



INVESTIGATION OF DYSLIPIDEMIAS ASSOCIATED WITH TYPE 2 DIABETES MELLITUS.

Musalula Sinkala¹, Trevor Kaile¹, Sandra Chileya², Clemence Marimo¹, Lydia Korolova³, Geoffrey Kwenda³, Gibson Sijumbila^{2*}

¹Department of Pathology and Microbiology Lusaka, Zambia

²Department of Physiological Sciences, Lusaka, Zambia

³Department of Biomedical Sciences, Lusaka, Zambia

Abstract: Diabetes mellitus is a complex metabolic disease which affects carbohydrate, protein and lipid metabolism. In view of the significant effect on lipid metabolism we investigated the relationships between type 2 diabetes mellitus and blood levels of triglycerides, cholesterol, high density lipoproteins (HDL) and low density lipoproteins (LDL). This was an analytical cross-sectional study where participants were adult patients with type 2 diabetes mellitus (cases) and without diabetes mellitus (controls). Diabetes mellitus patients had significantly higher levels of blood fatty acid synthase (FAS) and triglycerides than non diabetic controls. There was a weak insignificant positive correlation in blood levels of fatty acid synthase and triglycerides in both the patients and the controls. In addition the blood concentrations of cholesterol, HDL, and LDL were not significantly different between type 2 diabetes mellitus patients and non diabetic controls. Our conclusion was that type 2 diabetes mellitus patients had significantly higher levels of FAS and triglycerides than normal controls and that there was no significant positive correlation between FAS levels and triglyceride levels. In addition there was no significant difference in cholesterol metabolism between the two groups.

Key words: Diabetes mellitus, Fatty acid synthase, Hypertriglyceridemia

Introduction

Insulin is one of the most important hormones in regulation of carbohydrate, protein and lipid metabolism. In diabetes mellitus, a chronic metabolic disease that comes about because of

the body's inability to take up glucose in the cells due to either insulin resistance, reduced insulin secretion or both, there is marked derangement of lipid metabolism known as dyslipidemia. Dyslipidemia basically comprises hypertriglyceridemia, low concentration of blood HDL cholesterol, high concentrations of blood LDL cholesterol. Insulin resistance and dyslipidemia are features of the metabolic syndrome which in addition includes obesity, hypertension, impaired glucose tolerance, and hyperinsulinemia¹. The most common form of

For Correspondence:

gibson.sijumbila@Tunza.zm

Received on: October 2014

Accepted after revision: December 2014

Downloaded from: www.johronline.com

diabetes mellitus is referred to as type 2 diabetes mellitus (T2DM) and this is often associated with obesity and occurs in adults^{2,3}. T2DM individuals usually present with insulin resistance, hyperinsulinemia, and hyperglycemia⁴. The cardiovascular complications of diabetes mellitus are mostly a result of the abnormalities in lipid metabolism⁵. From an initial perception that a disorder of glucose metabolism was the primary event in the pathogenesis of T2DM, there is now a growing realization that chronic elevation of free fatty acid (FFA) levels is an early event that contributes to the development of this disease. This line of thought is supported by the fact that there is tight association between insulin resistance and dyslipidemia. It has also been demonstrated that if plasma FFA levels are elevated for more than a few hours, they will cause insulin resistance⁶. Later findings also showed that elevation of plasma free fatty acids (FFAs) plays a pivotal role in the development of T2DM by causing insulin resistance⁷. Other findings have also suggested that circulating levels of FFAs, which are often elevated in obese and diabetic individuals, have an important causative link in the association of obesity with insulin resistance and T2DM⁸. The mechanism by which FFAs cause insulin resistance, although not completely understood, may involve generation of lipid metabolites like diacylglycerol, proinflammatory cytokines such as TNF- α , IL1 β , IL6, MCP-1 and cellular stress including oxidative and endoplasmic reticulum stress⁹.

In order to investigate the lipid abnormalities associated with T2DM we carried out a cross sectional study in which we compared the lipid profiles in diabetics and non diabetic participants. And to elucidate further the possible mechanisms for dyslipidemia we also studied the association between fatty acid synthase (FAS), the main enzyme in the synthesis of fatty acids and the level of triglycerides.

This work was very important as it was going to give us an idea about the dyslipidemia profile in the general population and T2DM patients; information that would be vital to policy makers

in the fight against T2DM and its complications.

Materials and Methods

This was a cross-sectional study which was carried out at the University Teaching Hospital (UTH) in Lusaka, Zambia. The study population consisted adults aged between 18-75 years old with T2DM that reported to clinic 5 at UTH and control cases were adults who came to the hospital with minor ailments or for medical check-ups. A total of 44 participants (22 cases) and (22 controls) were enrolled in the study. 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 using a 5 ml syringe and a 20G needle from the antecubital vein of 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.

Blood for fasting blood sugar was collected from diabetics and controls. Circulating FAS concentration 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 elsewhere¹⁰. 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. Blood triglyceride, cholesterol, LDL, HDL levels were determined using the available test kits on the Pentra 400 Chemistry Analyser

available in UTH Clinical Chemistry Laboratory according to the manufacturer's recommendation and assay procedures for the automated analyser. All test protocols were calibrated and controls ran before samples could be assayed.

Data were expressed as mean \pm SEM for normally distributed continuous variables. The independent student's *t*-test was used to compare mean values for concentrations of FAS, triglycerides and other biochemical tests (Cholesterol, LDL, HDL,) between the two groups (T2DM and non-T2DM group). Data analysis was done using IBM SPSS Statistics version 22 for Mac and Microsoft. All statistical tests were performed at 5% significance level or 95% confidence interval and differences were considered significant if 2-tailed *p* value was less than 0.05 ($p < 0.05$). The study was approved by the University of Zambia Biomedical Research Ethics Committee (UNZABREC).

Results and Discussion

FAS and triglyceride conc. Mean difference

FAS concentration and triglyceride concentration were higher in T2DM participants (FAS; 22.8 ± 4.1 ng/mL and TG; 1.46 ± 0.17 mmol/L) than in control participant (FAS; 11.8 ± 1.4 ng/mL and 1.03 ± 0.1 mmol/L) with statistical significance, $t(41) = 2.471$, $p = 0.018$ and $t(41) = 2.14$, $p = 0.039$ respectively (Fig. 1a and Fig. 1b, respectively).

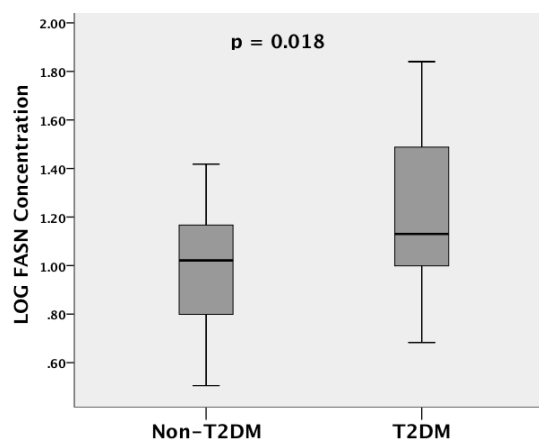


Fig 1a Serum Fatty acid synthase concentration difference between T2DM and non-diabetic controls

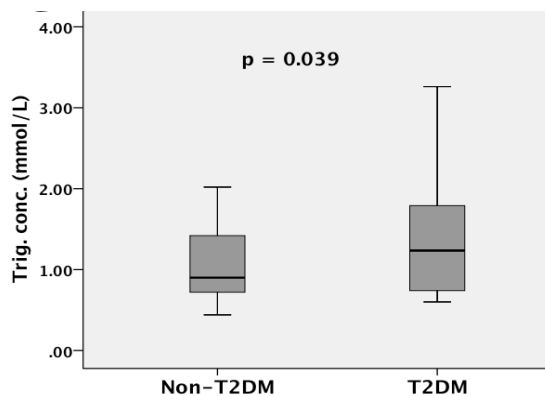


Fig 1b Serum triglyceride concentration difference between T2DM and non-diabetic controls

Bivariate linear regression analysis of FAS and triglycerides showed a weak correlation in both diabetic participants (Fig. 2a) and healthy controls (Fig. 2b) without statistical significance ($r = 0.288$, $p = 0.193$ and $r = 0.121$, $p = 0.612$ respectively)

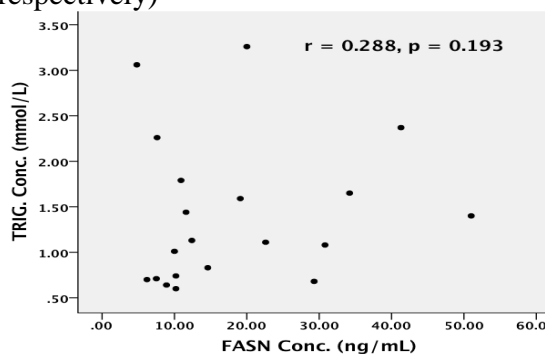


Fig. 2a Correlation between FAS and triglycerides in T2DM group

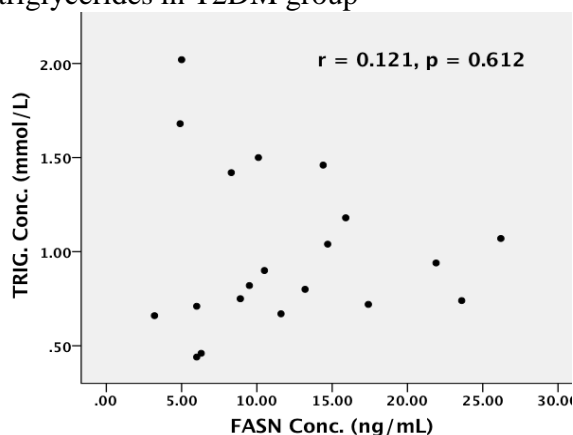


Fig. 2b Correlation between FAS and triglycerides in health non-diabetic control group

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 lower BMI ($27.6 \pm 1.5 \text{ kg/m}^2$) compared to healthy controls ($27.8 \pm 1.3 \text{ kg/m}^2$), but the differences was not significant, $t(42) = 0.071$; $p = 0.944$ (Table 1.0). Fasting blood glucose levels were higher in T2DM subjects ($9.6 \pm 0.9 \text{ mmol/L}$), than healthy controls (5.1 ± 0.1

mmol/L) with statistical significance, $t(36) = 4.89$, $p < 0.0001$ (Table 1.0). Total cholesterol, HDL-cholesterol, and LDL-cholesterol were higher in T2DM participants ($5.28 \pm 0.33 \text{ mmol/L}$, $1.31 \pm 0.06 \text{ mmol/L}$, and $1.14 \pm 0.08 \text{ mmol/L}$ respectively) than in the control group ($4.75 \pm 0.23 \text{ mmol/L}$, $1.39 \pm 0.12 \text{ mmol/L}$, and $1.31 \pm 0.13 \text{ mmol/L}$), but the differences were not statistically significant, $t(42) = 1.314$; $p = 0.196$, $t(39) = 1.118$; $p = 0.270$, and $t(42) = 0.678$; $p = 501$ respectively (Table 1.).

Table 1 Anthropometric and Metabolic characteristics of the study groups¹

	Ideal levels	Controls (n=22)	T2DM (n=22)	p
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)	3.1-6.1	5.1 ± 0.1	9.6 ± 0.9	< 0.0001
Total Cholesterol	Below 5.2	4.75 ± 0.23	5.28 ± 0.33	0.196
LDL – cholesterol (mmol/L)	Below 2.6 mmol/L-lower desirable	1.31 ± 0.13	1.14 ± 0.08	0.551
HDL – Cholesterol (mmol/L)	1.6 mmol/L and above –higher desirable	1.39 ± 0.12	1.31 ± 0.06	0.270

¹Health non-T2DM individuals and individuals with T2DM. ²P represents overall significant differences across groups. P-values were derived from independent sample student's t-test. Age and BMI were included in the model as covariates

The results show that individuals with T2DM have significantly higher mean serum FAS concentration than non-T2DM individuals ($p = 0.018$). This is rather surprising because insulin has been shown to increase activity of fatty acid synthase¹¹⁻¹³. So in the presence of relative insulin deficiency or resistance as in diabetes mellitus one would expect reduction in the levels of serum FAS. This can be partly explained in the context of development of diabetes mellitus. It has been observed that free fatty acids, a product of fatty acid synthase tend to promote the development of insulin resistance through poorly understood mechanisms which may involve generation of lipid metabolites (diacylglycerol), proinflammatory cytokines (TNF- α , IL1 β , IL6, MCP-1) and cellular stress including oxidative and endoplasmic reticulum stress⁹. Insulin

resistance would then stimulate increased secretion of insulin resulting in hyperinsulinemia establishing a multiplier effect in the development of diabetes. Increased fatty acids are converted into triglycerides which are then exported from the liver as very low density lipoproteins^{11,14,15}.

Blood triglyceride concentrations were significantly higher in T2DM patients than the controls. This is in line with what has been observed in other studies where it was observed that it is a common lipid abnormality in persons with visceral obesity, metabolic syndrome T2DM¹⁶⁻¹⁸. The cause of the hypertriglyceridemia in patients with diabetes mellitus is mainly due to increased mobilization of fatty acids from peripheral tissues which are conjugated into triglycerides in the liver and these triglycerides are released in circulation in

the form of very low density lipoproteins. Activation of fatty acid synthase may also be a contributing factor in the early stages of diabetes mellitus development.

There was a weak insignificant positive correlation between fatty acid synthase levels and triglyceride levels in diabetes mellitus patients and controls. This lack of significant correlation is not a surprise at all as the most important cause of hypertriglyceridemia is mobilization of fatty acids from peripheral tissues which are converted into triglycerides by the liver. Synthesis of fatty acids in the liver by FAS may not be a major factor in causation of hypertriglyceridemia in established T2DM.

The average age of T2DM patients was significantly higher than non diabetic controls which are to be expected as T2DM tends to be more common from early forties upwards. There was no significant difference in body mass index between diabetes mellitus patients and non diabetic controls. This could have been due to the inevitable weight loss diabetic patients tend to have especially in severe cases. This comes about because of rapid breakdown of and mobilization of muscle proteins and triglycerides from storage tissues. Fasting blood sugar was significantly higher in diabetic patients than in controls as hyperglycemia is one of the cardinal features of diabetes mellitus. In uncontrolled diabetes one would expect to see abnormalities of cholesterol metabolism. This was not the case in our study as there was no significant difference in total cholesterol, LDL-cholesterol and HDL-cholesterol between type 2 diabetes mellitus patients and non diabetic controls. This could have been due to improved management of diabetes mellitus in study subjects like provision of appropriate diet, regular exercises and oral medication as needed. The classical dyslipidemia found in uncontrolled diabetes mellitus comprises hypertriglyceridemia, low blood concentration of HDL- cholesterol and high blood concentration of LDL- cholesterol^{19,20}. It is the LDL-cholesterol (bad cholesterol) that is pathogenic to the cardiovascular system. All these lipid changes associated with diabetes

mellitus are attributed to increased free fatty acid flux secondary to insulin resistance. The fact the cholesterol metabolism in diabetics was not significantly different from the non diabetics, even though they had elevated blood sugar may not be as high as one would expect. Hence the need for effective control of T2DM is very important to minimize possible complications, many of which are as a result of dyslipidemias.

The study was limited in that the T2DM patients recruited had diabetes mellitus over different time intervals and duration of the disease was not taken into consideration. Even though a diagnosis of the disease within 10 years was the inclusion criteria, many people only present with complications after they have had the disease for sometime. The time of diagnosis may not necessarily coincide with the duration of the disease. In addition they were managed differently; some were on dietary control measures only and others were on different medications depending on what they responded best to or a combination of both.

Conclusion

The main findings were that type 2 diabetes mellitus patients had significantly higher levels of FAS and triglycerides than normal controls and that there was no significant positive correlation between FAS levels and triglycerides levels. In addition there was no significant difference in cholesterol metabolism between the two groups. Type 2 diabetes mellitus patients had significantly higher fasting blood sugar levels than non-diabetic controls and there was no significant difference in body mass index between the two study groups.

Funding/Support:

Southern Africa Consortium for Research Excellence (SACORE) Zambia and the University of Zambia (UNZA) supported this study.

Acknowledgment:

We thank the administration in the University Teaching Hospital (UTH) for their assistance during the research process. We also thank staff of the UNZA – UCL Endocrinology Research Laboratory for their support and hard work.

Additionally, we express great appreciation to the subjects who participated.

References

- Holz B, Paulweber B, Sandhofer F, Patsch JR. Hypertriglyceridemia and insulin resistance. *J Intern Med* 1998; 243:79-82.
- Sobngwi E, Mbanya JC, Unwin NC, Kengne AP, Fezeu L, Minkoulou EM, Aspray TJ, et al. Physical activity and its relationship with obesity, hypertension and diabetes in urban and rural Cameroon. *Int J Obes Relat Metab Disord* 2002; 26:1009-1016.
- Azimi-Nezhad M, Ghayour-Mobarhan M, Parizadeh MR, Safarian M, Esmaeili H, Parizadeh SM, Khodae G, et al. Prevalence of type 2 diabetes mellitus in Iran and its relationship with gender, urbanisation, education, marital status and occupation. *Singapore Med J* 2008;49:571-576.
- Joven J, Espinel E, Rull A, Beltran-Debon R, Aragonés G, Rodríguez-Gallego E, Camps J, et al. Serum fatty acid synthase concentration is increased in patients with hepatitis viral infection and may assist in the prediction of liver steatosis. *J Clin Virol*;51:199-201.
- McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. *Science* 1992;258:766-770.
- Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, Coleman E, et al. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. *J Clin Invest* 1991;88:960-966.
- Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Relation of high TG-low HDL cholesterol and LDL cholesterol to the incidence of ischemic heart disease. An 8-year follow-up in the Copenhagen Male Study. *Arterioscler Thromb Vasc Biol* 1997;17:1114-1120.
- Lam TK, Carpentier A, Lewis GF, van de Werve G, Fantus IG, Giacca A. Mechanisms of the free fatty acid-induced increase in hepatic glucose production. *Am J Physiol Endocrinol Metab* 2003;284:E863-873.
- Boden G. Obesity, insulin resistance and free fatty acids. *Curr Opin Endocrinol Diabetes Obes*;18:139-143.
- Ito T, Sato K, Maekawa H, Sakurada M, Orita H, Shimada K, Daida H, et al. Elevated levels of serum fatty acid synthase in patients with gastric carcinoma. *Oncol Lett*;7:616-620.
- Menendez JA, Vazquez-Martin A, Ortega FJ, Fernandez-Real JM. Fatty acid synthase: association with insulin resistance, type 2 diabetes, and cancer. *Clin Chem* 2009;55:425-438.
- Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 2008;118:829-838.
- Davalos A, Goedeke L, Smibert P, Ramirez CM, Warriar NP, Andreo U, Cirera-Salinas D, et al. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proc Natl Acad Sci U S A*;108:9232-9237.
- Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 1999;401:73-76.
- Van Obberghen E, Baron V, Delahaye L, Emanuelli B, Filippa N, Giorgetti-Peraldi S, Lebrun P, et al. Surfing the insulin signaling web. *Eur J Clin Invest* 2001;31:966-977.
- Fisher WR. Hypertriglyceridemia in diabetes. An approach to management. *J Fla Med Assoc* 1991;78:747-750.
- Jialal I, Amess W, Kaur M. Management of hypertriglyceridemia in the diabetic patient. *Curr Diab Rep*;10:316-320.
- Subramanian S, Chait A. Hypertriglyceridemia secondary to obesity and diabetes. *Biochim Biophys Acta*;1821:819-825.
- Najjar SM, Yang Y, Fernstrom MA, Lee SJ, Deangelis AM, Rjaily GA, Al-Share QY, et al. Insulin acutely decreases hepatic fatty acid synthase activity. *Cell Metab* 2005; 2:43-53.
- Ginsberg HN. Insulin resistance and cardiovascular disease. *J Clin Invest* 2000; 106:453-458.