insulin resistance that precedes type 2 diabetes, which is a diminished capacity for insulin response in some tissues (e.g., muscle, fat). It is common that morbidities, such as essential hypertension, obesity, type 2 diabetes, and cardiovascular disease (CVD) develop.
- Polycystic ovary syndrome – a complex syndrome in women in the reproductive years where an ovulation and androgen excess are commonly displayed as hirsutism. In many cases of PCOS, insulin resistance is present.

2-5 Glutamic acid



Glutamic acid (abbreviated as **Glu** or **E**) is one of the 20-22 proteinogenic amino acids, and its codons are GAA and GAG. It is a non-essential amino acid. The carboxylate anions and salts of Glutamic acid are known as **glutamates**. In neuroscience, glutamate is an important neurotransmitter that plays a key role in long-term potentiating and is important for learning and memory (Robert Sapolsky (2005).

2-5-1 Chemistry

The chain acid functional has a pK_a of 4.1 and therefore exists almost entirely in its negatively charged deprotonated carboxylate form at pH values greater than 4.1; therefore, it is negatively charged at physiological pH ranging from 7.35 to 7.45.

2-5-2 History

Although they occur naturally in many foods, the flavor contributions made by Glutamic acid and other amino acids were only scientifically identified early in the twentieth century. The substance was discovered and identified in the year 1866, by the German chemist Karl Heinrich Leopold Ritthausen who treated wheat gluten (for which it was named) with sulfuric acid. (R.H.A. Plimmer (2012). In 1907 Japanese researcher Kikunae Ikeda of the Tokyo Imperial University identified brown crystals left behind after the evaporation of a large amount of Kombu broth as Glutamic acid. These crystals, when tasted, reproduced the ineffable but undeniable flavor he detected in many foods, most especially in seaweed. Professor Ikeda termed this flavor umami. He then patented a method of mass-producing a crystalline salt of Glutamic acid, monosodium glutamate. (Renton, et al (2008)

2-5-3 function and uses

Metabolism

Glutamate is a key compound in cellular metabolism. In humans, dietary proteins are broken down by digestion into amino acids, which serve as metabolic fuel for other functional roles in the body. A key process in amino acid degradation is transamination, in which the amino group of an amino acid is transferred to an α-ketoacid, typically catalyzed by a transaminase. The reaction can be generalized as such:

R_1-amino acid + R_2-α-ketoacid⇌ R_1-α-ketoacid + R_2-amino acid

A very common α-keto acid is α-ketoglutarate, an intermediate in the citric acid cycle. Transamination of α-ketoglutarate gives glutamate. The resulting α-ketoacid product is often a useful one as well, which can contribute as fuel or as a substrate for further metabolism processes. Examples are as follows:

Alanine + α-ketoglutarate pyruvate + glutamate

Aspartate + α-ketoglutarate oxaloacetate + glutamate

Both pyruvate and oxaloacetate are key components of cellular metabolism, contributing as substrates or intermediates in fundamental processes such as glycolysis, gluconeogenesis, and the citric acid cycle.

Glutamate also plays an important role in the body's disposal of excess or waste nitrogen. Glutamate undergoes deamination, an oxidative reaction catalyzed by glutamate dehydrogenase, (Grabowska, et al (2011).)as follows:

Glutamate + H_2O + $NADP^+$ → α-ketoglutarate + NADPH + NH_3 + H^+

Ammonia (as ammonium) is then excreted predominantly as urea, synthesized in the liver. Transamination can, thus, be linked to deamination, effectively allowing nitrogen from the amine groups of amino acids to be removed, via glutamate as an intermediate, and finally excreted from the body in the form of urea.

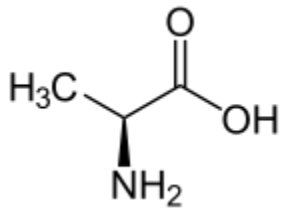
2-5-4 Flavor enhancer

Glutamic acid, being a constituent of protein, is present in every food that contains protein, but it can only be tasted when it is present in an unbound form. Significant amounts of free Glutamic acid are present in a wide variety of foods, including cheese and soy sauce, and is responsible for umami, one of the five basic tastes of the human sense of taste. Glutamic acid is often used as a food additive and flavor enhancer in the form of its salt, known as monosodium glutamate (MSG).

2-5-5 Nutrient

All meats, poultry, fish, eggs, dairy products, and Kombu are excellent sources of Glutamic acid. Some protein-rich plant foods also serve as sources. Thirty to 35% of the protein in wheat is Glutamic acid. Ninety-five percent of the dietary glutamate is metabolized by intestinal cells in a first pass (Reeds, P.J., *et al.* (1 April 2000).

2-6 Alanine



Alanine (abbreviated as **Ala** or **A**)((IUPAC-IUB Recommendations 1983) is an α-amino acid with the chemical formula $CH_3CH(NH_2)COOH$. The L-isomer is one of the 20 amino acids encoded by the genetic code. Its codons are GCU, GCC, GCA, and GCG. It is classified as a nonpolar amino acid. L-Alanine is second only to leucine in rate of occurrence, accounting for 7.8% of the primary structure in a sample of 1,150 proteins.(Doolittle, R. F. (1989), D-Alanine occurs in bacterial cell walls and in some peptide antibiotics.

2-6-1 Structure

The α-carbon atom of alanine is bound with a methyl group ($-CH_3$), making it one of the simplest α-amino acids with respect to molecular structure and also resulting in alanine's being classified as an aliphatic amino acid. The methyl group of alanine is non-reactive and is thus almost never directly involved in protein function.

2-6-2 Sources

Dietary sources

Alanine is a nonessential amino acid, meaning it can be manufactured by the human body, and does not need to be obtained directly through the diet. Alanine is found in a wide variety of foods, but is particularly concentrated in meats.

Good sources of alanine include:

- **Animal sources**: meat, seafood, caseinate, dairy products, eggs, fish, gelatin, lactalbumin

- **Vegetarian sources**: beans, nuts, seeds, soy, whey, brewer's yeast, brown rice, bran, corn, legumes, whole grains.

2-6-3 Biosynthesis

Alanine can be manufactured in the body from pyruvate and branched chain amino acids such as valine, leucine, and isoleucine.

Alanine is most commonly produced by reductive amination of pyruvate. Because transamination reactions are readily reversible and pyruvate pervasive, alanine can be easily formed and thus has close links to metabolic pathways such as glycolysis, gluconeogenesis, and the citric acid cycle. It also arises together with lactate and generates glucose from protein via the alanine cycle.

2-6-4 Physiological function

Glucose–alanine cycle

Alanine plays a key role in glucose–alanine cycle between tissues and liver. In muscle and other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. Glutamate can then transfer its amino group through the action of alanine aminotransferase to pyruvate, a product of muscle glycolysis, forming alanine and α-ketoglutarate. The alanine formed is passed into the blood and transported to the liver. A reverse of the alanine aminotransferase reaction takes place in liver. Pyruvate regenerated forms glucose through gluconeogenesis, which returns to muscle through the circulation system. Glutamate in the liver enters mitochondria and degrades into ammonium ion through the action of glutamate dehydrogenase, which in turn participate in the urea cycle to form urea.

The glucose–alanine cycle enables pyruvate and glutamate to be removed from the muscle and find their way to the liver. Glucose is regenerated from pyruvate and then returned to muscle: the energetic burden of gluconeogenesis is thus imposed on the liver instead of the muscle. All available ATP in muscle is devoted to muscle contraction.(Nelson, et al. (2005),).

2-6-5 Link to diabetes

Alterations in the alanine cycle that increase the levels of serum alanine aminotransferase (ALT) is linked to the development of type II diabetes. With an elevated level of ALT the risk of developing type II diabetes increases.

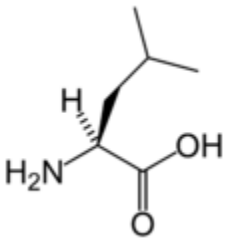
2-6-6 Chemical properties

Free radical stability

The deamination of an alanine molecule produces a stable alkyl free radical, $CH_3C^{\bullet}HCOO^-$. Deamination can be induced in solid or aqueous alanine by radiation.(Zagórski, et al. (1998),

This property of alanine is used in dissymmetric measurements in radiotherapy. When normal alanine is irradiated, the radiation causes certain alanine molecules to become free radicals, and, as these radicals are stable, the free radical content can later be measured by nuclear magnetic resonance in order to find out how much radiation the alanine was exposed to. Radiotherapy treatment plans can be delivered in test mode to alanine pellets, which can then be measured to check that the intended pattern of radiation dose is correctly delivered by the treatment system.

2-7 Leucine



Leucine (abbreviated as **Leu** or **L**)(Commission on Biochemical Nomenclature2007).(is a branched-chain α-amino acid with the chemical formula $HO_2CCH(NH_2)CH_2CH(CH_3)_2$. Leucine is classified as a hydrophobic amino acid due to its aliphatic isobutyl side chain. It is encoded by six codons (UUA, UUG, CUU, CUC, CUA, and CUG) and is a major component

of the subunits in ferritin, astacin and other 'buffer' proteins. Leucine is an essential amino acid, meaning that the human body cannot synthesize it, and it therefore must be ingested.

2-7-1 Biosynthesis

As an essential amino acid, leucine cannot be synthesized by animals. Consequently, it must be ingested, usually as a component of proteins. In plants and microorganisms, leucine is synthesized from pyruvic acid by a series of enzymes :(Nelson, et al ,.(2000).)

- Acetoactate synthase
- Acetohydroxy acid isomeroreductase
- Dihydroxyaciddehydratase
- α-Isopropylmalatesynthase
- α-Isopropylmalateisomerase
- Leucine aminotransferase

Synthesis of the small, hydrophobic amino acid Valine also includes the initial part of this pathway.

2-7-2 Biology

Leucine is utilized in the liver, adipose tissue, and muscle tissue. In adipose and muscle tissue, leucine is used in the formation of sterols, and the combined usage of leucine in these two tissues is seven times greater than its use in the liver.(J. Rosenthal(2008).)

Leucine is the only dietary amino acid that has the capacity to stimulate muscle protein synthesis.(Etzel MR (2004). As a dietary supplement, leucine has been found to slow the degradation of muscle tissue by increasing the synthesis of muscle proteins in aged rats.(L. Combaret et al(2008).,) While once seen as an important part of the three branch chained amino acids in sports supplements, leucine has since earned more attention on its own as a catalyst for muscle growth and muscular insurance. Supplement companies once marketed the "ideal" 2:1:1 ratio of leucine, iso-leucine and valine; but with furthered evidence that leucine

is the most important amino acid for muscle building, it has become much more popular as the primary ingredient in dietary supplements.("Leucine Awesome, Scientists Say". (2011).,)

Leucine potently activates the mammalian target of rapamycin kinase that regulates cell growth. Infusion of leucine into the rat brain has been shown to decrease food intake and body weight via activation of the mTOR pathway.(Cota et al.,(2006).

Leucine toxicity, as seen in decompensate Maple Syrup Urine Disease (MSUD), causes delirium and neurologic compromise, and can be life-threatening.

In yeast genetics, mutants with a defective gene for leucine synthesis (leu2) are transformed with a plasmid that contains a working leucine synthesis gene (LEU2) and grown on minimal media. Leucine synthesis then becomes a useful selectable marker.

2-7-3 Chemical properties

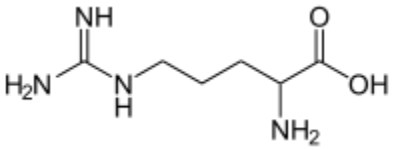
Leucine is a branched-chain amino acid (BCAA) since it possesses an aliphatic side-chain that is non-linear.

Racemic leucine had been subjected to circularly polarized synchrotron radiation in order to better understand the origin of bimolecular asymmetry. An enantiomeric enhancement of 2.6% had been induced, indicating a possible photochemical origin of biomolecules' homochirality.(Meierhenrich (2008).,)

2-7-4 Food additive

As a food additive, L-Leucine has E number**E641** and is classified as a flavor enhancer.

2-8 Arginine



Arginine (abbreviated as **Arg** or **R**)(IUPAC-IUBMB Joint Commission on Biochemical Nomenclature(2007).) is an α-amino acid. It was first isolated in 1886. The L-form is one of the 20 most common natural amino acids. At the level of molecular genetics, in the structure of the messenger ribonucleic acid mRNA, CGU, CGC, CGA, CGG, AGA, and AGG, are the triplets of nucleotide bases or codons that code for arginine during protein synthesis. In mammals, arginine is classified as a semi essential or conditionally essential amino acid, depending on the developmental stage and health status of the individual.(Tapiero, H.; *et al.* (November 2002).) Preterm infants are unable to synthesize or create arginine internally, making the amino acid nutritionally essential for them(Wu, G.; *et al.* (August 2004).)There are some conditions that put an increased demand on the body for the synthesis of L-arginine, including surgical or other trauma, sepsis and burns. Arginine was first isolated from a lupine seedling extract in 1886 by the Swiss chemist Ernst Schultz.

In general, most people do not need to take arginine supplements because the body usually produces enough.

2-8-1 Sources

Dietary sources

Arginine is a conditionally nonessential amino acid, meaning most of the time it can be manufactured by the human body, and does not need to be obtained directly through the diet. The biosynthetic pathway however does not produce sufficient arginine, and some must still be consumed through diet. Individuals who have poor nutrition or certain physical conditions may be advised to increase their intake of foods containing arginine. Arginine is found in a wide variety of foods, including:("L-Arginine Supplements Nitric Oxide Scientific Studies Food Sources". (2007).,)

- Animal sources

dairy products (e.g., cottage cheese, ricotta, milk, yogurt, whey protein drinks), beef, pork (e.g., bacon, ham), gelatin , poultry (e.g. chicken and turkey light meat), wild game (e.g. pheasant, quail), seafood (e.g., halibut, lobster, salmon, shrimp, snails, tuna)

- Plant sources

wheat germ and flour, buckwheat, granola, oatmeal, peanuts, nuts (coconut, pecans, cashews, walnuts, almonds, Brazil nuts, hazelnuts, pine nuts), seeds (pumpkin, sesame, sunflower), chick peas, cooked soybeans, Phalariscanariensis (canary seed or ALPISTE)

2-8-2 Biosynthesis

Arginine is synthesized from coralline by the sequential action of the cytosolic enzymes arginine succinatesynthetase (ASS) and arginine succinatelyase (ASL). In terms of energy, this is costly, as the synthesis of each molecule of arginine o succinate requires hydrolysis of adenosine triphosphate (ATP) to adenosine monophosphate (AMP), i.e., two ATP equivalents. Taking an excess of arginine essentially gives more energy by saving ATPs that can be used elsewhere.

Citrulline can be derived from multiple sources:

- from arginine via nitric oxide synthase (NOS)
- from ornithine via catabolism of proline or glutamine/glutamate
- from asymmetric dimethyl arginine (ADMA) via DDAH

The pathways linking arginine, glutamine, and proline are bidirectional. Thus, the net utilization or production of these amino acids is highly dependent on cell type and developmental stage.

On a whole-body basis, synthesis of arginine occurs principally via the intestinal–renal axis, wherein epithelial cells of the small intestine, which produce coralline primarily from glutamine and glutamate, collaborate with the proximal tubule cells of the kidney, which extract citrulline from the circulation and convert it to arginine, which is returned to the circulation. As a consequence, impairment of small bowel or renal function can reduce endogenous arginine synthesis, thereby increasing the dietary requirement.

Synthesis of arginine from citrulline also occurs at a low level in many other cells, and cellular capacity for arginine synthesis can be markedly increased under circumstances that also induce iNOS. Thus, citrulline, a co product of the NOS-catalyzed reaction, can be recycled to arginine in a pathway known as the citrulline-NO or arginine-citrulline pathway. This is demonstrated by the fact that in many cell types, citrulline can substitute for arginine to some degree in supporting NO synthesis. However, recycling is not quantitative because citrulline accumulates along with nitrate and nitrite, the stable end-products of NO, in NO-producing cells.(Morris Jr, SM (October 2004).)

2-8-3 Function

Arginine plays an important role in cell division, the healing of wounds, removing ammonia from the body, immune function, and the release of hormones. (Stechmiller*et al.* (2005).) (Witte, et al (2003).

The benefits and functions attributed to oral supplementation of L-arginine include:

- Precursor for the synthesis of nitric oxide (NO)(Andrew, et al(1999).)
- Reduces healing time of injuries (particularly bone)
- Quickens repair time of damaged tissue.(Stechmiller*et al.* (2005).) (Witte, et al (2003).
- Helps decrease blood pressure in clinical hypertensive subjects (Gokce, (2004).(Dong JY,et al. (2011). (Rajapakse, *et al.* (2008).

2-9 Amino Acid Metabolism, β-Cell Function, and Diabetes

Specific amino acids are known to acutely and chronically regulate insulin secretion from pancreatic β-cells in vivo and in vitro. Mitochondrial metabolism is crucial for the coupling of amino acid and glucose recognition to exocytosis of insulin granules. This is illustrated by in vitro and in vivo observations discussed in the present review. Mitochondria generate ATP, which is the main coupling messenger in insulin secretion, and other coupling factors, which serve as sensors for the control of the exocytosis process. Numerous studies have sought to identify the factors that mediate the key amplifying pathway over the Ca^{2+} signal in nutrient-stimulated insulin secretion. Predominantly, these factors are nucleotides (ATP, GTP, cAMP, and NADPH), although metabolites have also been proposed, such as long-chain acyl-CoA derivatives and glutamate. This scenario further highlights the importance of the key enzymes or transporters, e.g., glutamate dehydrogenase, the aspartate and alanine amino transferases, and the malate-aspartate shuttle in the control of insulin secretion. In addition, after chronic exposure, amino acids may influence gene expression in the β-cell, which subsequently alters levels of insulin secretion. Therefore, amino acids may play a direct or indirect (via generation of putative messengers of mitochondrial origin) role in insulin secretion.

Amino acids can, under appropriate conditions, enhance insulin secretion from primary islet cells and β-cell lines(Charles et al (1983)(Smith et al 1997). In vivo, l-glutamine and l-alanine are quantitatively the most abundant amino acids in the blood and extracellular fluids followed closely by the branched chain amino acids(Blau et al(2003) However, unlike glucose, individual amino acids do not provoke insulin secretion in vitro when added at physiological concentrations. Combinations of amino acids at physiological concentrations or high concentrations of individual amino acids are much more effective. In vivo, amino acids derived from dietary proteins and those released from intestinal epithelial cells, in combination with glucose, stimulate insulin secretion, thereby leading to protein synthesis and amino acid transport in target tissues such as skeletal muscle(Kimball et al(2003) These effects occur independently of the well-characterized effects of insulin on GLUT4 translocation and glucose uptake and storage. In periods of fasting or starvation, amino acid release from skeletal muscle (primarily l-glutamine and l-alanine(Chang et al (1978) may modulate glucagon release from pancreatic α-cells, which subsequently may influence insulin secretion from β-cells. Dietary

amino acids may also stimulate incretin release, e.g., GLP-1, from intestinal L-cells(Reimann et al(2004)(Gameiroet al (2005)and therefore stimulate insulin secretion via indirect mechanisms. The positive effect of administration of two amino acids to insulin secretion in vivo was reported in a recent clinical assessment of the effect of leucine and phenylalanine administered in the presence of a protein hydrolysates to type 2 diabetic patients and suitable control subjects, which resulted in a threefold increase in insulin secretion compared with carbohydrate alone(van Loon et al(2003) Because in vivo insulin secretion is normally determined by administration of an oral or intravenous glucose load, it is probable that in vivo insulin secretion measurements are an underestimate of that possible from a mixed nutritional load. Using in vitro mouse islet incubations with specific amino acid mixtures at physiological concentrations, insulin secretion was robustly stimulated (Bolea et al (1997)Four amino acids were found to be particularly important for stimulating β-cell electrical activity, essential for insulin secretion (leucine, isoleucine, alanine, and arginine).

Only a relatively small number of amino acids promote or synergistically enhance insulin release from pancreatic β-cells(Fajans et al (1967) (McClenaghan et al (1996) The mechanisms by which amino acids enhance insulin secretion are varied. The cationically charged amino acid, l-arginine, does so by direct depolarization of the plasma membrane at neutral pH but only in the presence of glucose, whereas other amino acids, which are co-transported with Na^+, can also depolarize the cell membrane as a consequence of Na^+ transport and thus induce insulin secretion by activating voltage-dependent calcium channels. Metabolism, resulting in partial oxidation, e.g., l-alanine(Brennan et al 2002), may initially increase the cellular content of ATP, leading to closure of the ATP-sensitive K^+ (K_{ATP}) channel, depolarization of the plasma membrane, activation of the voltage-activated Ca^{2+} channel, Ca^{2+} influx, and insulin exocytosis. Additional mitochondrial signals may be generated that affect insulin secretion ((Malaisseet al (1982) (Maechleret al (2002).

2-9-1 NUTRIENT-INDUCED INSULIN SECRETION

In vivo, the β-cell is constantly monitoring nutrient availability and metabolic status and can generate appropriate secondary stimulus-coupling signals in response to the most minor changes in the concentration of specific metabolites. This is coupled with regulatory input from other signaling pathways, including the gut-derived incretins, vagal signals, and neuropeptides. The β-cell is metabolically distinct from almost all other mammalian cell types in several respects: *1*) it can use glucose in the physiologically relevant range (2–20 mmol/l)

as it expresses a combination of GLUT2 (high K_m glucose transporter) and gluco kinase, *2*) low lactate dehydrogenase and plasma membrane mono-carboxylate pyruvate/lactate transporter activity and correspondingly high activity in the mitochondrial malate-aspartate shuttle so ensuring mitochondrial oxidation of NADH, and

A high activity of both pyruvate dehydrogenase and pyruvate carboxylase, ensuring both anaplerotic and oxidative metabolism of glucose/pyruvate can coexist. All these specific metabolic adaptations are geared to enhancing mitochondrial Tricarboxylic acid (TCA) cycle activity, oxidative phosphorylation, and efficient ATP production. An enhancement of the ATP-to-ADP ratio results in closure of the K_{ATP} channel, depolarization of the plasma membrane, opening of voltage-activated Ca^{2+} channels, influx of Ca^{2+}, and finally fusion of insulin-containing granules with the plasma membrane (Rutter et al (2001).

Lipid metabolism, via long-chain acyl-CoA formation, may also affect insulin secretion (Deeney et al (2000)Indeed, it is now recognized that citrate exported from the mitochondria to the cytosol is cleaved by ATP citrate lyase to generate oxaloacetate and acetyl-CoA, which subsequently forms malonyl-CoA in a reaction catalyzed by acetyl-CoA carboxylase, promoting fatty acid synthesis and accumulation of long-chain acyl-CoAs(Haber et al (2006)), thereby enhancing Ca^{2+}-evoked insulin exocytosis ((Deeney et al (2000). Amino acids also play a role as modulators of lipid metabolism. Acetyl-CoA carboxylase, responsible for malonyl-CoA synthesis, is activated by glutamate-sensitive protein phosphatase type 2A(Gaussin et al (1996), an effect demonstrated in islet β-cells(Kowluru et al (2001). Acetyl-CoA carboxylase is also regulated by phosphorylation via AMP kinase, an enzyme sensitive to amino acid concentration (Xianget al 2004). Recent work in our laboratory (see later gene expression section for details) has demonstrated that addition of 10 mmol/l l-alanine to the BRIN-BD11 β-cell line increased expression of ATP-citrate lyase by 2.0-fold. ATP citrate-lyase will convert citrate to acetyl-CoA in the cytosol, thus providing the key step in fatty acid synthesis, acetyl-CoA carboxylase, with substrate. In addition, we have also found that that addition of 10 mmol/l l-glutamine to BRIN-BD11 cells up regulated acetyl-CoA carboxylase expression at the mRNA and protein level (M. Corless, A. Kiely, N.H. McClenaghan, P.R. Flatt, P.N., unpublished data), thus stimulating fatty acid synthesis.

In the mitochondrial matrix, Ca^{2+} increases the activity of several dehydrogenase. In this manner, increased cytosolic Ca^{2+} occurring during cell activation is relayed to the mitochondria via a Ca^{2+}uniporter (Duchen et al (1999)). Such Ca^{2+} entry is favored by activation of the respiratory chain, for instance, by glucose in the β-cell. Therefore, hyper polarization of the mitochondrial membrane permits the rise in mitochondrial Ca^{2+}, further activating NADH-generating dehydrogenase (Kennedy et al (1999)). The primary actions of glucose are mediated by potentiating of ATP concentration by enhanced TCA cycle substrate (oxidative and anaplerotic) supply. Generation of other additive factors derived from glucose metabolism might also be promoted by mitochondrial Ca^{2+} elevation (Maechleret al (1997).

Amino acids may acutely influence insulin secretion via a number of possible mechanisms, including generation of metabolic coupling factors, depolarization of the plasma membrane, or enhancement of mitochondrial function. These mechanisms are discussed in detail later in this review, but in the first instance, essential aspects of amino acid–dependent effects on signaling, gene expression, and metabolism will be covered.

2-9-2 SIGNALING ROLE OF AMINO ACIDS

Certain amino acids are now known to play important nutrient-sensing roles involving the mammalian target of rapamycin (mTOR)-mediated signaling pathway (McDaniel et al (2002). mTOR is a component of a signaling pathway that couples insulin receptor stimulation and nutrient availability with protein synthesis via activation and phosphorylation of the ribosomal protein S6. Indeed, the mTOR pathway is an important regulator of cell size that coordinates the activity of the cell growth machinery with the levels of energy and nutrients. This is accomplished via activation of several downstream effectors including the 4E-BP1 (eukaryotic initiation factor 4E-binding protein) family of translational repressors and the protein kinases S6K1 and S6K2 (S6 kinases 1 and 2), which are sensitive to both mTOR and insulin signaling pathways. Leucine is the most effective amino acid in this regard. The activation of the mTOR pathway is likely to be important in the β-cell, where mTOR and growth factor/insulin signaling are likely to synergize so stimulating mitochondrial function, insulin secretion, and protein synthesis (Kwon et al (2004). Nutrients and cellular metabolism regulate mTOR effectors such as S6K1 through the interaction with the mTOR complex. In cells growing in

nutrient-rich conditions, the mTOR kinase activity is high. In cells growing in nutrient-poor conditions, the mTOR kinase activity is low. It is not known how amino acids activate the mTOR complex, but it is probable that stimulation of a kinase or inhibition of a phosphatase that act upon mTOR as a substrate is involved (Briaud et al (2003).

Recent data has highlighted the importance of AMPK activity in the regulation of insulin secretion in pancreatic β-cells (da Silva et al (2003). Amino acids have been shown to be important regulators of AMPK activity, e.g., there was a marked reduction in AMPK activity on addition of the amino acids leucine, glutamine, and arginine (Leclerc et al(2004). Indeed, AMPK activity and insulin secretion were inversely correlated for the amino acids investigated. It was proposed that metabolizable amino acids regulate AMPK via changes in the cytosolic ATP-to-AMP ratio and phosphorylation of LBKI kinase, a regulator of AMPK activity (Leclerc et al (2004).

2-9-3 AMINO ACID–DEPENDENT GENE EXPRESSION IN THE β-CELL

In mammals, the impact of nutrients, especially amino acids and fatty acids, on gene expression has become an important area of research. Control of gene expression by nutrient availability has been well documented in prokaryotes and lower eukaryotes, which are able to adjust their metabolic activity to variations in the nutrient supply by altering their pattern of gene expression. However, the mechanisms responsible for amino acid control of mammalian cell gene expression have only recently been investigated. Amino acids may exert influence via mTOR-dependent stimulation of protein synthesis and indirectly, gene expression. Amino acid starvation can lead to tRNA accumulation, transcriptional factor activation, and up regulation of several genes that are involved in amino acid synthesis (Averous et al (2003). Interestingly, supra-physiological concentrations of amino acids have been shown to regulate gene expression in hepatocytes via cell swelling–dependent events (Haussinger et al (1996).

Expression of genes related to β-cell signal transduction, metabolism, and apoptosis are chronically regulated by l-alanine. Analysis performed using the Affymetrix rat genome RGU34A microarray revealed that a total of 66 genes were increased ≥1.8-fold after 24-h culture with l-alanine (Cunningham et al (2005)). These genes were grouped according to

molecular function, and increased expression of some key metabolic genes, including ATP-citrate lyase and catalase, was confirmed by real-time PCR. L-alanine–and l-glutamine–dependent regulation of β-cell gene expression was recently reviewed (Newsholme et al (2005).

It is known that l-glutamine specifically regulates pro inflammatory cytokine gene expression in mononuclear cells of the immune system, as well as specific functional genes in the liver, kidney, muscle, lymphocytes, adipocytes, fibroblasts, and tumor cells (Curi et al (2005). In context to the work described here, whereas l-glutamine only weakly stimulated insulin secretion from BRIN-BD11 cells at basal (1.1 mmol/l) glucose ,the amino acid was actively metabolized by several different pathways (.Brennan et al (2003)). More recently, we have discovered that 10 mmol/l l-glutamine increased the chronic 24-h insulin secretion rate of this clonal β-cell line by 30% compared with 1 mmol/l glutamine, which was associated with up regulation of 148 genes at least 1.8-fold and down regulation of 18 genes (M. Corless, A. Kiely, N.H. McClenaghan, P.R. Flatt, P.N., unpublished data). Notably, BRIN-BD11 cells (in common with all transformed cell lines) required exposure to l-glutamine at a minimum concentration of 1 mmol/l, to avoid significant loss of viability during a chronic period of culture.

We additionally observed that 24-h exposure to l-glutamine strongly up regulated both the calcineurin catalytic and regulatory subunit mRNA expression in BRIN-BD11 cells. Calcineurin, or protein phosphatase 2B, is a calcium-binding protein that has been shown to contribute to the mechanism of somatostatin-induced inhibition of exocytosis in mouse pancreatic β-cells (Renstrom et al(1996). In addition, it is now appreciated that the cAMP response element binding (CREB) protein transcription factor regulates specific pro-survival genes in the β-cell. CREB translocation to the nucleus is regulated by specific Ca^{2+}-dependent dephosphorylation of transducers of regulated CREB (TORC) by calcineurin (Schuit et al (2005). We also determined significant glutamine-dependent up regulation of PDX-1 and acetyl-CoA carboxylase at the mRNA level. Elevated PDX-1 transcriptional binding was confirmed by an electrophoretic mobility shift assay, and increased acetyl-CoA carboxylase protein expression was demonstrated by Western blotting (M. Corless, A. Kiely, N.H. McClenaghan, P.R. Flatt, P.N., unpublished data). Thus, glutamine may be required for the

optimal in vivo and in vitro differentiation of pancreas-derived stem cells toward the β-cell phenotype and optimal lipid synthesis.

In summary, whereas l-alanine and l-glutamine may acutely regulate insulin secretion (as described in detail below), they also play a role in regulating β-cell gene expression, which will affect the ability of the β-cell to chronically respond to nutrient availability, metabolism, hormonal stimuli of insulin secretion, and regulators of functional integrity.

2-9-4 ROLE OF AMINO ACIDS IN NADH MITOCHONDRIAL SHUTTLES AND STIMULATION OF ENERGY METABOLISM

In pancreatic β-cells, the activities of the NADH shuttles play an important role in glucose metabolism. This is as a consequence of low lactate dehydrogenase activity resulting in β-cell dependence on NADH shuttles to regenerate cytosolic NAD^+. The transport of glycolysis-derived reducing equivalents from the cytosol to the mitochondrial matrix also results in the coupling of glycolysis to mitochondrial energy metabolism. Amino acids such as aspartate and glutamate play a key role in such shuttles. After transport into the mitochondria, glycolysis-derived electrons are transferred to the electron transport chain, which creates the proton electrochemical gradient driving ATP synthesis. The formation of a robust proton gradient limits the production of mitochondrial coupling factors.

In β-cells, NADH may be transported to the mitochondrial matrix by either the glycerol-phosphate or the malate-aspartate shuttle (Eto et al (1999). Inhibition of the malate-aspartate shuttle by amino-oxycetate (which acts on transamination reactions and inhibits cytosolic NADH re oxidation) attenuated the secretory response to nutrients, thus demonstrating the dominance of this latter shuttle in the β-cell. One key constituent of the malate-aspartate NADH shuttle is the mitochondrial aspartate-glutamate transporter with its two Ca^{2+}-sensitive isoforms Citrin and Aralar1, which are expressed in excitatory tissues. However, Aralar1 is the only aspartate-glutamate transporter isoform expressed in β-cells. Adenoviral-mediated over expression of Aralar1 in INS-1E β-cells and rat pancreatic islets enhanced glucose-evoked NAD (P)H generation, electron transport chain activity, and mitochondrial ATP formation (Rubi et al (2004). Aralar1 was demonstrated to exert its effect on insulin secretion

upstream of the TCA cycle (Rubi et al (2004). Indeed, the capacity of the aspartate-glutamate transporter appeared to limit NADH shuttle activity and subsequent mitochondrial metabolism. Our laboratory is now investigating the role of the Aralar1 transporter in β-cell amino acid metabolism and insulin secretion. We have demonstrated that in Aralar1-overexpressing INS-1E β-cells, an l-alanine addition resulted in increased NAD (P) H production, electron transport chain activity, and insulin secretion (K.B., P. Maechler, P.N., unpublished data).

2-9-5 MECHANISMS OF AMINO ACID–DEPENDENT STIMULATION OF INSULIN SECRETION

2-9-5-1 Glutamate.

L-glutamate is the most highly debated amino acid with respect to stimulation of insulin secretion and the possible molecular mechanisms of its action on promotion of secretion. Intracellular generation of l-glutamate has been proposed to participate in nutrient-induced stimulus-secretion coupling, as an additive factor in the amplifying pathway of glucose-stimulated insulin secretion (Maechler et al (1999). During glucose stimulation, total cellular glutamate levels have been reported to increase in human, mouse, and rat islets as well as in clonal β-cells (Maechler et al (1999). (Broca et al(2003)whereas in other studies, no change was detected (Danielsson et al (2003)(MacDonald et al (2000)The finding that mitochondrial activation in permeabilized β-cells directly stimulates insulin exocytosis (Maechler et al (1997)pioneered the identification of glutamate as a putative intracellular messenger(McClenaghan et al(1997)(Hsu By et al (2001). It has been suggested that glutamate could be transported into secretory granules, thereby promoting Ca^{2+}-dependent exocytosis (McClenaghan et al(1997).(Hsu By et al (2001). Such a model has been substantiated by demonstration that clonal β-cells express vesicular glutamate transporters and that glutamate transport characteristics are similar to neuronal transporters (54). Other evidence in support of the l-glutamate hypothesis comes from work with β-cells over expressing l-glutamate decarboxylase (GAD): over expression of GAD reduced l-glutamate content in INS-1E and islet β-cells and reduced secretory responses to high glucose(Henquin et al (1986).

In recent years, the role of l-glutamate in insulin secretion has been robustly challenged(Sener et al (2002) (MacDonald et al (1991).An increase in intracellular l-glutamate concentration on addition of glucose (16.7 mmol/l) in rat islets was not observed in a key study (MacDonald et al (1991).Incubation with l-glutamine (10 mmol/l) increased the l-glutamate concentration 10-fold but did not stimulate insulin release, leading the authors to cast doubt on the proposed role of l-glutamate. In a separate study, it was demonstrated that, on incubation with glucose, a significant increase in l-glutamate concentration occurred in depolarized mouse and rat islets (Sener et al (2002)). However, the latter authors argued against the glutamate hypothesis on the basis of experiments with l-glutamine: l-glutamine caused an increase in l-glutamate content with no effect on insulin secretion. Additionally, in this study, activation of GDH by BCH lowered l-glutamate levels but increased insulin secretion. However, addition of l-glutamine as a precursor for l-glutamate may lead to saturating concentrations of l-glutamate without activation of the K_{ATP}-dependent pathway and thus may not result in an increase in insulin secretion (Gylfe et al (1976).

2-9-5-2 Alanine.

l-Alanine is consumed at high rates in both BRIN-BD11 cells and rat islets (~2 and 8 μmol/mg protein/20 min for BD11 cells and islets, respectively (Dixonet al (2003)Addition of 10 mmol/l l-alanine to 16.7 mmol/l glucose significantly increased glucose consumption in BRIN-BD11 cells (Brennanet al (2002)suggesting a critical role for l-alanine in β-cell function.

Early studies have shown that l-alanine is taken up and oxidized by *ob*/*ob* mouse islets (Hellman et al (1971). Recently, l-alanine has been shown to have insulinotropic effects both in β-cell lines and in rat islets (Dixonet al(2003)(McClenaghanet al (1996)Addition of 10 mmol/l l-alanine to an incubation medium containing 1.1 mmol/l d-glucose increased insulin secretion 3- and 1.6-fold for BRIN-BD11 cells and islets, respectively (Dixonet al(2003)It was suggested that, in RINm5F cells, the insulinotropic action of l-alanine was due to co-transport with Na^+, which resulted in membrane depolarization that led to the generation of Ca^{2+} spike potentials and an increase in intracellular Ca^{2+} (Dunne et al (1990). Other studies using the pancreatic β-cell line BRIN-BD11 demonstrated that l-alanine influenced glucose-induced

insulin secretion by electrogenic Na^+ transport (McClenaghan et al (1998). More recently, using ^{13}C nuclear magnetic resonance, l-alanine was shown to undergo substantial metabolism in BRIN-BD11 cells (Brennan et al (2002), resulting in glutamate, aspartate, and lactate production. Additionally, by use of the respiratory poison oligomycin, the metabolism and oxidation of alanine was shown to be important for its ability to stimulate insulin secretion (Henquin et al 1986).

In contrast to our own work, others have reported that addition of l-alanine did not stimulate insulin secretion from rat islet cells. However, in the presence of l-leucine or 2-ketoisocaproate, alanine promoted insulin secretion (Sener et al (2002). Additionally, l-alanine induced an increase in Ca^{2+} uptake and was oxidized by the β-cell. It was concluded that l-alanine could stimulate insulin secretion under specific conditions of nutrient availability and that the mode of induction of insulin secretion may be a combination of increased ATP production and Na^+ co-transport (Sener et al (2002).

2-9-5-3 Leucine.

The proposed mechanisms by which l-leucine stimulates insulin release from pancreatic β-cells include *1*) increased mitochondrial metabolism by activation of GDH and *2*) increased ATP production (and subsequent K_{ATP} channel–dependent membrane depolarization) by transamination of leucine to α-ketoisocaproate and subsequent entry into the TCA cycle via acetyl-CoA (Pantenet al (1972)In the presence of high glucose, leucine-induced insulin secretion is inhibited (MacDonald et al (1991), since high glucose inhibits flux through glutaminase and GDH. Recently, there has been renewed interest in l-leucine metabolism as a result of the observation of hyper insulinism in patients with mutations in the regulatory site of GDH (Stanley et al (1998) Affected patients have increased β-cell responsiveness to leucine and develop hypoglycemia after a protein meal. Key mutations in the inhibitory allosteric site in GDH (GTP binding) result in the loss of negative allosteric regulation. Although one of the proposed mechanisms by which leucine induces insulin secretion is the conversion of leucine to α-ketoisocaproate, a recent report showed that leucine and α-ketoisocaproate stimulated insulin release via distinct mechanisms (Gao et al (2003). α-Ketoisocaproate was proposed to stimulate insulin release by a combination of mechanisms including its own catabolism and

transamination to leucine with production of 2-oxoglutarate (α-ketoglutarate). However, others have demonstrated that α-keto acids can directly inhibit K_{ATP} channel activity and therefore stimulate insulin secretion (Heissig et al (2005).

Prolonged culture with leucine resulted in increased ATP, cytosolic Ca^{2+}, and glucose-induced insulin secretion in rat islets (Yang et al (2006). Additionally, chronic periods of culture with leucine up regulated ATP synthase and gluco kinase leading to the proposal that this combined up regulation sensitizes the β-cell to glucose-induced insulin secretion (Yang et al (2006).

Leucine along with other members of the branched-chain amino acids activate the mTOR signaling pathway in β-cells as previously described. Mitochondrial signals generated by metabolism of leucine have been suggested to be important for activation of the mTORmitogenic signaling pathway in insulin-sensitive tissues (Kwon et al (2004).

2-9-5-4 Arginine.

The stimulation of insulin release by l-arginine has been proposed to involve the transport of the cationic amino acid into the β-cell, which leads to membrane depolarization (Charles et al (1983)Herchuelz et al (1984) (`Henquin et al (1981)A recent detailed study agreed with this argument (Seneret al (2000)l-Arginine was shown to cause an elevation in intracellular Ca^{2+} concentration as a result of its electrogenic transport into the β-cell via the amino acid transporter mCAT2A. Depolarization of the plasma membrane will then result in activation of voltage-dependent calcium channels, an increase in cytosolic Ca^{2+}, and subsequent stimulation of insulin secretion. Clinical assessment of administered l-arginine has revealed only limited beneficial effects, possibly due to rapid removal of the amino acid in the epithelial cells of the intestine, where it can be rapidly converted to ornithine and citrulline, then exported to the kidney or the liver, where it can be converted to proline for export (Brosnan et al (2003).

Alternatively, l-arginine metabolism in the β-cell can give rise to urea production via arginase activity, or nitric oxide production via nitric oxide synthase. Inducible nitric oxide synthase may be up regulated in the presence of pro inflammatory cytokines (Ortiset al (2006), or indeed specific fatty acids (Dixonet al (2004), and under these conditions, l-arginine consumption and metabolism may have a negative impact on β-cell function. Chronically

elevated nitric oxide levels will reduce insulin secretion, possibly by interfering in mitochondrial function and generation of key stimulus-secretion coupling factors. The impact of arginase activity and urea production are currently unknown. l-Arginine may alternatively be converted to l-glutamate and thus can influence insulin secretion as described above (Broca et al (2003), However, no studies have yet explored l-arginine metabolism in detail in the β-cell; thus, the potential for l-glutamate generation remains to be determined.

2-10Previous Studies:

Study the interfere of BCAAs and in particular leucine with insulin signaling through stimulation of mammalian target of rapamycin and its downstream effecter, S6 kinase, and phosphorylation of insulin receptor substrate-1 (IRS-1) on serine residues, some conflicting results have been reported regarding the role of BCAAs in the regulation of insulin resistance. For instance, the elevation of BCAAs was accompanied with increased energy expenditure and better insulin sensitivity in global knock-out of mitochondrial branched-chain aminotransferase in mice. **(**Xiaoping et al (2015)

Study an important role of leucine, and the branched-chain amino acids that must be supplied in daily diet, in controlling protein synthesis and regulating cell metabolism in various cell types. In pancreatic β cells, leucine acutely stimulates insulin secretion by serving as both metabolic fuel and allosteric activator of glutamate dehydrogenase to enhance glutaminolysis..Identified long-term treatment of leucine has been shown to improve insulin secretory dysfunction of human diabetic. (**Jichun et al (2011)**

Stud**y** the metabolic effects of ingested individual amino acids, to determine whether leucine stimulates insulin and/or glucagon secretion and whether, when it is ingested with glucose, it modifies the glucose, insulin, or glucagon response. They found that when leucine was ingested with glucose, it attenuated the serum glucose response and strongly stimulated additional insulin secretion. (Kalogeropoulou et al (2008).

Study that Protein induces an increase in insulin concentrations when ingested in combination with carbohydrate. Increases in plasma insulin concentrations have been

observed after the infusion of free amino acids. However, the insulinotropic properties of different amino acids or protein (hydrolysates) when co-ingested with carbohydrate have not been investigated**,** they observed strong initial increase in plasma glucose and insulin concentrations ,after which large differences in insulin response between drinks became apparent, and ingestion of the drinks containing free leucine, phenylalanine, and arginine the drinks with free leucine, phenylalanine, and wheat protein hydrolysates were followed by the largest insulin response. (Luc et al , 2002).

Study organized Pancreatic β-cells are continually monitor and respond to dietary nutrients, under the modulation of additional neurohormonal signals, in order to secrete insulin. β-cell nutrient sensing requires complex mechanisms of metabolic activation, resulting in production of stimulus-secretion coupling signals that promote insulin biosynthesis and release. The primary stimulus for insulin secretion is an elevation in blood glucose concentration and β-cells are particularly responsive to this important nutrient secretagogue via the tight regulation of glycolytic and mitochondrial pathways at steps such as gluco kinase, pyruvate dehydrogenase, pyruvate carboxylase, glutamate dehydrogenase and mitochondrial redoxshuttles. With respect to development of type-2 diabetes (T2DM), it is important to consider individual effects of different classes of nutrient or other physiological or pharmacological agents on metabolism and insulin secretion and to also acknowledge and examine the interplay between glucose metabolism and that of the two other primary nutrient classes, amino acids (such as arginine and glutamine) and fatty acids. It is the mixed nutrient sensing and outputs of glucose, amino and fatty acid metabolism that generate the metabolic coupling factors (MCFs) essential for signaling for insulin exocytosis. Primary MCFs in the β-cell include ATP, NADPH, glutamate, long chain acyl coenzyme A and diacylglycerol. It is the failure to generate MCFs in a coordinated manner and at sufficient levels that underlies the failure of β-cell secretion during the pathogenesis of T2DM**.** (Philip et al (2012)

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

Materials and Methods

Study Approach: quantitative.

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

Study Area: Samples were collected from different diabetes centers and hospitals in Khartoum state,(Academic hospital Khartoum, , Yestebsheron hospital Khartoum, Elbangadid hospital KhartoumEast,Elnow hospital Omdurman.)

3-1-1 Target Population and Sample Size: 167 Sudanese patients with type2 diabetes mellitus were enrolled in this study in contrast to 47 healthy volunteers (Age and sex matched) were involved as control.

The Period of Research:-

The duration of the research was commencing from August 2012 to August 2016.

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.

3-1-2 Ethical consideration:

-Permission of this study was obtained.

-The aims and the benefits of the study were explained to the participants with assurance of confidentiality.

-An informed consents were obtained from all participants.

-Health education was provided to all participants.

3-1-3 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.

3-2 Study Variables and Methods of measurement:

A total of 167 Sudanese patients with type2 diabetes mellitus were enrolled in this study in contrast to 47 healthy volunteers (Age and sex matched) were involved as control.. The study population was divided into males (n =116) and females (n = 98)

. 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 for 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 glutamate, alanine, luecine, arginine, were 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 this was done in Department of Biochemistry, central laboratory,Ministryof Higher Education and Scientific Research. .

- **Serum levels of amino acids were measured using amino acid auto analyzer.**

Sample preparation:

the unbound amino acids in plasma were analyzed because. high molecular weight compounds have to be removed as they would obstruct the separation column. The solution of proteins done by acid Precipitation.

Deproteinization of blood plasma carried out as soon as possible, within half an hour otherwise glutamine, asparagines and Cysteine will decompose and there will be an increase in Glutamic and. Aspartic acid. 5-sulfosalicylic acid was used for this purpose.

Plasma preparation for amino acid analysis was carried as follows:

- Pour 900µl of plasma in a centrifuge tube.
- Added 100µl of 20% 5-sulfosalicylic acid.
- Incubated at 4 C^o for 30 minutes.
- Centrifuged at 1300rpm for 10-15 minutes. Deproteinization was completed.
- Diluted the supernatant with sample diluting buffer in the ratio of 1:1. The pH of the sample should be within the range of 1.8: 2.0; if necessary, correct the pH-value by concentrated LiOH.
- The sample was ready to be analyzed. Used within short time or otherwise stored frozen at -20C^o.

Separation of samples and calculation:

100 µl of prepared plasma was injected into the column.

Calculation:

Concentration of each amino acid in plasma calculated automatically according to the following equation:

$$\frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{Concentration of standard (µmol/l)}$$

P.S:

Concentration of plasma amino acids multiplied by 2.

-Serum levels of insulin hormone were measured using ELIZA technique

providing a method for the quantitative determination of human insulin in plasma.

Principle of the procedure:

Insulin ELIZA is a solid phase two- site enzyme immunoassay .It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with peroxidase- conjugated anti- insulin antibodies bound to micro plate wells. A simple washing step removes unbound enzyme labeled antibodies. The bound conjugate is detected by reaction with3, 3,5,5-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

Test procedure:

All reagents and samples brought to room temperature before use .

calibration curve prepared for each assay run.

1. Enzyme conjugate **IX** solution prepared and washed buffer **IX** solution.
2. Sufficient micro plate wells prepared to accommodate calibrators and samples in duplicate.

3. 25 µl each of calibrators and sample pipette into appropriate wells.
4. 100 µl of enzyme conjugate **IX** solution added to each well.
5. Incubated on a plate shaker (700-900 rpm) for one hour at room temperature (18 c^o-25 c^o)
6. Washed 6 times with 700 µl wash buffer **IX** solution per well using an automatic plate washer with over flow-wash function, after final wash, inverted and taped the plate firmly against absorbent paper. Do not included soak step in washing procedure. Or manually discarded the reaction volume by inverting the micro plate over a sink. Added 350 µl wash buffer **IX** solution to each well .Discarded the wash solution, taped firmly several times against absorbent paper to remove excess liquid. Repeated 5 times.
7. Added 200 µl substrate TMB into each well.
8. Incubated for 15 minutes at room temperature (18 c^o-25 c^o).
9. Added 50 µl stop solution to each well. Placed plate on shaker for approximately 5 seconds to insure mixing.
10. Read optical density at 450 nm and calculated results. Read within 30 minutes.

Calculation of results:

Computerized calculation: The concentration of insulin was obtained by computerized data reduction of the absorbance for the calibrators, except for the calibrator 0, versus the concentration using spine regression.

- HbA1c percentage was measured by ion exchange resin chromatography:

Principle of the method:

After preparing the hemolysate,where the labile fraction is eliminated, hemoglobin's are retained by a cataionic exchange resin , HbA1c is specifically eluted after washing other types of hemoglobin's fractions and is quantified by direct photometric reading at 415 nm, the estimation of the relative concentration of HbA1c is made by the measure of total hemoglobin concentration by direct photometric reading at 415 nm.

Procedure of the method:

Hemolysate preparation and labile fraction elimination

1. Braught the columns and reagents to room temperature(21 c^o-26 c^o).
2. Pipette into the test tube:

Blood	50 µl
Reagent 1	200 µl

3. Shake thoroughly and let it stand at room temperature for 10- 15 minutes .This Hemolysate used in steps 6 an 11.
4. Column preparation: Remove the upper cap of the column and then snap the tip off the bottom.
5. Using the flat end of the pipette , push the upper disc down to the resin surface taking care not to compress it ,let the column drain completely to waste.
6. Separation and reading of HbA1c fraction: Carefully pipette on the upper filter

Hemolysate	50 µl	let the column drain to waste

7. In order to drain any sample residue left above the upper disc, pipette:

Reagent 2	200 µl	let the column drain to waste

8. Pipette:

Reagent 2	2.0 ml	let the column drain to waste

9. Place the column over a test tube and add:

Reagent 3	4.0 ml	Collect the elute(HbA1c fraction)

10. Shake thoroughly and read the absorbance(A) of the HbA1c fraction at 415 nm against distilled water, the absorbance is stable for at least one hour.

11. Reading of Hb total: pipette in to a test tube :

Reagent 3	12.0 ml
Hemolysate	50 µl

12. Shake thoroughly and read the absorbance (A) at 415 nm against distilled water the absorbance is stable for at least one hour.

Calculations:

The HbA1c relative concentration in the sample is calculated using the following general formula:

$$\frac{\text{Absorbance of HbA1c} \times \text{volume of HbA1c}}{\text{Absorbance of total Hb} \times \text{volume of total Hb}} \times 100 = \% \text{ HbA1c}$$

The volume of HbA1c is 4 ml , the volume of Hb total is 12 ml. The following formula is for the calculation of the concentration:

$$\frac{\text{Absorbance of HbA1c}}{\text{Absorbance of total Hb}} \times \frac{100}{3} = \% \text{ HbA1c}$$

3-3Data Collection and Statistical Analysis

Data collected in the tabulated database sheet and analyzed by statistical package for social science SPSS. The data included the age, gender, body mass index, insulin, HBA1C, glutamate, alanine, luecine arginine, findings, the data for numerical values were expressed in mean± standard deviation, differences between each investigated patient group whenever in glutamate, alanine, luecine arginine, mean value and control group were obtained. The results consider statistically significant when the differences show equal or more than standard deviation.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

4.1 Tables and Figures

Table (4-1) shows frequency distribution between male and female in the study

Percentage is (50.29 %) for male and (49.71%) for female.

Table (4- 1): Shows Frequency Distribution

Gender	Frequency	Percentage
Male	84	50.29 %
Female	83	49.71%
Total	167	100%

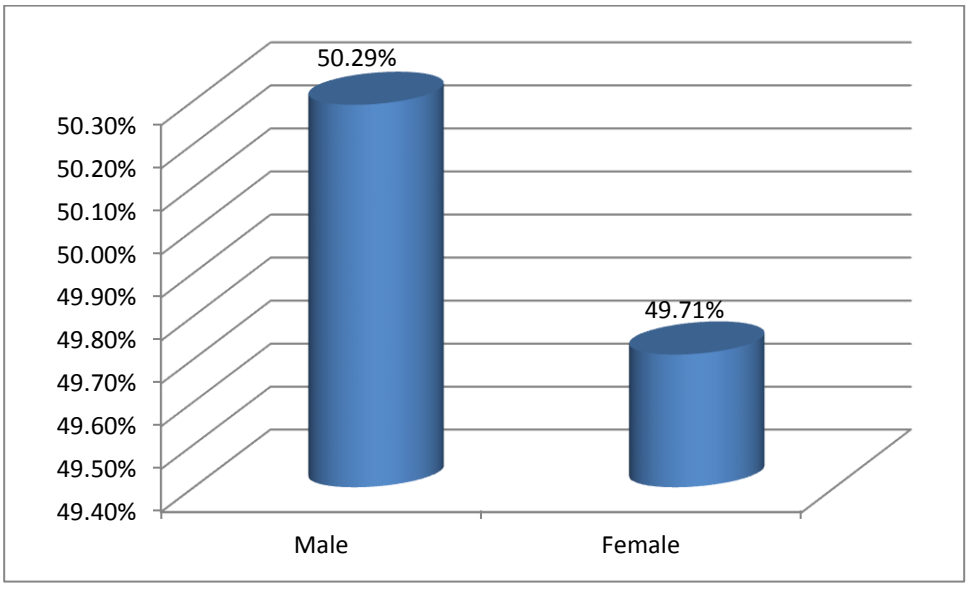
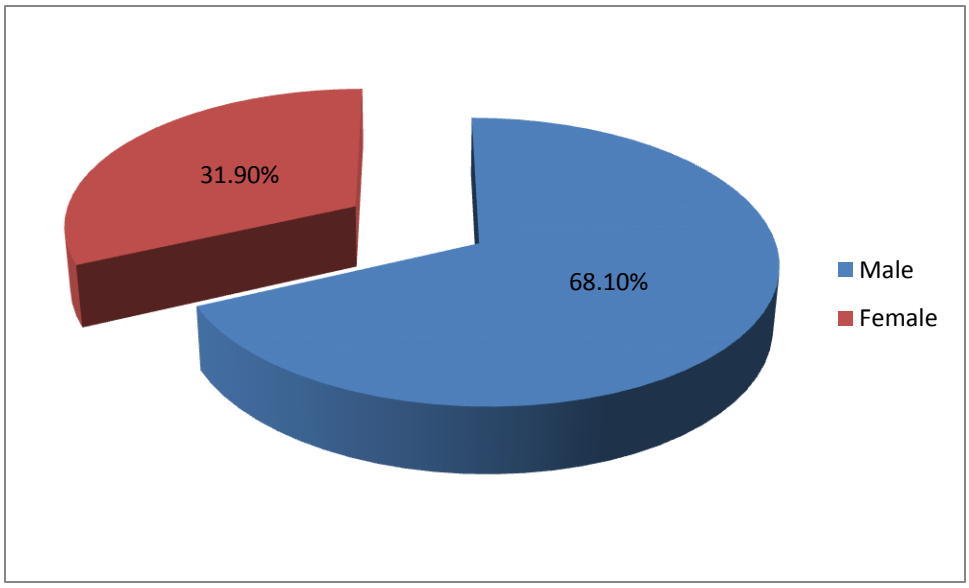


Figure (4-1) Shows Frequency Distribution

Table (4-2): Frequency of gender among control:

Gender	Frequency	Percentage
Male	32	68.1%
Female	15	31.9%
Total	47	100%



Figure(4-2) Frequency of gender among control

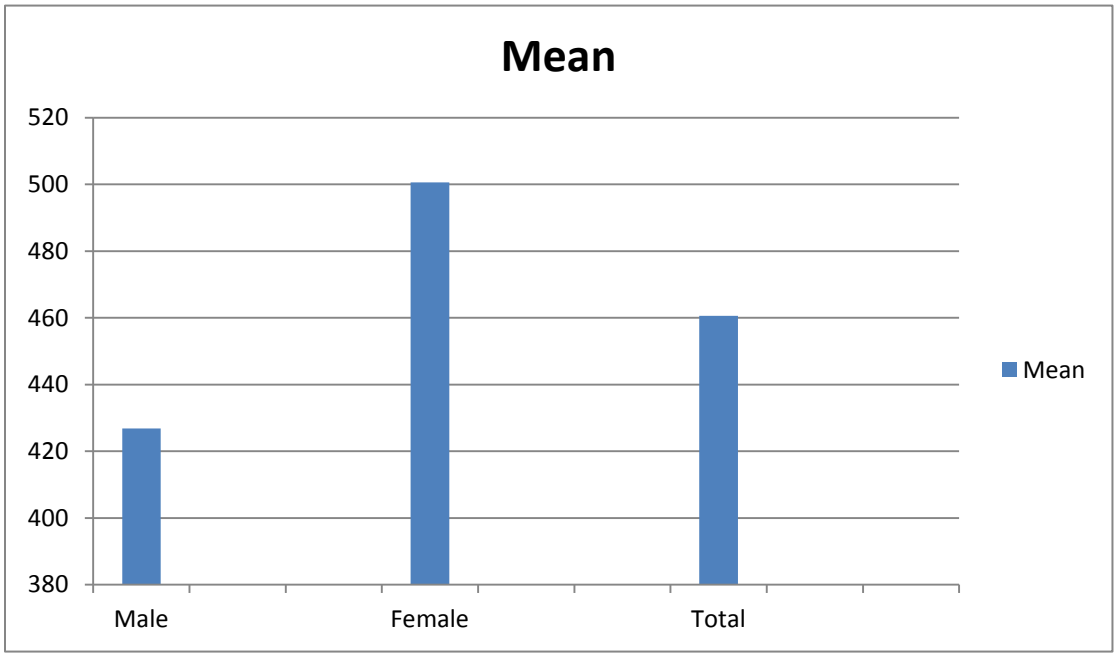
Table (4-3) shows significantly higher levels of alanine among diabetic patients (mean=496.51±242.19) compared to control group (mean=333.09±97.65±).

Table (4-3): The mean difference between case and control group for alanine:-

Variable	Study group	Mean	Std.Deviation	T test	p-value
alanine	Case	496.51	242.19	4.517	0.000
	control	333.09	97.65		

The value of (alanin) is (p-value =0.000< 0.05), this indicates that there is significant increase between case and control.

Figure (4-3) shows Significantly higher levels of alanine seen among female diabetic patients (mean=500.61±235.91)(p-value=0.018<0.05), compared to male diabetic patients(mean=426.83±224.39).



Figure(4- 3): Comparison of Alanine level between male and female diabetes mellitus (type 2)

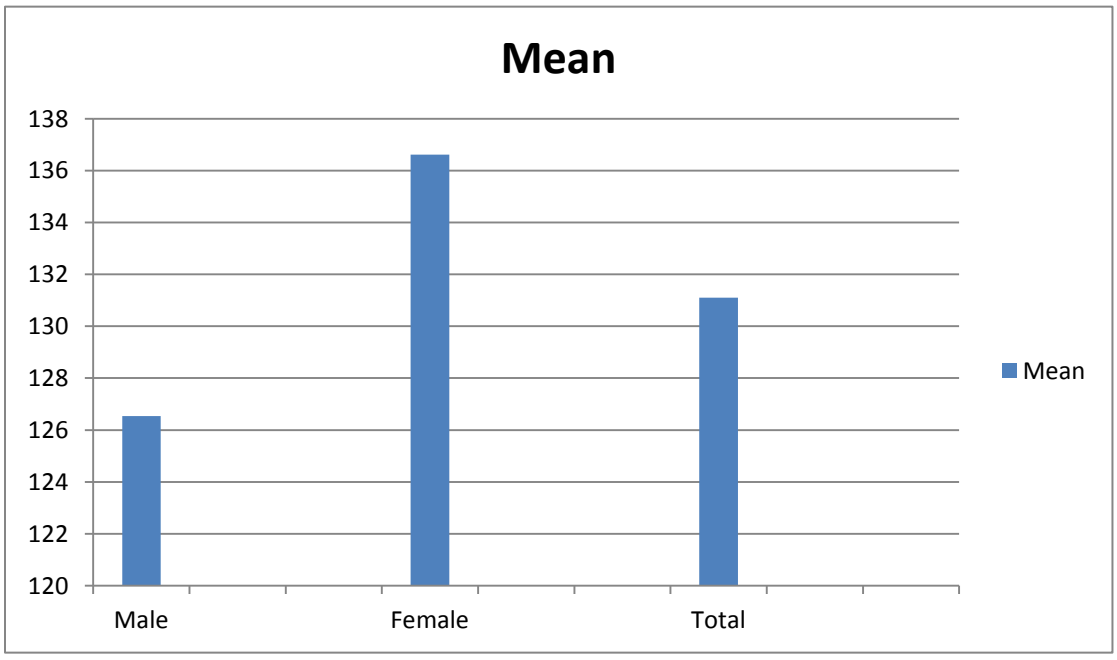
Table (4-4) shows significantly increased levels of luecine among diabetic patients(mean=137.54±46.42) compared to control group (mean=119.66±10.87).

Table (4-4): The mean difference between case and control group for leucine

Variable	Study group	Mean	Std.Deviation	T test	p-value
leucine	Case	137.54	46.42	2.617	0.010
	control	119.66	10.87		

The value of (leucine) is (p-value =0.010< 0.05), this indicates that there is significant increase between case and control.

Figure(4-4) shows insignificantly increase in leucine(mean=136.31±44.75)(p-value=0.068>0.05),compared to male diabetic patients(mean=126.81±40.4).



Figure(4- 4) Shows Comparison of leucine level between male and female

Table (4-5) shows significantly increased levels of glutamate found among diabetic patients (mean=129.34±65.90) compared to control group (mean=88.19±3.99).

Table (4-5): The mean difference between case and control group for glutamate:-

Variable	Study group	Mean	Std.Deviation	T test	p-value
glutamate	Case	129.34	65.90	4.271	0.000
	control	88.19	3.99		

The value of (glutamate) is (p-value =0.000< 0.05), this indicates that there is significant increase between case and control.

Figure (4-5) shows significant increase in glutamate(mean=132.41±63.01) (p-value=0.007<0.05),compared to male diabetic patients (mean=118.55±58).

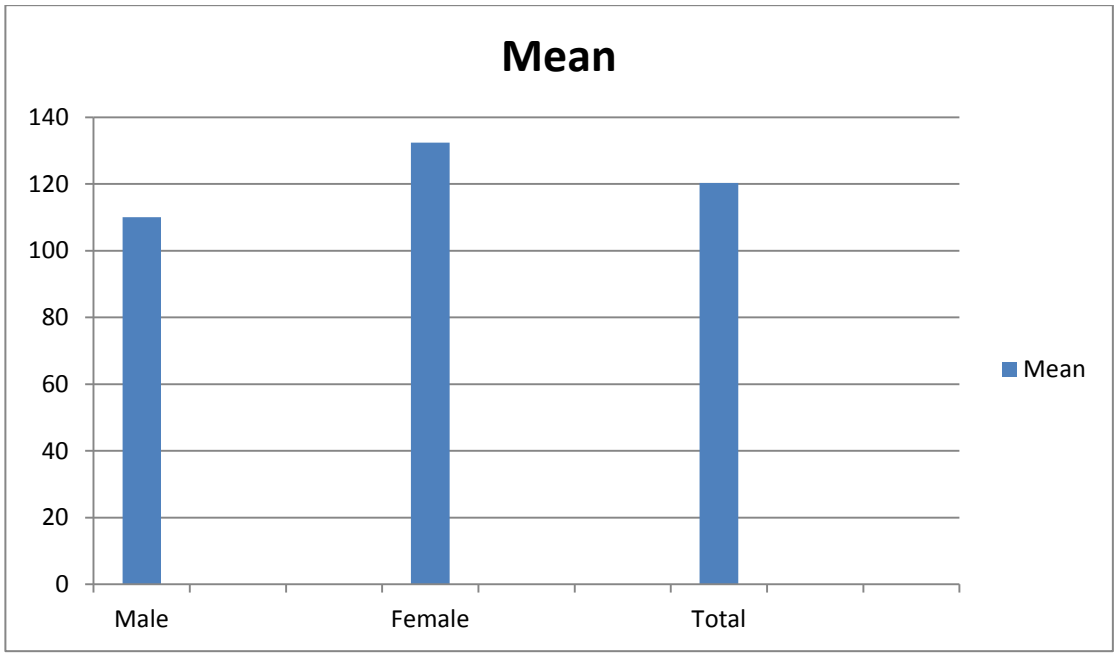


Figure (4-5) Shows Comparison of Glutamate level between male and female

Table (4-6) shows significantly increased levels of arginine found among diabetic patients (mean=88.66±31.13) compared to control group (69.23±19.76).

Table (4-6): **The mean difference between case and control group for arginine**

Variable	Study group	Mean	Std.Deviation	T test	p-value
Arginine	Case	88.66	31.13	4.051	0.000
	control	69.23	19.76		

The value of (Arginine) is (p-value =0.000< 0.05), this indicates that there is significant increase between case and control

Figure (4-6) shows insignificant increase in arginine (mean=88.28±30.79) (p-value=0.082>0.05) were seen among female diabetic patients compared to male diabetic patients (mean=81.11±28.56).

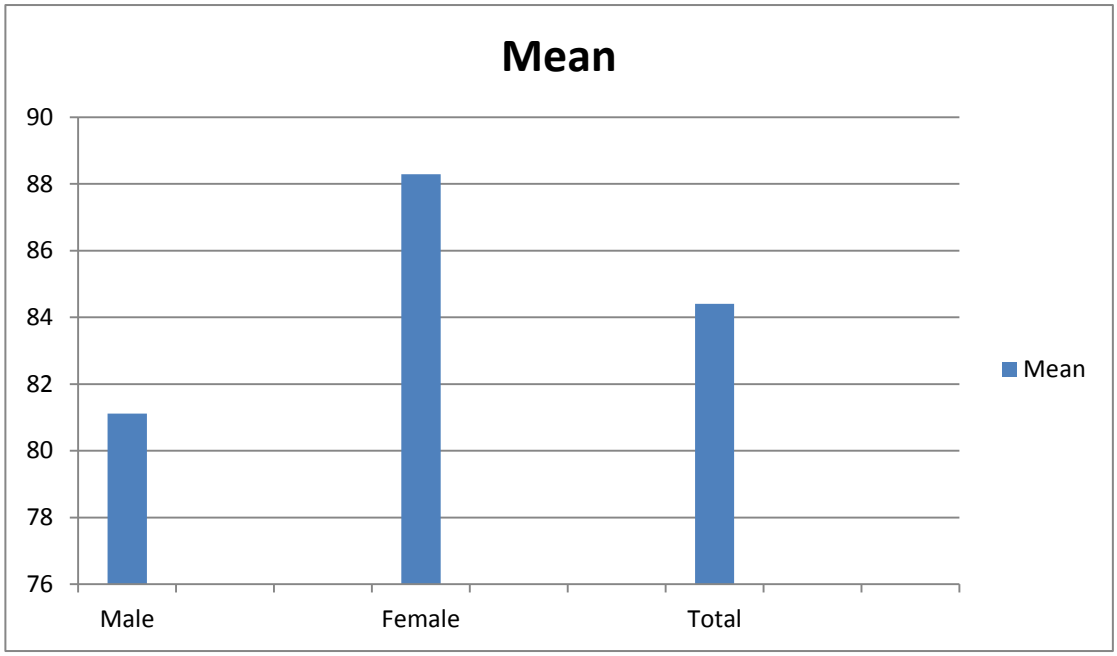


Figure (4-6) Shows Comparison of Arginine level between male and female

Table (4-7) shows significantly higher levels of insulin observed among diabetic patients (mean=15.96±2.52) compared to control group (mean=7.68±1.84),

Table (4-7): **The mean difference between case and control group for insulin**

Variable	Study group	Mean	Std.Deviation	T test	p-value
insulin	Case	15.96	2.52	20.919	0.000
	control	7.68	1.84		

The value of (insulin) is (p-value =0.000< 0.05), this indicates that there is significant between case and control

Figure (4-7) shows significantly higher levels of insulin seen among female diabetic patients (mean=15.29±3.98) compared to male diabetic patients (mean=13.17±4.12) (p-value =0.000<0.05),

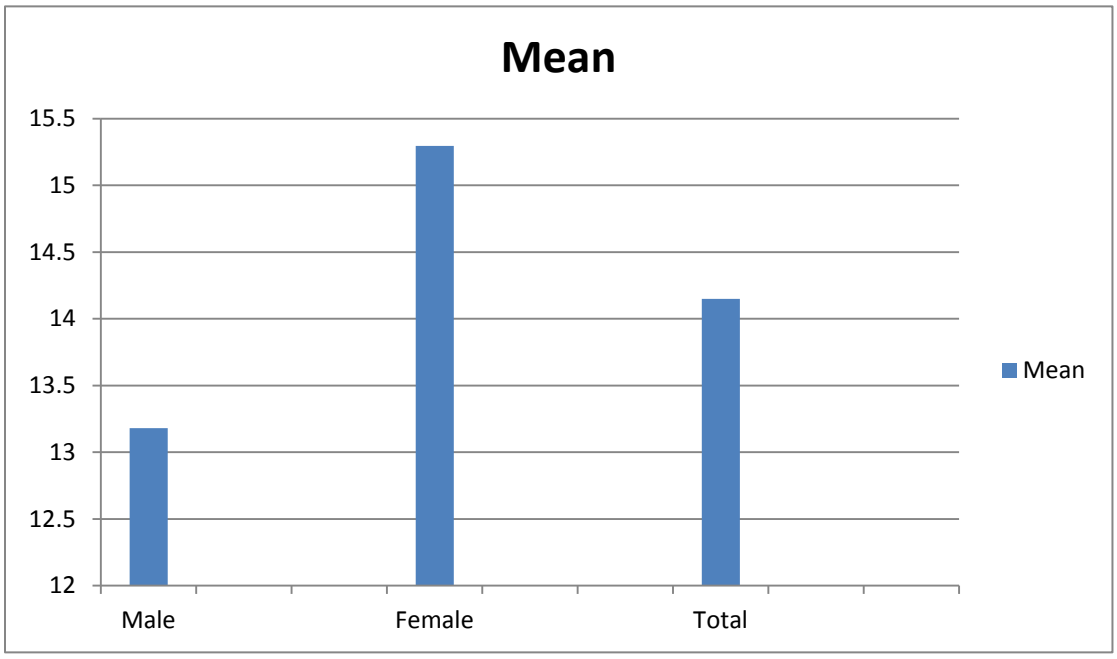


Figure (4-7) Shows Comparison of Insulin level between male and female

Table (4-8) shows significantly higher levels of HbA1C in diabetic patients (mean=8.97) compared to control group (mean=5.17) , the mean of the patients is more than the range expected for diabetic good control which is (7- 8)%.

Table (4-8): **The mean difference between case and control group for HbA1c**

Variable	Study group	Mean	Std.Deviation	T test	p-value
HbA1c	Case	8.97	1.53	16.359	0.000
	control	5.17	.78		

The value of (HbA1c) is (p-value =0.000< 0.05), this indicates that there is significant increase between case and control.

Figure (4-8) shows significantly higher levels of HbA1C seen among female diabetic patients (mean=8.57±2.32) compared to male diabetic patients (mean=7.77±1.83) (p-value =0.000<0.05) .

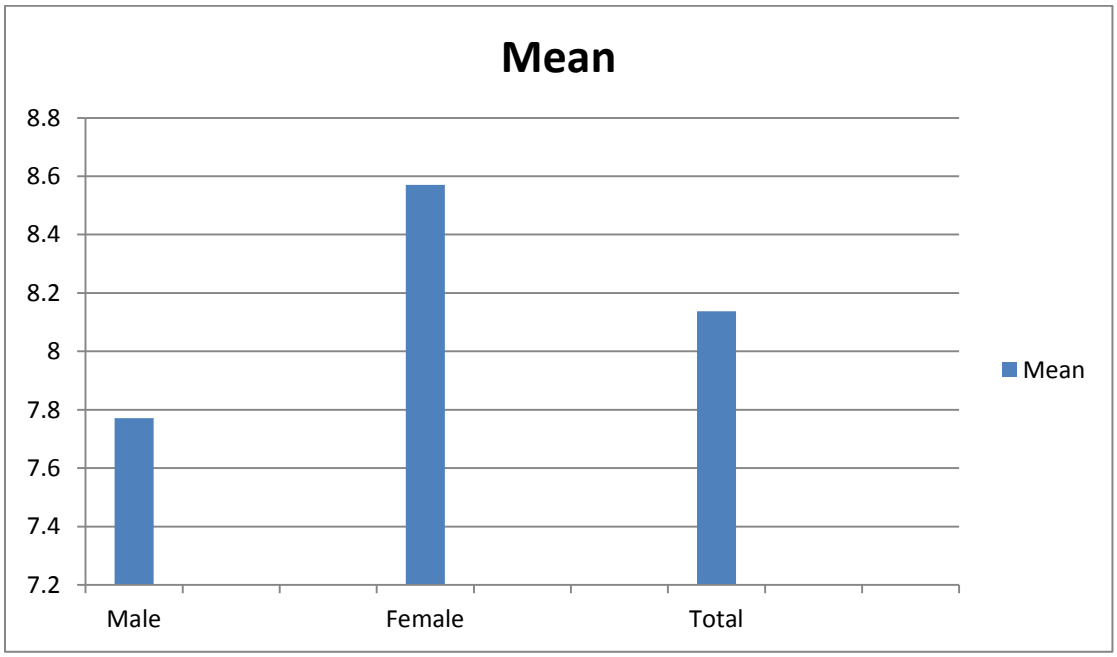


Figure (4-8) Shows Comparison of HbA1C level between male and female

Figure (4-9) shows comparison between BMI in patients with diabetes mellitus (type2) and control group indicates significant higher levels of BMI in diabetic patients (mean=25.18) compared to control group (mean=23.87) (p-value =0.000<0.05).

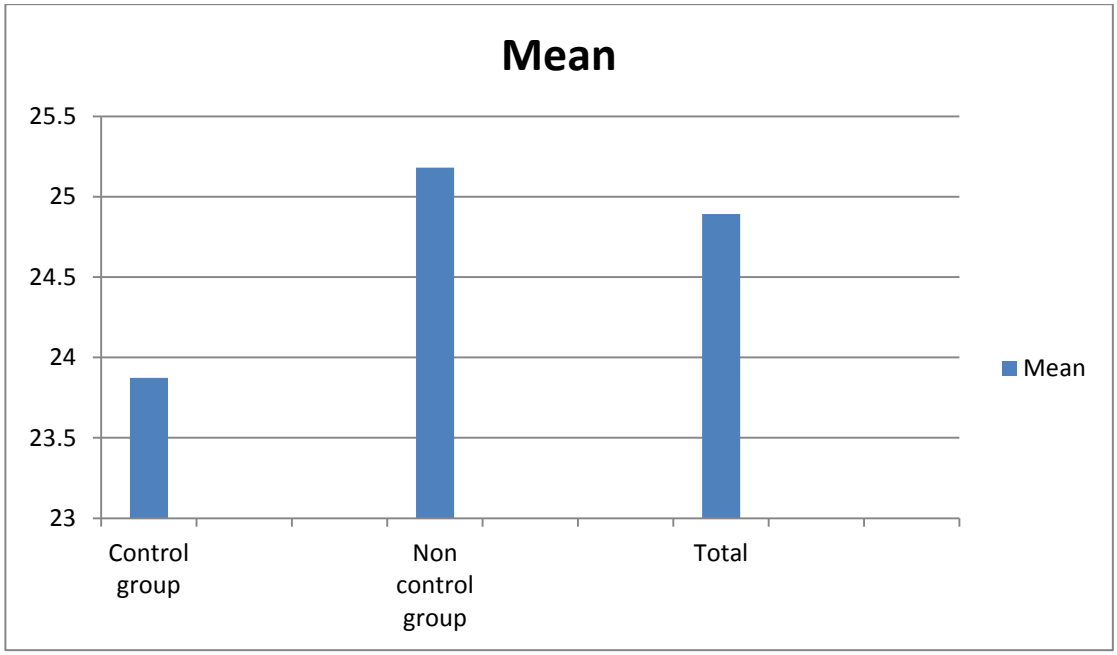


Figure (4-9) comparison between BMI in patients with diabetes mellitus (type2) and control group

Figure (4-10) shows comparison of BMI level between male and female diabetes mellitus (type2) indicates significant higher levels in female group(mean=25.59±3.97) compared to male group(mean=24.30±2.6) (p-value =0.005<0.05).

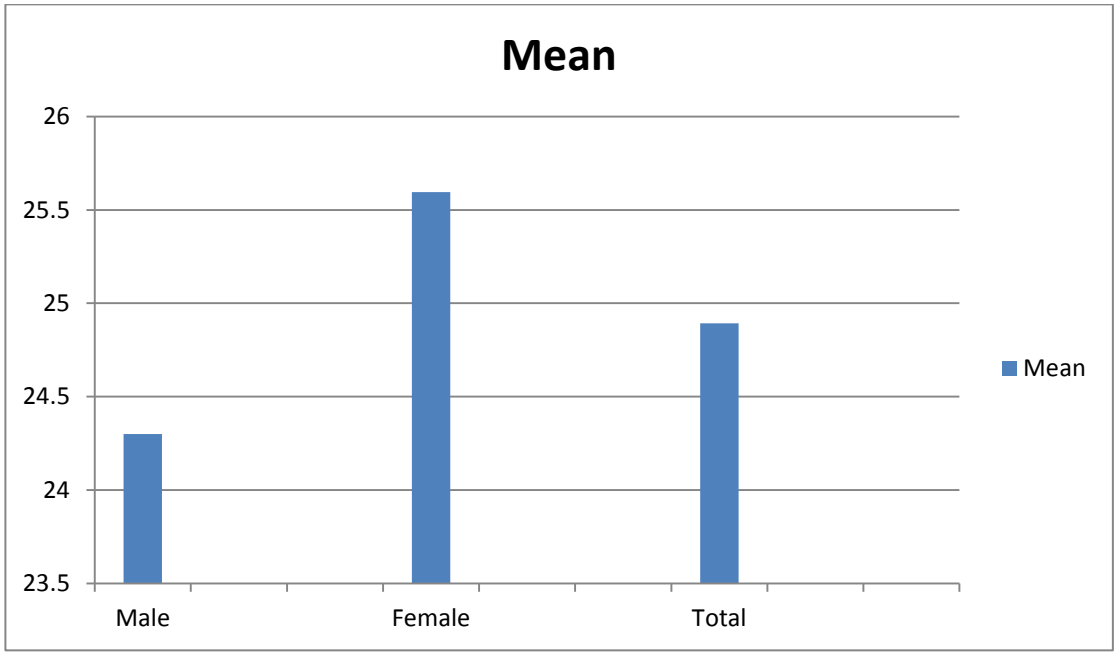


Figure (4-10) Shows Comparison of BMI level between male and female patients

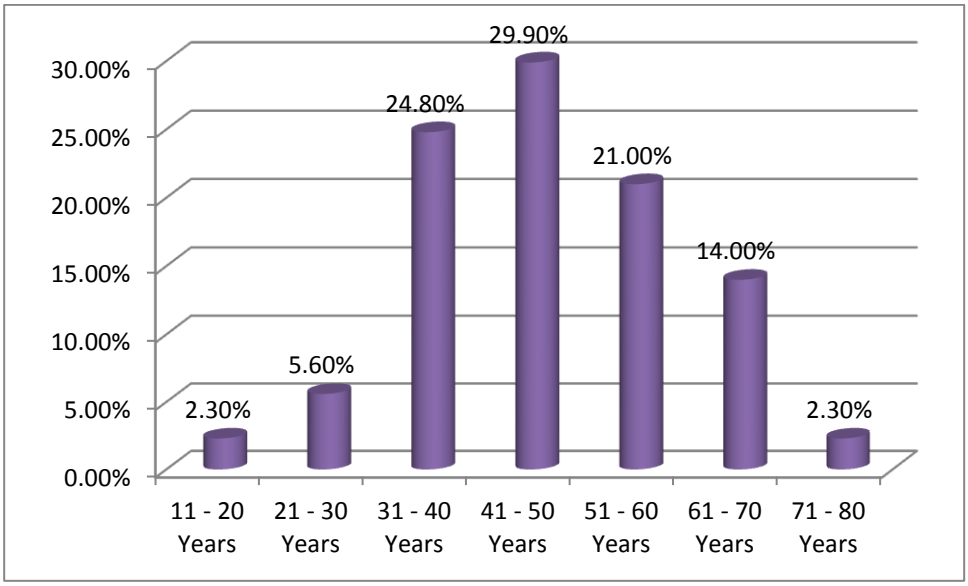
Table (4-9) correlation between Glutamate, alanine, Leucine, Arginine with HbA1c and insulin in diabetic patients show strong correlation and high significancy, but correlation between Glutamate, alanine, Leucine, Arginine with BMI in diabetic patients shows week correlation and low significancy.

Table (4-9):**Correlation: Glutamate, alanine, Leucine, Arginine with HbA1c, Insulin, and BMI**:

		HbA1c	Insulin	BMI
Glutamate	r	0.378	0.304	-0.058
	P -value	0.000	0.000	0.395
	r	0.196	0.271	0.051
alanine	P- value	0.004	0.000	0,462
	r	0.158	0.176	-0.041
Leucine	P -value	0.020	0.000	0.552
	r	0.303	0.237	0.056
Arginine	P -value	0.000	0.000	0.417

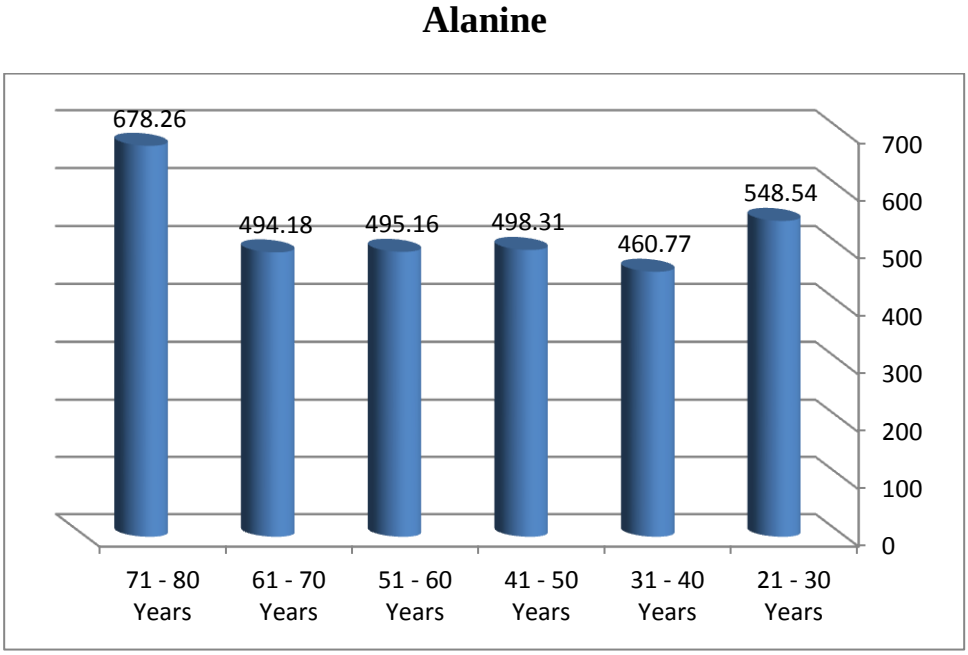
Table (4-10): **Frequency of age among case and control**:

age	Frequency	Percentage
11 - 20 Years	5	%2.3
21 - 30 Years	12	%5.6
31 - 40 Years	53	%24.8
41 - 50 Years	64	%29.9
51 - 60 Years	45	%21.0
61 - 70 Years	30	%14.1
71 - 80 Years	5	%2.3
Total	214	%100



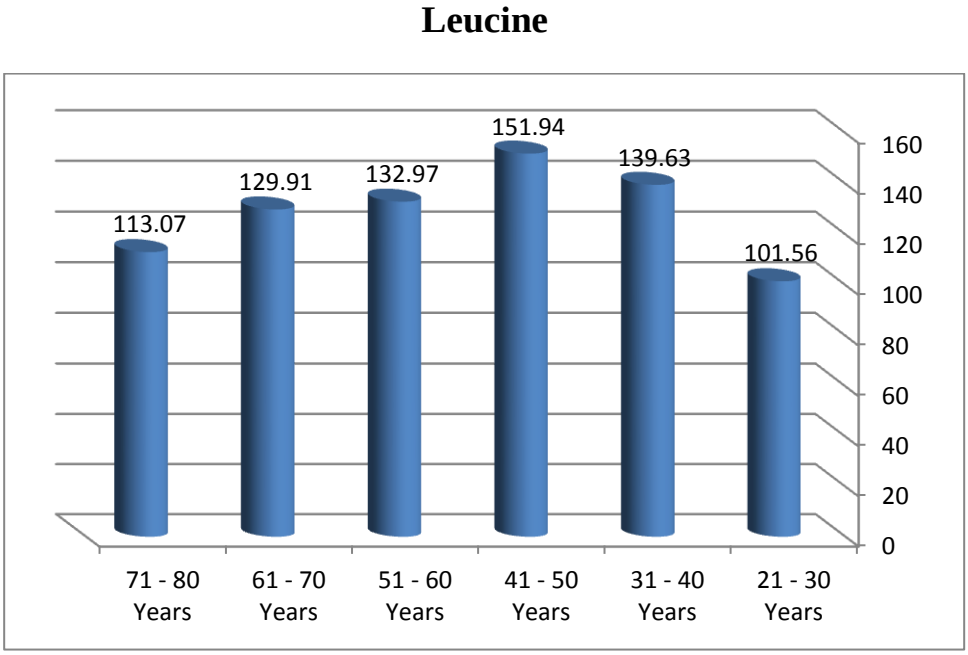
Figure(4-11) Frequency of age among case and control

Figure (4-12) shows comparison of alanine levels between age intervals indicated increased levels in elder patients (71-80 years age) (mean=678.26)



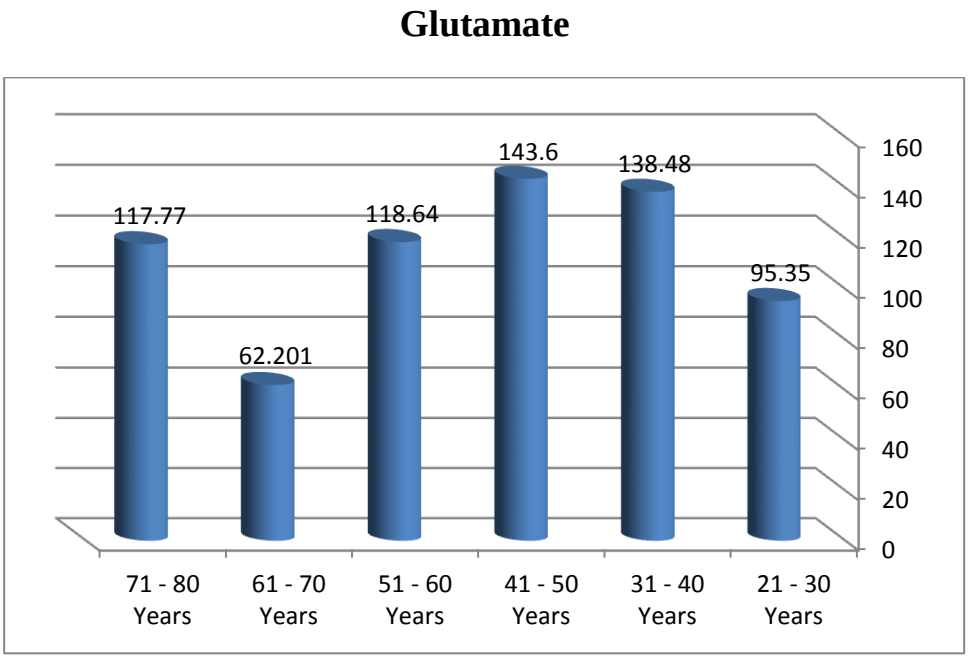
Figure(4-12): Shows comparisons of Alanine between Age intervals in diabetes mellitus (type2)

figure (4-13) shows comparison of leucine levels between age intervals shows increased levels in people aged (41-50 years) (mean=151.94),



Figure(4-13): Shows comparisons of Leucine between Age intervals in diabetes mellitus (type2)

figure (4-14) shows comparison of glutamate levels between age intervals shows increased levels in people aged (41-50 years) (mean=143.60),



Figure(4-14): Shows comparisons of glutamate between Age intervals in diabetes mellitus (type2)

figure (4-15) shows comparison of arginine levels between age intervals indicates increased levels in patients aged (41-50 years) (mean=92.20).

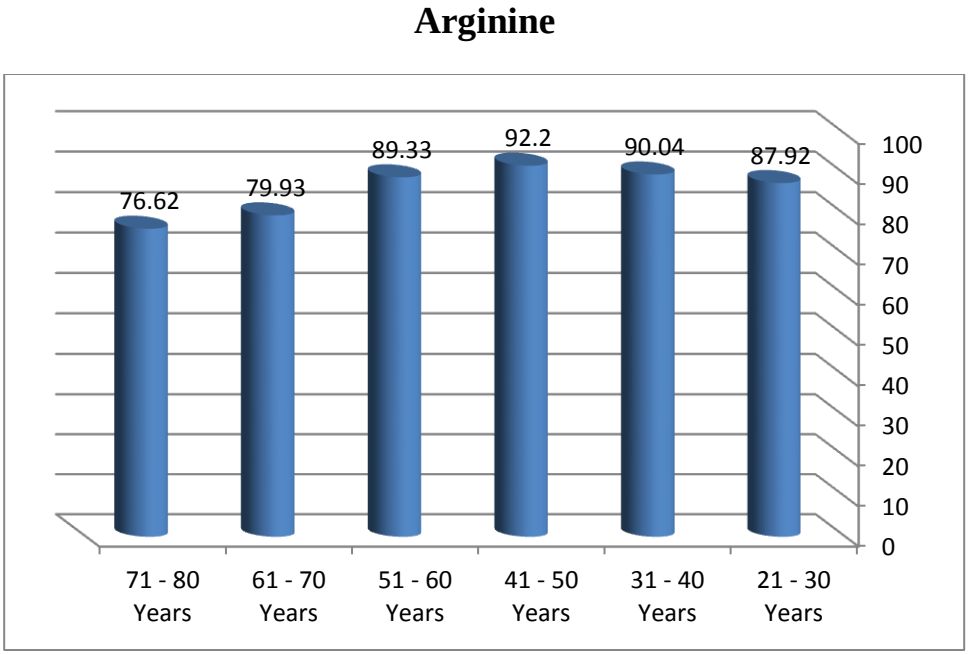


Figure (4-15): Shows comparisons of arginine between Age intervals in diabetes mellitus (type2)

figure (4-16) shows comparison of insulin levels between age intervals shows increased levels in patients aged (51-60 years) (mean=16.65)

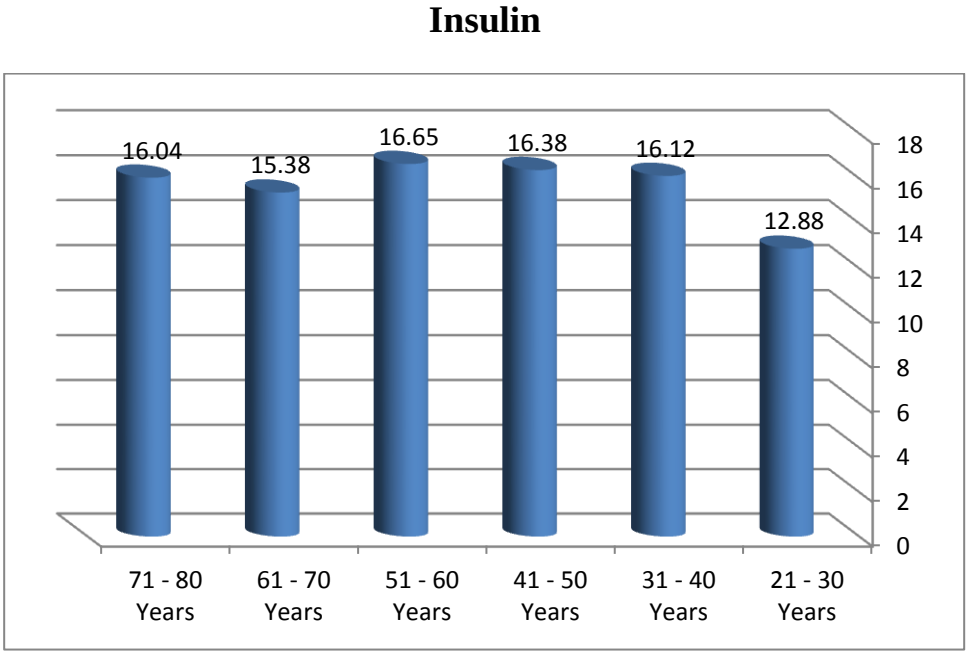


Figure (4-16): Shows comparisons of Insulin between Age intervals in diabetes mellitus (type2)

figure (4-17) shows comparison of HbA1C levels between age intervals indicated increased levels in patients aged (51-60 years) (mean=9.30),

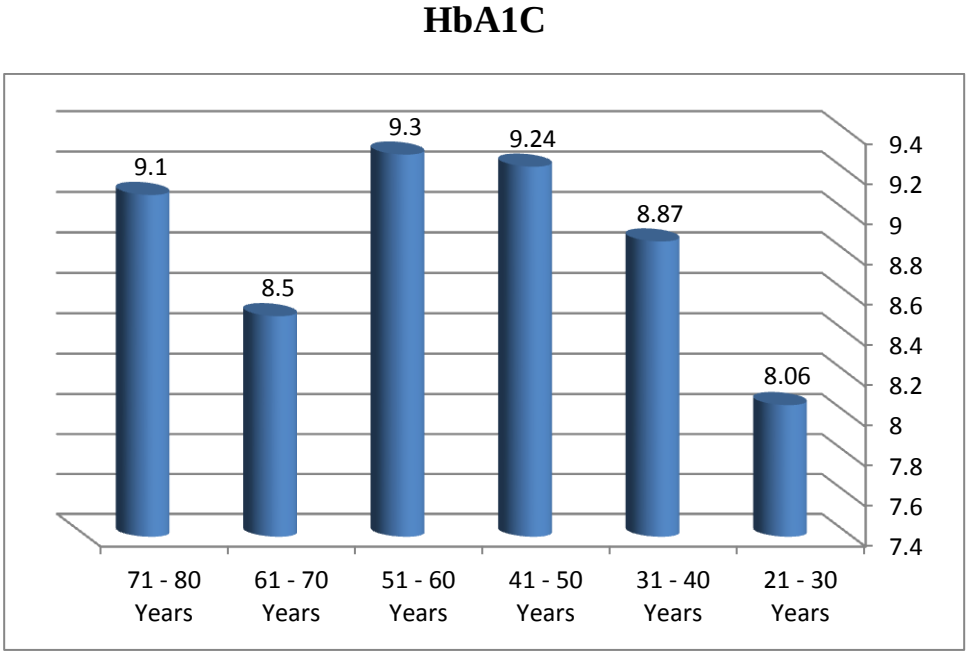


Figure (4-17): Shows comparisons of HbA1C between Age intervals in diabetes mellitus (type2)

figure (4-18) shows comparison of BMI levels between age intervals indicated increased levels in patients aged (41-50 years) (mean=26.51) .

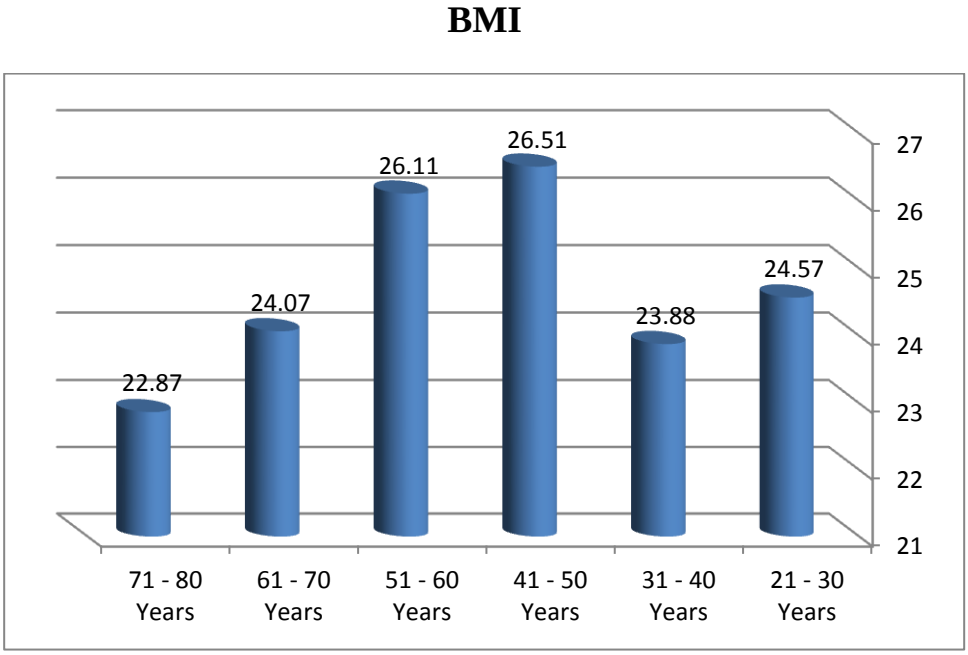


Figure (4-18): Shows comparisons of BMI between Age intervals in diabetes mellitus (type2)

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

5-1 Discussion:

The mechanisms by which amino acids confer their regulatory effects are complex and involve mitochondrial metabolism. Chronic effects of changes in amino acid concentration in vivo and in vitro on pancreatic β-cell function and integrity have not yet been investigated in detail, but initial experiments indicate an important role for amino acids in the regulation of pancreatic β-cell lipid metabolism and signal transduction. Therefore, changes can observe in the levels of amino acids in diabetics vs. non-diabetics.

A total of 167 with type 2 Sudanese diabetic patient where recruited in this study Males constituted 84 individuals (50.29%), and females 83 individuals (49.71%). in contrast to 47 healthy volunteers(Age and sex matched) as control the age range was from 20 years to 80 years. Results are shown in Tables and figures.

Significantly higher levels of alanine found among diabetic patients (mean=494.39±242.19) compared to control group (mean=330.00±97.65), significantly higher levels of alanine seen among female diabetic patients (mean=500.61±235.91) compared to male diabetic patients (mean=426.83±246.99), this is agreed with. (Newgard et al, 2009) who stated that BCAA contribute to insulin resistance but it is independent of body weight. The metabolism of this amino acid is associated with other amino acids – leucine and iso- leucine. and valine are referred to as branched-chain amino acids (BCAA). These amino acids have a different metabolism; unlike the other amino acids, they are degraded in muscles. Insulin resistance results in increased proteolysis and BCAA levels are elevated. The first step in the metabolism of BCAA is transamination withα-ketoglutarate to form branched-chain α-keto acids (BCKA) and glutamate. High accumulation of glutamate may lead to increased transamination of pyruvate to alanine. Similar results were found in obese subjects. One study reported that BCAA and aromatic amino acids were elevated 12 years before the onset of diabetes and the risk of diabetes was fourfold higher The authors assume that a combination of three amino acids (isoleucine, tyrosine and phenylalanine) could be a good predictor of diabetes (Wang et al,2011).

Significantly increased levels of luecine found among diabetic patients (mean=137.54±46.42) compared to control group (mean=119.66±10.87). insignificantly increase levels of luecine seen among female diabetic patients (mean=139.31±44.75) compared to male diabetic patients (mean=128.81±47.30). This is agreed with (Choo et al,2006) who said that to date, the mechanism by which luecine up regulates GK and ATP still remains unknown. However, recent studies have suggested that luecine signaling pathway may have crosstalk with some transcriptors or nuclear receptors including PDX-1(Moibi et al, 2007), LXR (Efanov et al,2004) and PPARγ(Kim SY et al,2004)(Kim HI et al,2004) in up regulation of GK and ATP.

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 ATP or other metabolic enzymes may represent a potential intervention strategy for treatment of islet dysfunction and type 2 diabetes.

Significantly increased levels of glutamate found among diabetic patients(mean=129.34±65.90) compared to a control group (mean=88.19±3.99), significantly higher levels of glutamate seen among female diabetic patients(mean=132.41±63.01) compared to male diabetic patients(mean=110.07±67.27) this result agreed with result carried by (Malaisse et al,1982) who stated that When islets are incubated with glutamine in the presence of BCH to activate GDH, there is stimulation of insulin release glutamine, after its conversion to glutamate by glutaminase, can also increase a-ketoglutarate production by GDH (Sener et al 1981) said the same, however, adding even a high concentration of glutamine alone to islets does not stimulate insulin release (Malaisse et al,1982) Glutamine alone probably does not promote insulin release because glutamate derived from glutamine would lower the level of oxaloacetate and pyruvate via reversing the mitochondrial alanine aminotransferase and aspartate aminotransferase reactions. Lowering the levels of oxaloacetate and pyruvate would diminish insulin release because there would be insufficient levels of these metabolites for their conversion into citrate and acyl-CoA .The concept that adding glutamine alone to islets leads to depletion of oxaloacetate and pyruvate by reversing these aminotransferase reactions is consistent with the fact that adding glutamine alone increases the level of alanine (from 14.3 to 26.3 pmol/islet) and the level of aspartate (from 17.2 to 28.4 pmol/islet) (Sener et al 1981)(Malaisse et al,1982) the a-ketoglutarate produced by GDH enables the production of pyruvate catalyzed by mitochondrial

alanine aminotransferase and oxaloacetate catalyzed by mitochondrial aspartate aminotransferase.

When glutamine alone is incubated with islets, the glutamate that is generated can also be decarboxylated to g-aminobutyrate (GABA) via the glutamate decarboxylase reaction (Pizarro et al,2010). Although GABA can be used for the production of succinate via the GABA shunt, and succinate is insulinotropic in fresh pancreatic islets (MacDonald et al,1990), the GABA pathway by itself is apparently not sufficiently active to promote insulin release (Pizarro et al,2010). When leucine is added with glutamine to activate a-ketoglutarate production by GDH, however, there is insulin release. This could be because sufficient a-ketoglutarate is generated for the transamination of GABA by GABA aminotransferase (Pizarro et al, 2010).

Significantly increased levels of arginine among diabetic patients(mean=88.66±31.13)found compared to control group (mean=69.23±19.76), insignificantly increase levels of arginine seen among female diabetic patients(mean=88.28±30.79) compared to male diabetic patients(mean=81.11±31.54) this is agreed with(Jensen et al,2008) , who said that glucose and other nutrients such as amino acids and fatty acids exert some of their effects on insulin secretion via their metabolism in β-cells to generate stimulus/secretion coupling factors, including a rise in the ATP/ADP ratio, which serves to suppress ATP-sensitive potassium (K_{ATP}) channels and activate voltage-gated Ca^{2+} channels, leading to stimulation of insulin granule exocytosis. In addition to the primary stimulus of glucose, specific amino acids may acutely and chronically regulate insulin secretion from pancreatic β-cells in vivo and in vitro.(Newsholme et al,2007) Mitochondrial metabolism is crucial for the coupling of glucose, alanine, glutamine and glutamate recognition with exocytosis of insulin granules. The positively charged amino acid L-arginine is now recognized as not only a powerful secretagogue, but also an essential synergic compound for nutrient-dependent insulin secretion.(Krause et al,2011) In addition to the known acute effects of some amino acids on β-cells, chronic exposure to specific amino acids may influence gene expression in the β-cell, which has an impact on insulin secretion and cellular integrity. Therefore amino acids may play a direct or indirect (via generation of putative messengers of mitochondrial origin) role in insulin secretion. . (Newsholme et al, 2007)

Significantly higher levels of insulin observed among the diabetic patients(mean=15.96±2.52) compared to a control group(mean=7.68±1.84), also significantly

higher levels of insulin seen among female diabetic patients(mean=16.67±2.33) compared to male diabetic patients(mean=15.26±2.52) this result agreed with results carried by (Danker et al,2009) who confirmed that hyperinsulinemia is a condition in which there are excess levels of insulin circulating in the blood relative to the level of glucose. While it is often mistaken for diabetes or hyperglycemia, Hyperinsulinemia can result from a variety of metabolic diseases and conditions. While Hyperinsulinemia is often seen in people with early stage type 2 diabetes mellitus, it is not the cause of the condition and is only one symptom of the disease. Type 1 diabetes only occurs when pancreatic beta-cell function is impaired. Hyperinsulinemia can be seen in a variety of conditions including diabetes mellitus type 2, in neonates and in drug induced hyperinsulinemia. It can also occur in congenital hyperinsulism.

Hyperinsulinemia is associated with hypertension, obesity, dyslipidemia, and glucose intolerance.(Modan et al,1985) These conditions are collectively known as Metabolic syndrome.(This close association between Hyperinsulinemia and conditions of metabolic syndrome suggest related or common mechanisms of pathogenicity.(Wang et al,2011) Hyperinsulinemia has been shown to "play a role in obese hypertension by increasing renal sodium retention.(Modan et al,1985)

In type 2 diabetes, the cells of the body become resistant to the effects of insulin as the receptors which bind to the hormone become less sensitive to insulin concentrations resulting in Hyperinsulinemia and disturbances in insulin release. (Shanik et al,2008) With a reduced response to insulin, the beta cells of the pancreas secrete increasing amounts of insulin in response to the continued high blood glucose levels resulting in Hyperinsulinemia. In insulin resistant tissues, a threshold concentration of insulin is reached causing the cells to uptake glucose and therefore decreases blood glucose levels. Studies have shown that the high levels of insulin resulting from insulin resistance might enhance insulin resistance.(Shanik et al,2008)

Significantly higher levels of HbA1C seen among diabetic patients(mean=8.97±1.53) compared to a control group(mean=5.17±.78) also significantly higher levels of HbA1C seen among female diabetic patients(mean=9.27±1.74) compared to male diabetic patients(mean=8.66±1.23) and agreed with.*(* Miedema et al,2005) who stated that 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..

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*)(* Peterson et al,1998*).*

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 *(*Larsen et al, 1990).

comparison between BMI in patients with diabetes mellitus (type2) and a control group indicated significant higher levels of BMI in diabetic patients (mean=25.18±3.64) compared to control group (mean=23.87±1.95) this is agreed with (H. E. Bays et al,2007)who said that An increase in body fat is generally associated with increased risk of metabolic diseases such as type 2 diabetes mellitus also comparison of BMI level between male and female diabetes mellitus (type2) indicated significant higher levels in female group(mean=25.86±4.03) compared to male group(mean=24.50±3.09) this is agreed with(Henderson et al,2009) who stated that higher protein turnover rates in women throughout adult life adiposity can accelerate protein turnover, comparison of alanine levels between age intervals indicated increased levels in elder patients (71-80 years age) (mean=678.2686) this agreed with (Smith et al,2008) who stated that amino acids levels significantly increased in elder patients ,comparison of leucine levels between age intervals showed increase levels in people aged (41-50 years) (mean=151.9431), comparison of glutamate levels between age intervals showed increase levels in people aged (41-50 years) (mean=143.6059), comparison of arginine levels between age intervals indicated increased levels in patients aged (41-50 years) (mean=92.2005) this was not agreed with (Smith et al,2009) who stated that amino acids level significantly increased in elder patients, comparison of insulin levels between age intervals showed increased levels in patients aged (51-60 years)

(mean=16.6500) this agreed with (Henderson et al,2009)who said that, hyperinsulinemia due to insulin resistance increased with age, comparison of HbA1C levels between age intervals indicated increased levels in patients aged (51-60 years) (mean=9.3033) this agreed with(Dubowitz et al 2014)who said that both glucose intolerance and HbA1c levels increased with age , comparison of BMI levels between age intervals indicated increased levels in patients aged (41-50 years) (mean=26.5146) this agreed with study done by (Smith et al,2009) who stated that BMI was reported to be high in middle-aged women and men (Smith et al,2009) .

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 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, however, another paper reported higher protein turnover rates in women throughout adult life adiposity can accelerate protein turnover (Gougeon et al,2008)(Henderson et al,2010)(Guillet et al,2009'). 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

Also comparison of alanine levels between patients with renal insufficiency and patients without renal insufficiency indicated increase levels of alanine in patients with renal insufficiency(mean=646.79±314.61) compared to patients without renal disease (mean=486.93±234.88) , comparison of leucine levels between patients with renal insufficiency in diabetes mellitus (type2) and patients without renal insufficiency indicated decrease levels of leucine in patients with renal insufficiency(mean=102.80±27.54) compared to patients without renal disease (mean=139.76±46.55), comparison of glutamate level between patients with renal insufficiency in diabetes mellitus (type2) and patients without renal insufficiency indicated increase levels of glutamate in patients with renal insufficiency(mean=141.50±50.19) compared to patients without renal disease (mean=128.56±66.82), comparison of arginine level between patients with renal insufficiency and arginine in patients without renal insufficiency indicated increase levels of arginine in patients with renal insufficiency(mean=88.82±34.32) compared to patients without renal disease (mean=88.65±31.04), also comparison between alanine levels in

diabetes mellitus (type2) with hypertension and patients without hypertension showed decrease levels of alanine in patients with hypertension (mean=445.32±206.51) compared to patients without hypertension (mean=511.58±250.47) , comparison between leucine levels in diabetes mellitus (type2) with hypertension and patients without hypertension, there was decrease levels of leucine in patients with hypertension (mean=124.66±43.85) compared to patients without hypertension (mean=141.34±46.64) ,comparison between glutamate levels in diabetes mellitus (type2) with hypertension and patients without hypertension showed decrease levels of glutamate in patients with hypertension (mean=103.47±56.89) compared to patients without hypertension (mean=136.96±66.62), comparison between arginine levels in diabetes mellitus (type2) with hypertension and patients without hypertension showed decrease levels of arginine in patients with hypertension (mean=82.46±27.82) compared to patients without hypertension (mean=90.49±31.91) , a comparison between alanine in diabetes mellitus (type2) with heart disease and patients without heart disease showed increase levels of alanine in patients with heart Disease (mean=737.52±227.06) compared to patients without heart disease (mean=489.07±239.45) , comparison of leucine in diabetes mellitus (type2) with heart disease and patients without heart disease showed decrease levels of leucine in patients with heart disease (mean=119.02±12.20) compared to patients without heart disease (mean=138.12±46.98) , comparison of glutamate in diabetes mellitus (type2) with heart disease and patients without heart disease showed increased levels of glutamate in patients with heart disease (mean=176.99±20.18) compared to patients without heart disease (mean=127.87±66.29), comparison of arginine in diabetes mellitus (type2) with heart disease and patients without heart disease showed increased levels of arginine in patients with heart disease (mean=112.19±16.66) compared to patients without heart disease (mean=87.94±31.22)

5-2 Conclusions:

- Among investigated adults in both genders, generally the results showed higher levels of alanine, luecine, glutamate, and arginine among diabetic patients compared to a control group ,also higher levels of alanine, luecine, glutamate, and arginine seen among female diabetic patients compared to male diabetic patients.
- higher levels of insulin, HbA1C, BMI was observed in this study among diabetic patients compared to a control group, also higher levels of insulin, HbA1C, BMI seen among female diabetic patients compared to male diabetic patients.
- Comparisons of alanine levels between age intervals showed increase levels in elder people (71-80 years age) . Comparisons of leucine levels between age intervals showed increase levels in people aged (41-50 years). Comparisons of glutamate levels between age intervals showed increase levels in people aged (41-50 years) comparisons of arginine levels between age intervals showed increase levels in people aged (41-50 years) . Comparisons of insulin levels between age intervals showed increase levels in people aged (51-60 years) ,comparisons of HbA1C levels between age intervals showed increase levels in people aged (51-60 years) Comparisons of BMI levels between age intervals showed increase levels in people aged (41-50 years) . .

5-3Recommendations:

In this study some of the known effects of the nutritional compounds on insulin secretion and β-cell metabolism reviewed. Understanding the molecular mechanisms by which glucose, amino acids regulate insulin secretion and cell integrity may identify novel targets for future diabetes therapies. Although there is growing evidences suggesting the beneficial effects of nutrients such as amino acids for the treatment of diabetes, With respect to the treatment of T2DM, more research is needed to investigate and identify the potential effects of individual nutrient (specific amino acid) supplementation in human clinical trials.

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APPENDIX

بسم الله الرحمن الرحيم

Sudan University of science and technology

College of graduate studies

Questionnaire

For PH.D degree

Association of some amino acids in serum with insulin secretion in Sudanese patients with type 2 diabetes mellitus

Name:..... الأسم

Age:..... العمر

Gender:..... (ذكر) الجنس (انثى)

Weight:.....الوزن

Height:.....الطول

Complications of diabetes mellitus مضاعفات مرض السكري

1) Ischemic heart disease نعم ( )لا( )
أمراض القلب

2) Hypertension نعم ( )لا( )
الضغط

3) Renal insufficiency نعم ( ) لا ( )

أمراض الكلي

Ministry of Higher Education Scientific Research
Central Laboratory
Department of Biochemistry

sample ID: S.C-S.P

Sample type: Blood

Name: Saida Mohammed Khair

Age:

Date of rec sample: 1/1/2014

Analysis required:
Glutamic acid
L-Alanine
Leucine
Arginine

Results: See attached Sheet and Chromatogram

Comments:

Head Department Dr. Nagwa Mohamed Ahmed **Sign.**

Gen.Director Dr.Dina A.H.Ibrahim **Sign.**

DRG

DRG® Insulin ELISA (EIA-1825)

CE

Revised 28 Jan. 2013 rm (Vers. 8.1)

IVD

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Insulin ELISA provides a method for the quantitative determination of human insulin in serum or plasma.

2 SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the β-cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain.

Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. Its principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, epinephrine (adrenaline), growth hormone and cortisol.

Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

3 PRINCIPLE OF THE PROCEDURE

Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microplate wells. A simple washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

4 WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- The contents of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop solution in this kit contains 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All samples should be handled as of capable of transmitting infections.

5 MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100, 200 and 1000 µl. (repeat pipettes preferred for addition of enzyme conjugate 1x solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Microplate reader (450 nm filter)

- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device

6 REAGENTS

Each Insulin ELISA kit contains reagents for 96 wells, sufficient for 42 samples and one calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers.
The expiry date for the complete kit is stated on the outer label.
The recommended storage temperature is 2 °C - 8 °C.

Coated Plate Mouse monoclonal anti-insulin For unused microplate wells completely reseal the bag using adhesive tape and use within two months.	1 plate	96 wells 8-well strips	Ready for use
Calibrators 1, 2, 3, 4, 5 Recombinant human insulin Color coded yellow Concentration indicated on vial label	5 vials	1000 µL	Ready for use
Calibrator 0 Color coded yellow	1 vial	5 mL	Ready for use
Enzyme Conjugate 11X (peroxidase conjugated mouse monoclonal anti-insulin)	1 vial	1.2 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	12 mL	Ready for use
Wash Buffer 21X Dilute with 1000 mL redistilled water to make wash buffer 1X solution Storage after dilution: 2 °C - 8 °C for 8 weeks.	1 bottle	50 mL	
Substrate TMB Colorless solution Note! Light sensitive!	1 vial	22 mL	Ready for use
Stop Solution 0.5 M H_2SO_4	1 vial	7 mL	Ready for use

6.1 Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer according to the table below.
When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently. Use within one day.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µL	7.0 mL
4 strips	350 µL	3.5 mL

7 SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation.
Samples can be stored at 2 °C - 8 °C up to 24 hours. For longer periods, store samples at –20 °C.
Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing heparin or EDTA as anticoagulant, and separate the plasma fraction.
Samples can be stored at 2 °C - 8 °C up to 24 hours. For longer periods store samples at -20 °C.
Avoid repeated freezing and thawing.

7.1 Preparation of samples

No dilution is normally required, however, samples containing > 200 mU/L should be diluted 1+9 v/v with Calibrator 0.

8 TEST PROCEDURE

All reagents and samples must be brought to room temperature before use.
Prepare a calibration curve for each assay run.

1. Prepare enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
3. Pipette 25 µL each of Calibrators and samples into appropriate wells.
4. Add 100 µL of Enzyme Conjugate 1X solution to each well.
5. Incubate on a plate shaker (700-900 rpm) for 1 hour at room temperature (18 °C – 25 °C)
6. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function function, after final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.
   Or manually,
   Discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash buffer 1X solution to each well. Discard the wash solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
7. Add 200 µL Substrate TMB into each well
8. Incubate for 15 minutes at room temperature (18 °C – 25 °C)
9. Add 50 µL Stop Solution to each well.
   Place plate on a shaker for approximately 5 seconds to ensure mixing.
10. Read optical density at 450 nm and calculate results.
    Read within 30 minutes

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

9 INTERNAL QUALITY CONTROL

Commercial controls such as Diabetes-antigen Control (Human) (Cat. No. CTL-5132) and/or internal serum pools with low, intermediate and high insulin concentration should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number; preparation date of components; OD values for the blank, Calibrators and controls.
Laboratories should follow government regulations or accreditation requirements for quality control frequency.

10 CALCULATION OF RESULTS

Computerized calculation

The concentration of insulin is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the concentration using cubic spline regression.

Manual Calculation

1. Plot the absorbance values obtained for the Calibrators, except for Calibrator 0, against the insulin concentration on a log-log paper and construct a calibration curve.
2. Read the concentration of the samples from the calibration curve.

Example of results

Wells	Identity	A_{450}	Mean conc. mU/L
1 A–B	Calibrator 0	0.070/0.071	
1 C–D	Calibrator 1 *	0.105/0.106	
1 E–F	Calibrator 2 *	0.202/0.204	
1 G–H	Calibrator 3 *	0.434/0.470	
2 A–B	Calibrator 4 *	1.348/1.351	
2 C–D	Calibrator 5 *	2.451/2.476	
2 E–F	Sample 1	0.222/0.214	11.1
2 G–H	Sample 2	0.546/0.538	35.6
3 A–B	Sample 3	1,941/1.978	153

* Concentration indicated on the vial label.

Conversion factor

1 µg/L = 23 mU/L;
1 mU/L = 6.0 pmol/L

11 LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.

Application of this test to individuals already undergoing insulin therapy is complicated by formation of anti-insulin antibodies that are capable of interfering in the assay.
Grossly lipemic, icteric or hemolysed samples do not interfere in the assay.
However, hemolysis in serum and plasma samples may result in a degradation of insulin which could give falsely low values and contributes to higher inter assay variation. The degradation is dependent on time, temperature and the hemoglobin concentration. Keep hemolyzed samples cold or on ice to prevent the insulin degradation.

12 EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own.

Fasting levels for 137 tested, apparently healthy individuals, yielded a mean of 9.2 mU/L, a median of 6.9 mU/L and a range, corresponding to the central 95% of the observations, of 2–25 mU/L.

13 PERFORMANCE CHARACTERISTICS

13.1 Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured. The detection limit is 1 mU/L as determined by the methodology described in ISO11843- Part 4.
Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

13.2 Recovery

Recovery upon addition is 94–113% (mean 104%).
Recovery upon dilution is 101-110% (mean 106%).

13.3 Hook effect

Samples with a concentration of up to 30 000 mU/L can be measured without giving falsely low results.

13.4 Precision

Each sample was analysed in six replicates on six different occasions.

Sample	Mean value mU/L	Coefficient of variation		
		Within assay %	Between assay %	Total assay %
1	11	3.4	3.6	5.0
2	36	4.0	2.6	4.7
3	80	2.8	2.8	4.0
4	154	3.2	2.9	4.4

13.5 Specificity

The following cross reactions have been found:

C-peptide < 0.01%

Proinsulin	< 0.01%
Proinsulin des (31–32)	< 0.5%
Proinsulin split (32–33)	< 0.5%
Proinsulin des (64–65)	98%
Proinsulin split (65–66)	56%
Insulin aspart	< 3.2%
Insulin detemir	<0.0000007%
Insulin glargine	19%
Insulin glulisine	<0.000003%
Insulin lispro	<0.000003%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	0.7%
Mouse insulin	0.3%
Porcine insulin	374%
Ovine insulin	48%
Bovine insulin	31%

14 CALIBRATION

The Insulin ELISA kit is calibrated against 1st International Reference Preparation 66/304.

15 WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by DRG may affect the results, in which event DRG disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.
DRG and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

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CE

HEMOGLOBIN A1C

BioSystems
REAGENTS & INSTRUMENTS

COD 11044 20 tests	COD 11045 100 tests
STORE AT 15-30°C	
Reagents for measurement of hemoglobin A_{1c} concentration Only for *in vitro* use in the clinical laboratory	

HEMOGLOBIN A1C

Chromatographic - spectrophotometric
ION EXCHANGE

PRINCIPLE OF THE METHOD

After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Hemoglobin A_{1c} (HbA_{1c}) is specifically eluted after washing away the hemoglobin A_{1a+b} fraction[1] (HbA_{1a+b}), and is quantified by direct photometric reading at 415 nm. The estimation of the relative concentration of HbA_{1c} is made by the measure of total hemoglobin concentration by direct photometric reading at 415 nm.

CONTENTS

	COD 11044	COD 11045
1. Reagent	1 x 30 mL	1 x 30 mL
2. Reagent	1 x 50 mL	1 x 240 mL
3. Reagent	1 x 450 mL	4 x 450 mL
4. Microcolumns	1 x 20	1 x 100

COMPOSITION

1. **Reagent.** Potassium phtalate 50 mmol/L, detergent 5 g/L, pH 5.0, sodium azide 0.95 g/L.
2. **Reagent.** Phosphate buffer 30 mmol/L, pH 6.5, sodium azide 0.95 g/L.
3. **Reagent.** Phosphate buffer 72 mmol/L, pH 6.5, sodium azide 0.95 g/L.
4. **Microcolumns.** Contain a pre-weighted amount of resin equilibrated with phosphate buffer 72 mmol/L, pH 6.5, sodium azide 0.95 g/L.

Use only microcolumns (4) and reagents 2 and 3 of the same kit.

STORAGE

Store at 15-30°C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity.
- Microcolumns (4): Absence of buffer over the resin bed.

ADDITIONAL EQUIPMENT

- Spectrophotometer or photometer with a 415 nm filter (405-425)

SAMPLES

Whole blood collected by standard procedures.

Hemoglobin A_{1c} is stable for 7 days at 2-8°C. Heparin or EDTA may be used as anticoagulants.

PROCEDURE

Hemolysate Preparation and Labile Fraction Elimination

1. Bring the columns and reagents to room temperature (21-26°C) (Note 1).
2. Pipette into a test tube:

Blood	50 µL
Reagent 1	200 µL

3. Shake thoroughly and let it stand at room temperature for 10-15 minutes. This hemolysate will be used in steps 6 and 11.

Column Preparation (Notes 2 and 3)

4. Remove the upper cap of the column and then snap the tip off the bottom.
5. Using the flat end of a pipette, push the upper disc down to the resin surface taking care not to compress it. Let the column drain completely to waste.

Separation and Reading of HbA_{1c} fraction

6. Carefully pipette on the upper filter:

Hemolysate	50 µL	Let the column drain to waste

7. In order to drain any sample residue left above the upper disc, pipette:

Reagent 2	200 µL	Let the column drain to waste

8. Pipette:

Reagent 2	2.0 mL	Let the column drain to waste

9. Place the column over a test tube and add:

Reagent 3	4.0 mL	Collect the eluate (HbA_{1c} Fraction)

10. Shake thoroughly and read the absorbance (A) of the HbA_{1c} fraction at 415 nm against distilled water (A_{HbA1c}). The absorbance is stable for at least one hour.

Reading of Hb_{TOTAL}

11. Pipette into a test tube:

Reagent 3	12.0 mL
Hemolysate	50 µL

12. Shake thoroughly and read the absorbance (A) at 415 nm against distilled water ($A_{HbTOTAL}$). The absorbance is stable for at least one hour.

CALCULATIONS

The HbA_{1c} relative concentration in the sample is calculated using the following general formula:

$$\frac{A_{HbA1c} \times V_{HbA1c}}{A_{HbTOTAL} \times V_{HbTOTAL}} \times 100 = \%HbA_{1c}$$

The volume of HbA_{1c} (V_{HbA1c}) is 4 mL, the volume of Hb total ($V_{HbTOTAL}$) is 12 mL. The following formula is deduced for the calculation of the concentration:

$$\frac{A_{HbA1c}}{A_{HbTOTAL}} \times \frac{100}{3} = \%HbA_{1c}$$

The results obtained with the present method are equivalent to a US National Glycohemoglobin Standardization Program certified method (NGSP) and can be converted into equivalent to the International Federation of Clinical Chemistry standardized method (IFCC), using the internationally recommended master equation[2,3]:

HbA_{1c}-IFCC (mmol/mol) = 10.93 x HbA_{1c}-NGSP-DCCT (%) – 23.5

REFERENCE VALUES

The following cut-off points have been established by the Diabetes Control and Complications Trial Research Group (DCCT) and have been adopted by many countries for a reference population (Non diabetic) and for the evaluation of the degree blood glucose control in diabetic patients[4,5].

NGSP-DCCT (%)	IFCC (mmol/mol)	Reference values / Degree of control
4.0 - 6.5	20 - 48	Non Diabetic
6.0 - 7.0	42 - 53	Goal
7.0 - 8.0	53 - 64	Good Control
> 8.0	> 64	Action suggested

QUALITY CONTROL

It is recommended to use the Hemoglobin A_{1c} Controls, Normal (cod. 18001) and Elevated (cod. 18002), to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: Lower than 4.0 % = 20 mmol/mol.
- Linearity limit: At least 17.0 % = 162 mmol/mol.
- Repeatibility (within run):

Mean Concentration	CV	n
7.2 % = 55 mmol/mol	5.4 %	25
9.9 % = 85 mmol/mol	6.3 %	25

- Reproducibility (run to run):

Mean Concentration	CV	n
7.2 % = 55 mmol/mol	7.3 %	25
9.9 % = 85 mmol/mol	5.9 %	25

- Trueness: Results obtained with this method did not show systematic differences when compared with reference methods. Details of the comparison experiments are available on request.
- Interferences: Bilirubin (20 mg/dL) and lipemia (triglycerides 10 g/L) do not interfere. Some drugs and other substances may interfere[6].

  In the ionic exchange chromatographic methods, the presence of hemoglobin C or S in the sample may slightly alter results, but differences are not clinically significant[7]. Other hemoglobin variants like HbE, HbF, carbamyl-Hb and acetyl-Hb can interfere[7,8]. The incubation with Reagent (1) eliminates the interference due to HbA_{1c}-labile.

  In hemolytic anemia, iron deficiency anemia and transfusion, the average age of erythrocytes is altered. Caution should be used when interpreting the HbA_{1c} results from patients with these conditions.

DIAGNOSTIC CHARACTERISTICS

HbA_{1c} is the product of the irreversible condensation of glucose with the N-terminal residue of the β-chain of hemoglobin A.

The HbA_{1c} concentration in blood is directly proportional to the mean concentration of glucose prevailing in the previous 6-8 weeks, equivalent to the lifetime of the erythrocytes[4], and the estimated average glucose (eAG) during this period can be calculated with the formulas below[9].

$$eAG\ (mg/dL) = 28.7 \times HbA_{1c}\text{-NGSP-DCCT}\ (\%) - 46.7$$

$$eAG\ (mmol/L) = 1.59 \times HbA_{1c}\text{-NGSP-DCCT}\ (\%) - 2.59$$

$$eAG\ (mg/dL) = 2.64 \times HbA_{1c}\text{-IFCC}\ (mmol/mol) + 15.0$$

$$eAG\ (mmol/L) = 0.146 \times HbA_{1c}\text{-IFCC}\ (mmol/mol) + 0.843$$

HbA_{1c} levels are a valuable adjunct to glucose determinations in the assessment and follow up of individuals with diabetes mellitus, providing much more reliable information for glycemia monitoring than do determinations of glucose. Numerous studies have shown that diabetes related complications may be reduced by the long term monitoring and tight control of blood glucose levels.

The HbA_{1c} concentration may also be a useful tool in the diagnosis of diabetes[10].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. The obtained values are temperature-independent when working in the recommended interval (21-26°C). If working temperature is out of range, multiply the obtained value by the corresponding factor showed in the following table:

Working temperature	Factor
18-20°C	1.15
27-30°C	0.90

2. The storage of the columns may lead to an excessive packing of the resin, diminishing the flow rate and lengthening the elution. To avoid it, invert the column, do a gentle spin movement, let it stand upside down for 10 minutes, then place it back to its upright position and let the resin settle for a few minutes before of opening the column.
3. Some air bubbles may occasionally appear inside the resin bed. Their presence does not alter the test performance.

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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.

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

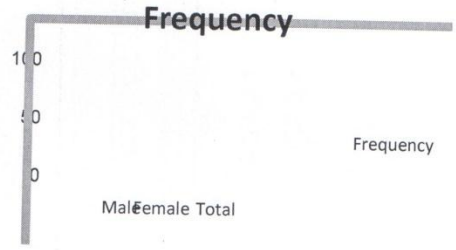
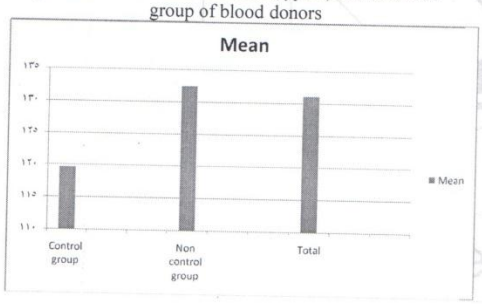


Table 2: Comparison of leucine level between patients with diabetes mellitus (type 2) and a control group of blood donors

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



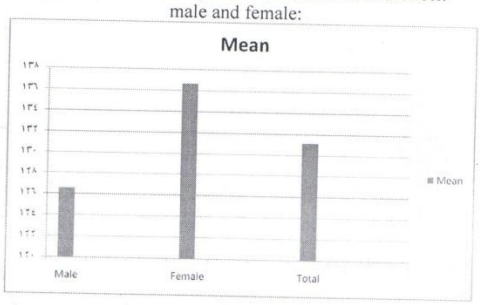
To date, the mechanism by which leucine up regulates GK and ATPβ still remains unknown. However, recent studies have suggested that leucine signaling pathway may have crosstalk with some transcriptors or nuclear receptors including PDX-1 (16), LXR (17) and PPARγ (18) (19) (20) in up regulation of GK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

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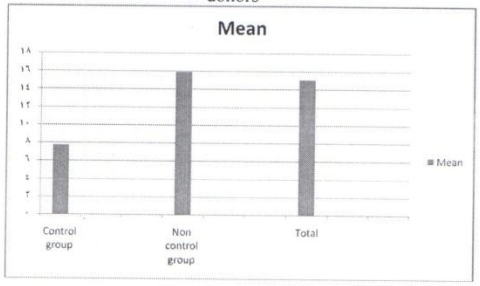
Figure 4: Correlations between insulin in patients with diabetes mellitus (type2) and a control group of blood donors

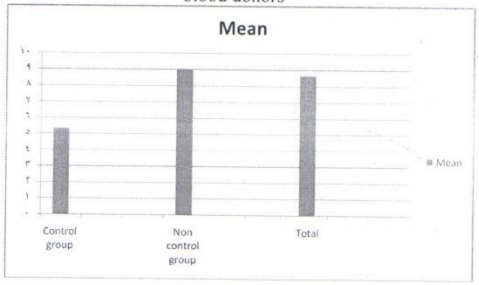


Ingestion of proteins or amino acids together with carbohydrates leads to strong insulin secretion in humans and animal models (29) (30) (31) Leucine is one of the most potent insulin secret agog among the branched-chain amino acids that facilitates glucose-induced insulin release from pancreatic β-cells (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

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



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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Association of Alanine in Serum with Insulin secretion among Sudanese Patients with Type 2 Diabetes Mellitus (A study in Khartoum State)

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Abstract:

Initial experiments indicate an important role for alanine in the regulation of β-cell lipid metabolism and signal transduction. Therefore, we can observe changes in the levels of alanine in diabetics vs. non-diabetics. To our knowledge no research have been done to investigate alanine amino acid associated with insulin secretion in diabetic patients type two in Sudan. This study was aimed to find differences in the levels of alanine 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 alanine and the serum levels of insulin in Sudanese patients with diabetes mellitus type 2. To assess the relationship between the serum levels of alanine 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 alanine 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 alanine 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 metabolism of alanine between diabetics and non-diabetics were observed. The altered levels of alanine in diabetic patients could be a suitable predictor of diabetes, also Hyperinsulinemia, is a condition in which there are excess levels of insulin circulating in the blood relative to the level of glucose. While it is often mistaken for diabetes or hyperglycemia, hyperinsulinemia can result from a variety of metabolic diseases and conditions.

Key words: Alanine, Insulin, Diabetes mellitus, Beta cells, Pancreas, Hyperinsulinemia

INTRODUCTION:

Diabetes mellitus:

Diabetes mellitus is a condition in which the body either does not produce enough, or does not properly respond to, insulin, a hormone produced in the pancreas. Insulin enables cells to absorb glucose in order to turn it into energy. In diabetes, the body either fails to properly respond to its own insulin, does not make enough insulin, or both, this causes glucose to accumulate

in the blood, often lead to various complications. Many types of diabetes are recognized.

Diabetes mellitus type 1:

Results from the body's failure to produce insulin .Presently almost all persons with type 1 diabetes must take insulin injections.

Diabetes mellitus type 2: –

Diabetes mellitus type 2 or non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes – is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency(1) Diabetes is often initially managed by increasing exercise and dietary modification. If the condition progresses, medications may be needed. Diabetes mellitus type 2 often affecting the obese.

Unlike type 1 diabetes, there is very little tendency toward ketoacidosis(2) One effect that can occur is nonketonic hyperglycemia. Long-term complications from high blood sugar can include increased risk of heart attacks, strokes, amputation, and kidney failure. For extreme cases, circulation of limbs is affected, potentially requiring amputation. Loss of hearing, eyesight, and cognitive ability has also been linked to this condition.

Due to aging, accelerated population growth, urbanization and high prevalence of obesity and an inactive lifestyle, the number of people with diabetes is increasing globally at a rapid speed. Important differences have been reported in the occurrence of DM and its complications between countries and between ethnic, cultural and even age groups within the same country. The prevalence of DM worldwide was estimated at 4% in 1995 and is expected to rise to 5.4% by the year 2025 . Consequently, the number of adults with DM will rise from 139 million to 300 million by the year 2025(3) The

major part of this increase will occur in developing countries. There will be 70% increase, from 84 to 128 million, in developing countries, and a 42%increase from 51 to 72 million in the developed countries. According to WHO estimates in 2000 the burden of diabetes is massive globally, with 20-35% of the diabetic patients suffering from neuropathy,30-45% with retinopathy, 10-20% with nephropathy, and from 10 to 25%having cardiovascular disease . Thus, the effect of diabetes on mortality and morbidity, its rapidly growing prevalence, and the high economic and human cost give emphasis on diabetes as a major global public health problem

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 ß-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.

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.

Amino acids are important modulators of glucose metabolism, insulin secretion and insulin sensitivity. However, little is known about the changes in alanine amino acid metabolism in patients with diabetes.

L-Alanine:-

L-alanine could stimulate insulin secretion under specific conditions of nutrient availability and that the mode of induction of insulin secretion may be a combination of increased ATP production and Na^+ co-transport (6).

The aim of this study was to find differences in the levels of alanine between patients with diabetes (type 2) and a control group

MATERIALS AND METHODS:-

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.

-An informed consents were obtained from all participants.

-Health education were 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 alanine 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.

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.

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RESULTS:

A total of 87 with type 2 Sudanese diabetic patient were 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 4. We found significantly increased levels of alanine 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)

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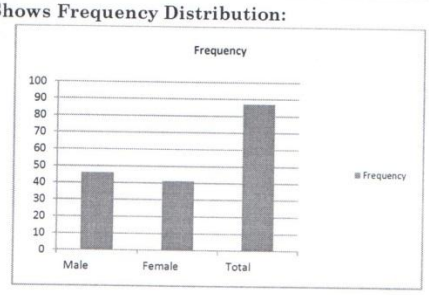
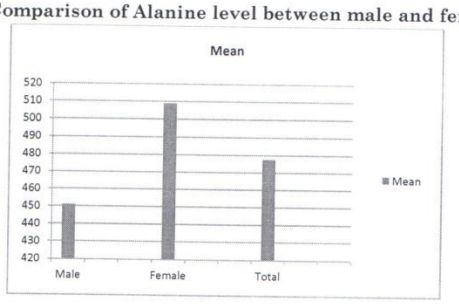


Table 2: Comparison of Alanine level between male and female:

Sex		Alanine
Male	Mean	451.170642
	N	53
	Std. Deviation	239.1775110
Female	Mean	509.107614
	N	44
	Std. Deviation	239.1096877
Total	Mean	477.451330
	N	97
	Std. Deviation	239.1096877

Figure 2: Comparison of Alanine level between male and female:



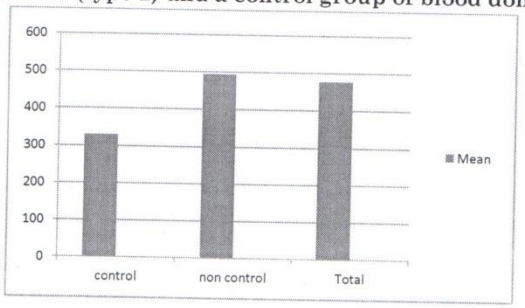
Only a few papers have directly addressed the question of sex dimorphism in protein metabolism in older persons. Surprisingly, two of these papers reported a higher muscle protein synthesis rate in older women as compared to BMI-

matched and age-matched men (7)(12)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 (8)However, another paper reported higher protein turnover rates in women throughout adult life (7) adiposity can accelerate protein turnover (9)(10)(11)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 3: Comparison of alanine level between patients with diabetes mellitus (type 2) and a control group of blood donors

Control, Noncontrol group		Alanine
Control group	Mean	330.077000
	N	10
	Std.Deviation	99.5356971
Non control group	Mean	494.390908
	N	87
	Std.Deviation	245.4833917
Total	Mean	477.451330
	N	97
	Std.Deviation	239.6582405

Figure 3 Shows Comparison of alanine level between patients with diabetes mellitus (type 2) and a control group of blood donors:

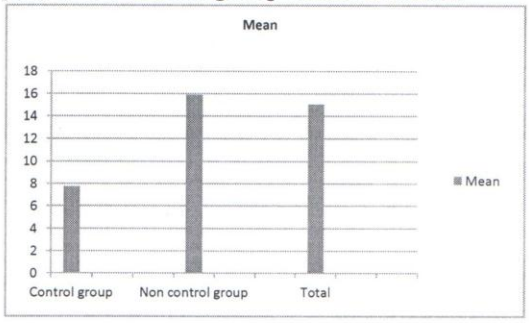


We found in patients with T2DM increased levels of alanine amino acid. The metabolism of this amino acid is associated with other amino acids – leucine and iso- leucine. and valine are referred to as branched-chain amino acids (BCAA). These amino acids have a different metabolism; unlike the other amino acids, they are degraded in muscles. Insulin resistance results in increased proteolysis and BCAA levels are elevated. The first step in the metabolism of BCAA is transamination withα- ketoglutarate to form branched-chain α-keto acids (BCKA) and glutamate. High accumulation of glutamate may lead to increased transamination of pyruvate to alanine. Similar results were found in obese subjects (13) The same authors state that BCAA contribute to insulin resistance but it is independent of body weight. One study reported that BCAA and aromatic amino acids were elevated 12 years before the onset of diabetes and the risk of diabetes was fourfold higher The authors assume that a combination of three amino acids (isoleucine, tyrosine and phenylalanine) could be a good predictor of diabetes (14)

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



Hyperinsulinaemia is a condition in which there are excess levels of insulin circulating in the blood relative to the level of glucose. While it is often mistaken for diabetes or hyperglycemia, hyperinsulinemia can result from a variety of metabolic diseases and conditions. While hyperinsulinemia is often seen in people with early stage type 2 diabetes mellitus, it is not the cause of the condition and is only one symptom of the disease. Type 1 diabetes only occurs when pancreatic beta-cell function is impaired. Hyperinsulinemia can be seen in a variety of conditions including diabetes mellitus type 2, in neonates and in drug induced hyperinsulinemia. It can also occur in congenital hyperinsulism, including nesidioblastosis.

Hyperinsulinemia is associated with hypertension, obesity, dyslipidemia, and glucose intolerance.(15) These conditions are collectively known as Metabolic syndrome.(16) This close association between hyperinsulinemia and conditions of metabolic syndrome suggest related or common mechanisms of pathogenicity.(14) Hyperinsulinemia has been shown to "play a role in obese hypertension by increasing renal sodium retention".(15)

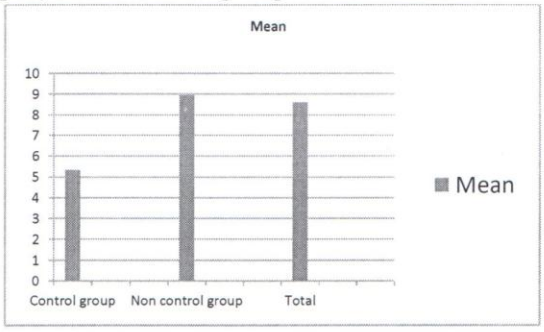
In type 2 diabetes, the cells of the body become resistant to the effects of insulin as the receptors which bind to the hormone become less sensitive to insulin concentrations

resulting in hyperinsulinemia and disturbances in insulin release.(17) With a reduced response to insulin, the beta cells of the pancreas secrete increasing amounts of insulin in response to the continued high blood glucose levels resulting in hyperinsulinemia. In insulin resistant tissues, a threshold concentration of insulin is reached causing the cells to uptake glucose and therefore decreases blood glucose levels. Studies have shown that the high levels of insulin resulting from insulin resistance might enhance insulin resistance.(17)

Table 5. Shows 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

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



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.(18)(19). 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(20).

CONCLUSION AND RECOMMENDATIONS:

Our results and the results of other studies dealing with the determination of amino acids levels in patients with T2DM suggest that the levels of amino acids in patients with T2DM are different from those in the control group and in patients with T1DM.

In conclusion, significant difference in metabolism of alanine amino acid between diabetics and non- diabetics were observed. Our results are in agreement with other studies(21) and support the statement that the altered levels of alanine amino acid in diabetic patients type2 could be a suitable predictor of diabetes in the future.

For people with type 2 diabetes, the problem of insulin resistance means there is plenty of insulin but the body does

not respond to it effectively. While most people associate this resistance with sugar levels in the blood, diabetes is also a problem with excess fat, especially too much fat inside skeletal muscle, which leads to the insulin resistance. If the level of fat in muscles can be reduced then, theoretically, insulin resistance can be prevented

A report published in 2009 by an International Expert Committee on the role of HbA1c in the diagnosis of diabetes recommended that HbA1c can be used to diagnose diabetes and that the diagnosis can be made if the HbA1c level is ≥6.5%(22).

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