



Plasma insulin and fatty acid synthase levels in patients with type 2 diabetes mellitus.

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Abstract

Fatty acid metabolism is generally under regulation of hormones and nutritional status. Insulin being an anabolic hormone promotes synthesis of fatty acids and triglycerides, whereas insulin deficiency tends to increase mobilisation of fatty acids from triacylglycerols and increase fatty acid oxidation. Fatty acids are synthesized by fatty acid synthase (FASN) complex in mammalian cells. We carried out an analytical cross sectional study to find out the link between serum insulin and serum FASN levels. A total of 44 participants, 22 with type 2 diabetes mellitus (T2DM) and 22 normal non-diabetic controls were recruited. Serum insulin and circulating FASN were assessed in T2DM patients and non-diabetic control subjects. Our results showed that diabetics have significantly higher insulin and FASN levels ($p < 0.0001$ and $p = 0.018$ respectively) than non-diabetics. Insulin levels were found to be significantly correlated to FASN in controls ($r = 0.476$, $p = 0.034$). A non-significant correlation between serum insulin and circulating FASN was observed in diabetic participants ($r = 0.333$) ($p = 0.139$). In conclusion our results suggest that hyperinsulinaemia is a feature of T2DM and insulin increases serum concentration of FASN; hence the increased levels of serum FASN in diabetics with hyperinsulinaemia. Increased levels of FASN in turn stimulate lipogenesis which is responsible for some of the complications of diabetes mellitus. Therefore increased serum FASN may be a useful marker of glucose intolerance due to insulin resistance.

Key words: Diabetes mellitus, Insulin, Fatty acid synthase, Triglycerides

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. Blood glucose levels are controlled in part by insulin, a hormone in the body that helps move glucose from the blood to muscles and other tissues. The two types of diabetes are referred to as type 1 diabetes mellitus (T1DM) and type 2 diabetes

mellitus (T2DM). T1DM results from inadequate insulin secretion by the pancreas and T2DM results from either deficiency or lack of responsiveness to insulin.

An estimate of 371 million people had diabetes in 2012, which accounts for approximately 5.3% of the global population. The number of people with T2DM is increasing in every country and its has been estimated that by 2030 this will have risen to 552 million. There has been substantial migration of people from rural to urban areas in Zambia. This movement of people from rural to urban areas in part accounts for the increasing cases of T2DM as other studies suggested[2, 3]. Obesity due to inactivity or unhealthy diet is one of the factors why rural-urban migration has seen increased prevalence of T2DM.

Uncontrolled diabetes mellitus leads to serious long-term complications which include cardiovascular diseases (doubled risk), chronic renal failure, retinal damage (which can lead to blindness), nerve damage (of several kinds), and microvascular damage, which may cause impotence and poor wound healing. T2DM individuals are usually obese, and they manifest with insulin resistance,

hyperinsulinaemia, and hyperglycemia[4]. Chronic insulin resistance leading to hyperinsulinaemia, is a major contributor to glucose and lipid metabolism abnormalities that culminate in pathophysiologic changes with ravaging consequences throughout the body [5].

Insulin is the major fed state hormone produced by the pancreatic cells. The effect of insulin on cellular metabolism is vast, though the most emphasized aspect of insulin effect is the hormone's ability to facilitate glucose uptake by skeletal muscles and fat tissue. Therefore, insulin does not only lower blood glucose concentration, but also supplies the required fuel necessary for normal function of tissues that are dependent on insulin for glucose uptake. However, the less emphasized point is that insulin is the major lipogenic hormone and it is an important effector of fatty acid production in the liver via activation of FASN [6]. FASN is a cytosolic multi-enzyme complex that functions normally in the liver and is minimally expressed in other tissues [7]. FASN a key enzyme in *de novo* lipogenesis [7, 8], is involved in the formation of fatty acids that are esterified to glycerol to yield triacylglycerols. It is biochemically predictable that hyperinsulinaemia may lead to up-regulation of FASN expression in the liver which could lead to an increase in the triacylglycerol content of the liver resulting in increased very low density lipoprotein (VLDL) and free fatty acids secretion into the systemic circulation. In circulation there would therefore be of hypertriglyceridaemia, increased plasma non-esterified fatty acids (NEFAs) and high serum LDL-cholesterol that are usually observed in T2DM. These derangements in lipid metabolism contribute significantly to the pathogenesis of chronic complication of T2DM[9].

Our study therefore aimed to investigate the relationship between serum insulin and serum FASN in T2DM. Our hypothesis was that hyperinsulinaemia is associated with increased concentration of FASN, which gives rise to observed dyslipidemia.

Materials and methods

To investigate hyperinsulinaemia and its association with serum FASN concentrations among T2DM individuals at UTH, an analytical cross sectional study was proposed. This involved adult patients with T2DM (cases) and without diabetes mellitus (controls).The study was conducted at the University Teaching Hospital (UTH) Outpatient Department (Clinic 5), Lusaka, Zambia. Adults aged between 18- 75 years old with T2DM who reported to clinic 5 at UTH and met the inclusion criteria were

enrolled into the study. A study control group consisted of individuals without clinically or laboratory diagnosed diabetes mellitus who met the inclusion criteria. The study sample and the control group were categorically matched for age and sex in order to minimize bias.

A total sample size of 44 participants (22 patients and 22 controls) had been calculated using the formula for determination of sample size for comparative research studies between two groups as given below;

$$N = \frac{4\sigma^2(z_{crit} + z_{pwr})^2}{D^2},$$

Where, N is the total sample size (the sum of the sizes of both comparison groups); σ is 8, the assumed SD of each group (assumed to be equal for both groups); the z_{crit} value is 1.960 as given in tables for Standard Normal Deviate (z_{crit}) corresponding to the desired significance criterion of 0.05 or 95% confidence interval (CI); the z_{pwr} value is 1.282 as given in Standard Normal Deviate (z_{pwr}) tables corresponding to 90% statistical power; and D is the minimum expected difference between the two means which has been estimated at 7.

Systematic sampling in which consecutive individuals with T2DM that had reported to clinic 5 and met the inclusion criteria (given below) were included into the study sample. The control group was selected by means of frequency matching of the same proportional characteristics (age and sex) as the study sample.

Those individuals with T2DM that had been diagnosed within the last 10 years and not on exogenous insulin treatment, aged between 18 and 75 years old and gave a written consent without undue duress were included in the study. Individuals who were on insulin, pregnant, non-negroid Zambian, had chronic inflammatory conditions or declined to give consent were excluded. Participants were thoroughly examined by the Medical Officer and their clinical and demographic data recorded in confidential files. At least 4mls of blood was collected from the antecubital vein from each participant in plain vacutainers. The blood specimens were centrifuged at 3000 revolutions per minute (3000 rpm) in order to separate the serum (supernatant) from the blood cellular component (sediment). Only serum was then meticulously collected from the vacutainers using pipettes and transferred to 2ml plastic cryovial containers with sealable screw caps, which were stored in a freezer at -80°C until the specimens were required for analysis. Serum insulin concentration

was determined with the NeoBioLab™ Human INS ELISA Kit using a quantitative competitive Elisa for measurement of Human Insulin in cell culture fluid, body fluid, tissue homogenate, serum or plasma according to the manufacturer's instructions.

Circulating FASN concentrations was measured in serum without additives by NeoBioLab sandwich enzyme immunoassay (FAS ELISA) for the quantitative measurement of samples in serum, plasma, cell culture supernatants and urine as described elsewhere[10]. Briefly the standard curve was constructed using Microsoft Excel 2011 and from the standard curve concentrations of samples were determined. Optical density of the samples and controls were read at 450 nm. Data were expressed as mean ± SEM for normally distributed continuous variables or median (interquartile range) for non-normally distributed variables. Normality was assessed using the Shapiro and Wilk statistic and the normality plots. Skewed variables were log-transformed prior to analysis. The independent student's *t*-test was used to compare mean values of plasma insulin concentration to FASN concentration, between the two groups (T2DM and control groups). The data was cleaned and thereafter showed no violation of normality as assessed using the Shapiro and Wilk statistic, and also showed homoscedasticity as assessed using Levene test for equality of variance. Bivariate linear regression and correlation coefficients were used to assess correlation between insulin and FASN. The bivariate linear regression data on insulin vs. FASN were plotted and presented on scatter graphs. Data analysis was done using IBM SPSS Statistics version 22 for Mac and Microsoft Excel 2011 for Mac. Results were summarised on to tables and graphs as given below. All statistical tests were performed at 5% significance level or 95% confidence interval and differences were considered significant if 2-tailed $p < 0.05$. The study was approved by the University of Zambia Biomedical Research Committee (UNZABREC).

Results and discussion

Insulin, FASN mean difference: The study found that T2DM participants had statistically significant higher insulin concentration ($35.9 \pm 3.6 \mu\text{IU/mL}$) compared to non-diabetic participants ($10.8 \pm 1.4 \mu\text{IU/mL}$), $t(40) = 6.518$, $p < 0.0001$ (Fig. 1a). FASN concentration was higher in T2DM participants (FASN; $22.8 \pm 4.1 \text{ ng/mL}$) than in control participant (FASN; $11.8 \pm 1.4 \text{ ng/mL}$) with statistical significance, $t(41) = 2.471$, $p = 0.018$ (Fig. 1b).

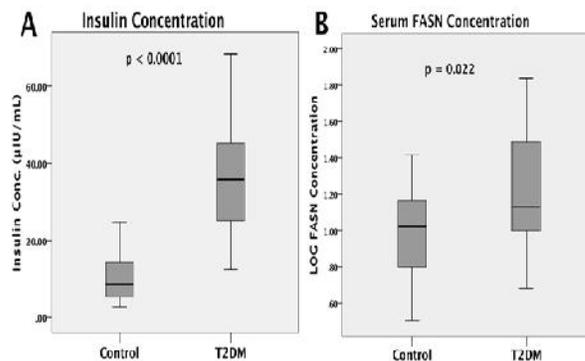
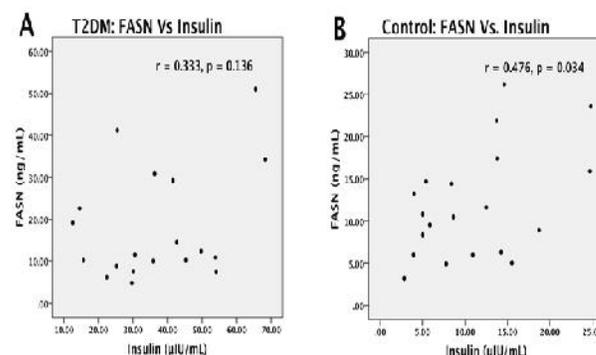


Figure 1: Insulin, FASN mean difference

(a) Insulin mean concentration difference between T2DM group and non-diabetic controls. (b) FASN mean concentration difference between T2DM and non-diabetic controls. Difference assessed using independent sample *t*-Test with *p*-value significance at 95% confidence level.

Linear regression analysis of insulin Vs. FASN: Bivariate linear regression analysis of FASN vs. insulin showed moderate positive correlation in T2DM participants though not statistically significant ($r = 0.333$, $p = 0.163$) (Fig. 2a). FASN Vs. insulin were moderately correlated in healthy controls participants with statistical significance ($r = 0.476$, $p = 0.034$) (Fig. 2b).



a): Linear association between insulin and FASN in T2DM group. b): Linear association between insulin and FASN in health non-diabetic control group. *r* = the correlation coefficient and *p* = *p*-value. Statistical significance determined at 95% confidence level.

Anthropometrics and Biochemicals Mean Difference: The T2DM participants were older (41.3 ± 2.2 years) than healthy control participants (37.1 ± 1.8 years) with statistical significance, $t(42) = 4.314$; $p < 0.0001$. Diabetic participants had similar BMI ($27.6 \pm 1.5 \text{ kg/m}^2$) to healthy controls ($27.8 \pm 1.3 \text{ kg/m}^2$), $t(42) = 0.071$; $p = 0.944$ (Table 1). Fasting blood glucose levels were higher in T2DM subjects ($9.6 \pm 0.9 \text{ mmol/L}$), than healthy control ($5.1 \pm 0.1 \text{ mmol/L}$) with statistical significance, $t(36) = 4.89$, $p < 0.0001$ (Table 1). Alanine amino transferase (ALT),

aspartate amino transferase (AST) and albumin levels had shown no statistically significant difference, $t(42) = -.887$; $p = 0.38$, $t(42) = -0.722$; $p = 0.475$ and $t(39) = 0.602$; $p = 0.551$ respectively between diabetic participants (ALT; 22.4 ± 4.0 U/L, AST 27.2 ± 2.3 U/L and ALB 25.4 ± 0.37 mmol/L), and control participants (ALT; 18.1 ± 2.8 U/L, AST; 25.1 ± 1.8 U/L, and ALB; 25.7 ± 0.35 U/L (Table 1).

	¹ Controls (n=22)	² T2DM (n=22)	<i>p</i>
Age (yr)	37.1 ± 1.8	41.3 ± 2.2	< 0.0001
BMI (kg/m ²)	27.8 ± 1.3	27.6 ± 1.5	0.944
Fasting Glucose (mmol/l)	5.1 ± 0.1	9.6 ± 0.9	< 0.0001
ALT (U/L)	18.1 ± 2.8	22.4 ± 4.0	0.38
AST (U/L)	25.1 ± 1.8	27.2 ± 2.3	0.475
Albumin (mmol/l)	25.7 ± 0.35	25.4 ± 0.37	0.551

Table 1: Anthropometric and metabolic characteristics of the study groups¹

¹ Health control individuals and individuals with T2DM. ²*P* represents overall significance of differences across groups. *P*-values were derived from independent sample student's *t*-test. Age and BMI were included in the model as covariates. AST, ALT and ALB were used to assess the association between hepatic function with serum insulin, FASN and triglycerides concentration.

Our results show that T2DM patients had statistically higher levels of insulin concentration than normal controls. This finding has also been observed in many studies and is generally one of the hallmarks of the condition. It has been postulated that the hyperinsulinaemia that is observed in this condition is brought about by insulin resistance on target tissues; and the body tries to offset this by producing more insulin. Increase in insulin resistance in a way is positively related to the magnitude of hyperinsulinaemia and this has been observed in the absence of conditions likely to affect insulin responsiveness like excess secretion of growth hormone[11]. The increase in insulin resistance also tends to worsen the glucose tolerance. It has also been observed that in the later stages of the condition impairment of insulin secretion may set in due to toxic damage by radical species on pancreatic cells or exhaustion pancreatic -cells giving rise to a full blown picture of diabetes mellitus[12].

Insulin facilitates glucose uptake in skeletal muscles by translocating glucose transporters from intracellular location to the plasma membrane where they pick up glucose and internalize it in cells. For

translocation to take place there is an intracellular signalling pathway that is activated when insulin binds to its receptors on cell membranes. Insulin action involves a series of signaling cascades initiated by insulin binding to its receptor, eliciting receptor autophosphorylation and activation of the receptor tyrosine kinase, resulting in tyrosine phosphorylation of insulin receptor substrates (IRSs). Phosphorylation of IRSs leads to activation of phosphatidyl inositol 3-kinase (PI3K) and, subsequently, to activation of Akt and its downstream mediator AS160, all of which are important steps for stimulating glucose transport induced by insulin. Human studies have shown that insulin resistance is caused by dysregulation of signalling by IRS1 and IRS2 proteins as a common underlying mechanism[13, 14]. Fatty acids appear to cause this defect in glucose transport by inhibiting insulin-stimulated tyrosine phosphorylation of IRS and IRS-1 associated phosphatidylinositol 3-kinase activity[15]. Abnormalities in lipid metabolism lead to deposition and accumulation of lipids in muscles, liver and pancreatic cells. Accumulation of these lipids in the muscle and liver lead to insulin resistance whereas in the pancreatic -cells the result is impaired function.

This brings us to our second important finding of significantly higher FASN concentrations in T2DM than in controls. Mammalian FASN consists of 2 identical 270-kD polypeptide chains, each comprising all 7 enzyme activities. Our findings were in agreement with other studies, which showed that individuals with T2DM have significantly higher fasting serum insulin concentration and mean serum FASN concentration than non-T2DM individuals ($p < 0.0001$; and $p = 0.018$). Increased serum FASN affirms the insulin-FASN axis, which describes insulin-stimulated up-regulation of FASN gene expression[16-18]. Evidence of the insulin-FASN axis was in part explained in this study by the moderate correlation between insulin and plasma FASN concentration in control participants with statistical significance ($r = 0.476$, $p = 0.0034$). However, the correlation between insulin and circulating FASN in individuals with T2DM was not statistically significant ($r = 0.115$, $p = 0.619$). The lack of statistically significant correlation in the T2DM participant group may have been due to diabetic pathophysiologic changes and/or drug treatment effect[19, 20]. These may have altered the metabolic interplay between insulin and FASN in diabetic individuals. However, any definitive conclusion on this discrepancy should await more studies and research that are statistically powered to access the above-mentioned effects. Nevertheless, the

T2DM group showed statistically significant higher FASN concentration ($p = 0.018$) than the non-diabetic group. But then, what are the implications of increased FASN expression? Up-regulation of FASN expression in the liver may lead to an increase in the triglycerides content of the liver which culminates in increased VLDL and free fatty acids secretion into the systemic circulation [16, 21, 22], contributing to the development of hypertriglyceridaemia. This metabolic link between insulin and FASN implies that, under hyperinsulinaemic conditions, the liver is turned into lipid ‘biosynthesis factory’ with all of its negative downstream effects, including the genesis of hypertriglyceridaemia [23, 24]. It is this derangement in lipid metabolism that is believed to be responsible for insulin resistance.

Furthermore, since hyperinsulinaemia is an early event [13, 25] in the disease pathology and correlated to FASN, we may postulate that over expression of hepatic FASN (and other lipogenic enzymes) and the outcome dyslipidaemias in T2DM must also be early events of T2DM pathogenesis. With respect to the above observations, further conclusions are made that the molecular lesions that culminate in chronic complications of T2DM such as macrovascular disorders may also be early events in the progression of T2DM. Furthermore, the findings of this research may corroborate other finding that overproduction of VLDL and FFA by the liver takes place early in the development of overt T2DM [26-28] suggesting that pancreatic β -cell failure as a results of islets lipotoxicity is a progressive mechanism that begins from the onset of T2DM [29, 30]. This takes us to a reasonable conclusion that β -cell demise maybe the end result of untreated and/or poorly managed diabetic dyslipidaemias, which in themselves stem from increased FASN activity. It must also be stated that the regulatory step in fatty acid synthesis is catalysed by acetyl-CoA carboxylase [31]. This enzyme carboxylates acetyl-CoA to form malonyl CoA; this malonyl CoA is the substrate for FASN. Acetyl-CoA carboxylase is inactive when it is phosphorylated and active when it is dephosphorylated. Insulin activates acetyl-CoA carboxylase by stimulating a pathway that dephosphorylates it [32]. Therefore apart from increasing the level of FASN, insulin also promotes dyslipidaemia by activating the enzyme which gives FASN the substrate.

In addition, if vascular complications that lead to cardiovascular disease in T2DM are early events that stem from FASN-driven lipogenesis and progress over many years then we may postulate that, diabetic cardiovascular complications, which are by

far the major cause of mortality among diabetics, may not be averted by rigorous glycaemic control alone. This view is supported by various evaluations of insulin-regulated metabolic pathways and the molecular pathogenesis of T2DM [33]. In keeping with this view, the United Kingdom Prospective Diabetes Study (UKPDS), the American Diabetes Association and the European Association for the Study of Diabetes and others have shown that dietary intervention and/or glycaemic control to the non-diabetic range have major benefits for diabetic related microvascular and neuropathic complications, but no benefits on cardiovascular complications and major macrovascular events [34, 35].

Here we propose that manipulation of FASN through inhibitors may be one important mode for the control of dyslipidaemias associated with hyperinsulinaemia because FASN inhibitors may help to reduce insulin resistance and diabetic dyslipidaemias.

The fact that data on lipid profiles of both T2DM and controls was not collected and there was no follow up the participants were limiting factors. In addition it would have been appropriate to determine the phosphorylation status or activity of acetyl-CoA carboxylase since insulin is known to modulate its activity.

Conclusions

In conclusion therefore our study showed that T2DM patients in Lusaka, Zambia have significantly higher levels of insulin and FASN than non-diabetic controls. T2DM patients also have significantly higher fasting blood sugar level. The high insulin levels may be a result of insulin resistance in peripheral tissues; and the associated high FASN levels may be the main cause of dyslipidaemias responsible for some of the long term complications of T2DM.

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