EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 3/ June 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

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

SAYDA MOHAMMED KHEIR OSMAN
College of Applied Medical Sciences
King Khalid University, Abha, Saudi Arabia
Prof. OMER FADL IDRIS
Department of Biochemistry
College of Science and Technology
University of Al Nilain, Khartoum, Sudan
Dr. NOHA AL JAILY ABOBKER
College of Medical Laboratory Science
SUST Khartoum, Sudan

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.

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 alanine 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. Data Collection and Analysis Data collected in the tabulated database sheet and analyzed by SPSS. The data included the age,gender, weight, height, body mass index, insulin, HBA1C, alanine findings.

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)

Table 1: Shows Frequency Distribution

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

Figure (1) Shows Frequency Distribution:

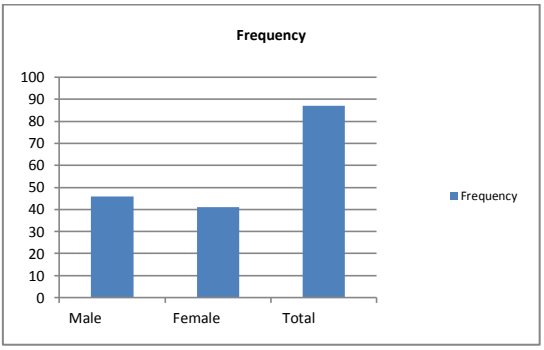
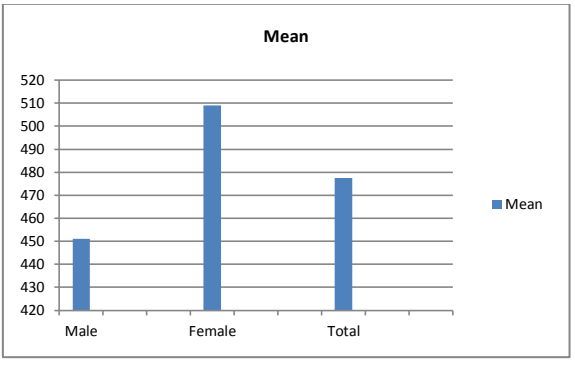


Table 2: Comparison of Alanine level between male and female:

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:



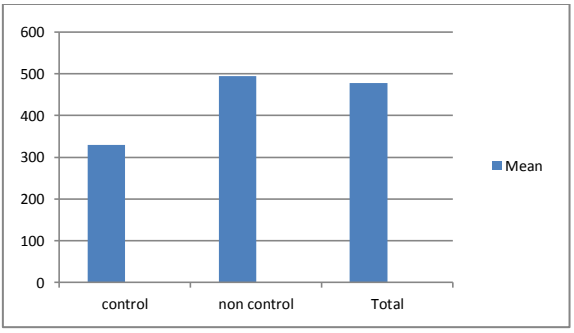
Only a few papers have directly addressed the question of sex dimorphism in protein metabolism in older persons. Surprisingly, two of these papers reported a higher muscle protein synthesis rate in older women as compared to BMI-

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:

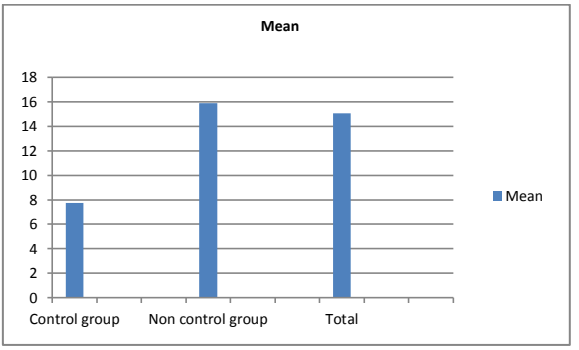


We found in patients with T2DM increased levels of alanine amino acid. The metabolism of this amino acid is associated with other amino acids – leucine and iso- leucine. and valine are referred to as branched-chain amino acids (BCAA). These amino acids have a different metabolism; unlike the other amino 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)

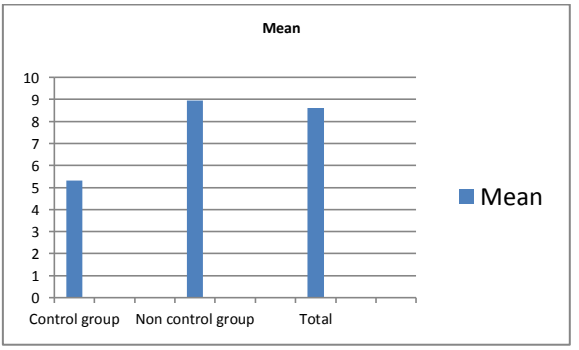
In type 2 diabetes, the cells of the body become resistant to the effects of insulin as the receptors which bind to the hormone become less sensitive to insulin concentrations

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).

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.

REFERENCE:

1. International Diabetes Federation Diabetes Blue Circle Symbol". 17 March 2006..
2. Kumar, Vinay; Fausto, Nelson; Abbas, Abul K.; Cotran, Ramzi S.; Robbins, Stanley .L. (2005). Robbins and Cotran Pathologic Basis of Disease (7th ed.). Philadelphia, Saunders. pp. 1194–1195. ISBN 0-7216-0187-1.
3. Murray Cj, Lopez (1996) AD. Harvard School of Public Health on behalf of the World Health Organization and the World Bank .The Global Burden of Disease. Harvard University Press. London
4. Marchetti P, Bugliani M, Boggi U, Masini M, Marselli L: The pancreatic ß cells in human type 2 diabetes. Adv Exp Med Biol 2012, 771, 288–309.

5. Zhang X, Wang Y, Hao F, Zhou X, Han X, Tang H, Ji L: Human serum metabonomic analysis reveals progression axes for glucose intolerance and insulin resistance statuses. J Proteome Res 2009, 8,5188–5195.
6. Curi R, Lagranha CJ, Doi SQ, Sellitti F, Procopio J, Pithon-Curi TC, Corless M, Newsholme P:(2005) Molecular mechanisms of glutamine action. J Cell Physiol204 :392 –401
7. Henderson G, Dhatariya K, Ford G, et al. Higher muscle protein synthesis in women than men across the lifespan, and failure of androgen administration to amend age-related decrements. FASEB J. 2009;23:631–641. This paper shows significant sex differences in protein metabolism. However, differences could be attributable to differences in body composition. The methodology employed is also somewhat different as compared to other papers.
8. Smith G, Atherton P, Reeds D, et al. No major sex differences in muscle protein synthesis rates in the postabsorptive state and during hyperinsulinemia–hyperaminoacidemia in middle-aged adults. J Appl Physiol. 2009;107:1308–1315. This paper reports a lack of sexual dimorphism in protein metabolism in middle-aged patients
9. Gougeon R, Morais J, Chevalier S, et al. Determinants of whole-body protein metabolism in subjects with and without type 2 diabetes. Diabetes Care. 2008;31:128–133.
10. Henderson G, Nadeau D, Horton E, Nair K. Effects of adiposity and 30 days of caloric restriction upon protein metabolism in moderately vs. severely obese women. Obesity (Silver Spring) 2010;18:1135–1142.This paper is important for understanding the influence of fat mass on protein metabolism.

11. Guillet C, Delcourt I, Rance M, et al. Changes in basal and insulin and amino acid response of whole body and skeletal muscle proteins in obese men. J Clin Endocrinol Metab. 2009; 94:3044–3050. This paper is important for understanding the influence of fat mass on protein metabolism
12. Smith G, Atherton P, Villareal D, et al. Differences in muscle protein synthesis and anabolic signaling in the postabsorptive state and in response to food in 65–80 year old men and women. PLoS One.2008;3: 1875.
13. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP: A branched-chain amino acid-related metabolic signature that differentiates obese and lean human and contributes to insulin resistance. Cell Metab 2009, 9, 311–326.
14. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnel CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE: Metabolite profiles and the risk of developing diabetes. Nat Med 2011, 17, 448–453.
15. Modan, Michaela; Halkin H; Almog S; Lusky A; Eshkol A; Shefi M; Shitrit A; Fuchs Z. (March 1985). "Hyperinsulinemia: A link between hypertension obesity and glucose intolerance". J. Clin. Invest. 75 (3): 809–
817. doi:10.1172/JCI111776. PMC 423608.PMID 3884667.
16. Danker, Rache; Chetrit A; Shanik MH; Raz I; Roth J (August 2009). "Basal-stat hyperinsulinemia in healthy normoglycemic adults is predictive of type 2 diabetes over a 24-year follow-up". Diabetes Care 32 (8): 1464–

1466. doi:10.2337/dc090153.PMC 2713622. PMID 19435 961.

17. Shanik, M.H.; Yuping, X.; Skrha, J.; Danker, R.; Zick, Y.; Roth, J. (2008). "Insulin Resistance and Hyperinsulinemia". Diabetes Care 31 (2): S262–S268. doi:10.2337/dco8-s264.
18. Miedema K (2005). "Standardization of HbA1c and Optimal Range of Monitoring".SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION 240: 61–*72*. doi*:*10.1080/00365510500236143. PMID 16112961.
19. Peterson KP, Pavlovich JG, Goldstein D, Little R, England J, Peterson CM (1998). "What is hemoglobin A1c? An analysis of glycated hemoglobins by electrospray ionization mass spectrometry". Clinical Chemistry (journal) *44 (9): 1951–1958*. PMID 9732983.
20. Larsen ML, Hørder M, Mogensen EF (1990). "Effect of long-term monitoring of glycosylated haemoglobin levels in insulin-dependent diabetes mellitus". N. Engl. J. Med.323 (15): 1021–*5*. doi*:*10.1056/NEJM199010113231503. PMID 2215560.
21. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP: A branched-chain amino acid-related metabolic signature that differentiates obese and lean human and contributes to insulin resistance. Cell Metab 2009, 9, 311–326.
22. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care, 2009, 32:1327-1334.