CHANGES IN THE LEVELS OF SIALIC ACID AMONG ORGANS DURING THE COURSE OF STREPTOZOTOCIN-INDUCED TYPE I AND TYPE II DIABETES

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ZARIA

NOVEMBER, 2016

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P13SCBC8083

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD

OF A

MASTER OF SCIENCE DEGREE IN BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

SEPTEMBER, 2016

DECLARATION

I declare that the work in this dissertation entitled "**Changes in the Levels of Sialic Acid among Organs during the Course of Streptozotocin-Induced Type I and Type II Diabete**s" has been carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project report was previously presented for another degree or diploma at this or any other Institution**.**

…… …… …….

Peter OBOCHI Signature Date

CERTIFICATION

This dissertation entitled CHANGES IN THE LEVELS OF SIALIC ACID AMONG ORGANS DURING THE COURSE OF STREPTOZOTOCIN-INDUCED TYPE I AND TYPE II DIABETES by Peter OBOCHI meets the regulations governing the award of the degree of master of science in Biochemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

DR. M.A. Ibrahim. …….

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Chairman, Supervisory Committee Signature Date

Professor. M.N. Shuaibu. …….

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Member, Supervisory Committee Signature Date

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Head of Department Signature Date

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Dean, School of Postgraduate Studies Signature Date

DEDICATION

This project work is dedicated to Almighty God the creator of the heavens and the earth for giving me the grace, strength and opportunity to make this dream come true.

ACKNOWLEDGEMENT

Though as a matter of requirement, only my name appears at the cover of this project report, but a great many individuals contributed to this success. I sincerely owe a huge gratitude to all those that made this project possible and because of whom my experience in ABU is a cherishable one.

My deepest gratitude goes to my supervisor, Dr. Mohammed Auwal Ibrahim. I have been so fortunate to have the best supervisor; he gave me freedom to explore and all the guidance in many difficult situations. Today, the success I have is anchored on Dr. Auwals patience and support. I sincerely hope, one day I too would become a good supervisor to my students as Dr. Auwal is to me.

My co-supervisor, Professor, M. N.Shuaib was always there to listen and give advice, you were always there during the difficult times. My sincere gratitude Sir.

I am also indebted to the workers in the animal house of Department of Pharmacology for their practical advice in animal handling. I retain a huge gratitude also to the laboratory scientist in Department of Biochemistry post -graduate laboratory; Mr. Apeh and Mr Aliyu, without them my analysis would have been hell.

My friends and colleagues that helped me stayed sane and focused through the period, I say thank you for keeping on the track.

Most importantly, none of these would have been possible without the love, patience and support from my immediate family; I dedicate this report to you. Most importantly and above all, I reserve my greatest thanks to God Almighty for making all these reality.

ABSTRACT

This study was undertaken to investigate the changes of sialic acid levels in some tissues: liver, kidney, pancreas, skeletal muscle and brain of sreptozotocin-induced diabetic rats over a period of 9 weeks and 8 weeks respectively for types I and II so as to understand the actual source of serum sialic acid upsurge during diabetes. The animals were classified into type I diabetic group (TIDG), type II diabetic group (TIIDG) and the normal control group (NCG). The type I diabetic condition was induced using 60 mg/kg body weight of streptozotocin while the type II diabetes was induced with 10% fructose along with 40 mg/kg body weight of streptozotocin. Fasting blood glucose, free serum sialic acid level and total sialic acid level in the tissues were monitored at 3 and 2 weeks intervals for a period of 9 and 8 weeks for type I and type II diabetes, respectively. There was a significant ($P<0.05$) decrease in free serum sialic acid level at the early stage at (week 2) for the TIIDG. The same pattern was observed in the TIDG at week 3 but insignificant ($P>0.05$). Also, there was an increase in free serum sialic acid level with the progression of the disease in TIDG and TIIDG, though insignificant ($P>0.05$) compared to the NCG. There was a significant ($P<0.05$) increase in total sialic acid level in the following tissues; brain, kidney, pancreas, skeletal muscle except the liver. Correlation analysis performed on the relation between levels of sialic acids in the serum and tissues shows that there was a level of varying negative correlation between the level of free serum sialic acids and the total sialic acid in the entire tissues; liver, kidney, pancreas, skeletal muscle and brain over the course of the experiment. The correlations show a relationship of increasing level of serum free sialic acid with decreasing total sialic in the tissues. This pattern of relationship, suggests that sialic acids in the serum were possibly liberated from the tested individual tissues. Increase in sialic acids in these tissues: liver, kidney, pancreas, skeletal muscle and brain is responsible for increased level free serum sialic acids seen in diabetes mellitus.

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ABBREVIATIONS

FSA: free serum sialic acid

TSA: total sialic acid

TNFα: Tumor necrosis factor alpha

FBG: Fasting blood glucose

Homa-IR: Homeostasis model assessment insulin resistance

GLUT4: Glucose transporter 4

ELISA: Enzyme linked immunosorbent assay

STZ: Streptozotocin

CVD: Cardiovascular diseases

PAD: Peripherial artery diseases

CTP: Cytidine tryphosphate

CMP: Cytidine monophosphate

NANA: N-acetyl neuraminic acid

AGES: Advanced glycation end products

RAGES: Receptor for advanced glycation end products

HRP: Horseradish peroxidase

mRNA: Messenger ribonuclic acid

TCA cycle: Tricarboxylic acid cycle or the citric acid cycle

NIDM: Non insulin dependent diabetes mellitus

IDDM: Insulin dependent diabetes mellitus

DNA: Deoxyribonuclic acid

PEP*:* Phosphoenulpyruvate.

CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus is one of the most important chronic diseases that worries both developed and developing countries. It is a metabolic disorder that is characterised by elevated blood glucose, resulting from defect in insulin production, secretion and action. According to a report by the International Diabetes Federation (IDF), about 382 million people are living with diabetes and the figure is expected to rise to 592 million by the year 2035, (IDF, 2015). There are majorly two types of diabetes; Type I diabetes also called the insulin dependent diabetes mellitus (IDDM) and the type II diabetes also called the non-insulin dependent diabetes mellitus (NIDDM).

The Type I diabetes is caused by autoimmune condition. The body's own antibodies recognise and attack the beta cells of the pancreas there by rendering it inefficient in producing and secreting insulin. This condition usually has genetic basis and the condition is characterised by low insulin secretion. The type II diabetes is caused by insulin resistance in the liver, fat and skeletal muscles which denotes the inability of the insulin receptors to recognise circulating insulin and react by sending signals. This condition is characterised by high insulineamia but reduces with progression as a result of beta cell failure.

In a report by Cade, *(*2008*),* diabetes is associated with a lot of macrovascular complications such as cerebrovascular disease, peripheral artery disease and coronary artery disease and microvascular complications including diabetic retinopathy, nephropathy and neuropathy. The development and severity of the diabetes associated complications is dependent on the duration and management of the condition.

Sialic acid is an acetylated 9- carbon neuraminic acid (Wang and Brand-Miller, 2003), which exists mainly as part of terminal end of glycoproteins and glycolipids oligosaccharides

side chains. They have some vital functions in cell to cell recognition and immunity and also confer negative charge to cells which gives cells a repulsive charge. The activation of the compliment system is a sialic acid mediated reaction. However, the concentration of these vital glycans is reported to be altered in several disease conditions, (Chetena *et al.,* 2015, Gruszewska *et al., 2014,* Mahendran *et al.,* 2013 and Taqi, 2012).

Sialic acid is proposed to play a role in diabetes related complications as its concentration has been reported to be altered in the disease conditions (Divija *et al*., 2014, Divija *et al*., 2013, Eraslan *et al*., 2013, Crook *et al*., 2001, Khurshid and Munir, 2008). A study carried out by Prajnak *et al.,* (2013) shows that there is a significant rise in serum sialic acid in diabetic patients with and without nephropathy. The rise is as a result of association of sialic acid with most of the acute phase proteins. These acute phase reactants are increased as a result of stimulation by pro inflammatory cytokines secreted by inflammatory cells at sites of damages. Also, in a similar report by Mahendran *et al.,* (2013), there was a strong relationship between elevated serum sialic acid in type II diabetes and cardiovascular diseases risk. Another report by Yokoyama *et al*., (1995) demonstrated that serum sialic acid is elevated in insulin dependent diabetes mellitus (IDDM). It is believed, according to Prajnak *et al*., (2013) and Cade, (2008) that injury caused to tissues as a result of high glucose level in the serum is primarily responsible for the initiation of diabetic vascular complications that result in initiation of inflammation and subsequent mobilisation of the acute phase proteins. It was speculated that these overall reactions lead to increased level of serum sialic acid.

Though much had been done and reported on the increased level of serum sialic acid in diabetes, dependence or correlation of increased sialic acid level and diabetes mellitus, but nothing has been reported about the actual source and specific role of increased sialic acid in diabetes and related complications. In this research work, an attempt has been

made to understand the changes in the levels of sialic acid occurring in different organs during type I and type II diabetic condition over a period of time.

1.1 Statement of Research Problem

According to the report by World Health Organization, globally, the menace of non-communicable diseases including diabetes is a big challenge. Non-communicable diseases are a major cause of morbidity and mortality in both developed and underdeveloped countries of the world. These non-communicable diseases kill about 38 million people each year, and that 80 % of this mortality is in the low and middle income countries. According to the same report, vascular diseases and diabetes account for most of the non-communicable diseases deaths, (World Health Organization, 2011).

Sialic acid has been established to play an important role in the pathogenesis of atherosclerosis which is key to development of the vascular complications in diabetic condition, (Nigam *et al.,* 2006). This sialic acid has also been reported to increase in diabetic patients with or without complications but the source of the increased sialic acid in diabetes and the specific role of this sialic acid in the pathology of diabetes and its related complications is yet unknown.

1.2 Justification

Diabetes mellitus is a serious public health problem. Hence, the need for more understanding of the pathogenetic process involved in diabetes, which may open novel intervention and therapeutic strategies to combat the diseases.

Though much work and report have been documented on both serum free and bound sialic acid levels in diabetes types I and II, a lot has been reported on the relationship between sialic acid levels and vascular conditions in diabetes, but no report has been made on the specific source of the increased sialic acid in the pathogenesis of developing diabetes. Therefore, there is the need to progressively monitor the changes in level of sialic acid in

diabetic condition with respect to serum and tissues over time to better understand the metabolism of sialic acid among the tissues during diabetes. This will be a step to understanding the actual source and role of sialic acid in the pathogenesis of diabetes and its related complications.

1.3 Aim and Objectives

Aim

To investigate the changes in the levels of sialic acid in serum and tissues: liver, kidney, pancreas, skeletal muscles and brain of streptozotocin-induced type I and type II diabetes in rats.

Objectives

1. To assess the changes in level of free serum sialic acid and total sialic in tissues (liver, kidney, pancreas, skeletal muscles and brain) of streptozotocin-induced type I diabetes over a period of 9weeks.
2. To assess the changes in level of free serum sialic acid and total sialic acid in tissues (liver, kidney, pancreas, skeletal muscles and brain) of streptozotocin-induced diabetes type II over a period of 8 weeks.
3. To investigate the pattern of relationship between the concentrations of serum sialic acid and the sialic acid levels of the liver, kidney, pancreas, skeletal muscles and brain in types I and II diabetes.

CHAPTER TWO

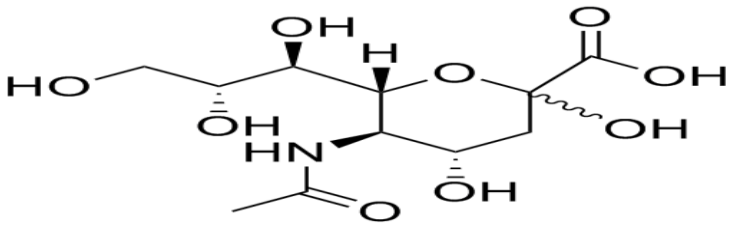
2.0 LITERATURE REVIEW

2.1 Sialic Acid

These are group of carbohydrates that are not commonly used as source of energy in the living cells. They are derivatives of neuraminic acid. These acidic sugars decorate all cell surfaces and exist as non-reducing ends of most secreted glycoconjugates of, usually, proteins and long chains carbohydrates in vertebrates. These acidic sugars contribute to a variety of normal and pathological process (Varki, 2008). They are more than 50 derivates of sialic acids in nature (Li and Chen, 2012); the most occurring of all is the N-acetylneuraminic acid (Neu5Ac or NANA). Eukaryotes and several prokaryotes have expressed sialic acids in their systems, some pathogens such as bacteria and viruses express these acids on their cell surfaces as components of lipopolysaccharides. Examples are *Escherichia coli* K1, *Haemophilus influenza, Haemophilusducreyi, Pasteurellamultocida, Neisseria gonorrhoeae, and Neisseria meningitides (*Li and Chen, 2012). In mammals, N-acetylneuraminic acids are found to be distributed in various organs such as the brain, liver, pancreas, kidney, muscles, adrenal glands and heart and in several fluids such as serum, cerebrospinal fluid, saliva, urine, amniotic fluid, and breast milk (Li and Chen, 2012).

2.2 Chemical Structure of Sialic Acid

Sialic acids have a cyclic nine-carbon structure with a carboxylic acid group at the C1 position which confers negative charge on the compound.



Sialic acid
(*N*-acetyl neuraminic acid)

2.3 Sialic Acid Metabolism

All the tissues of humans have the capacity to produce its own sialic acid from simple sugars. The synthesis of sialic acids in mammals occurs in the cytosol. There is a variation in metabolism of sialic acids in eukaryotes and bacteria (Li and Chen, 2012). The metabolism of sialic acids involves four major activities;

1. Synthesis
2. Transportation and Activation
3. Modification
4. Detachment and Recycling/Degradation

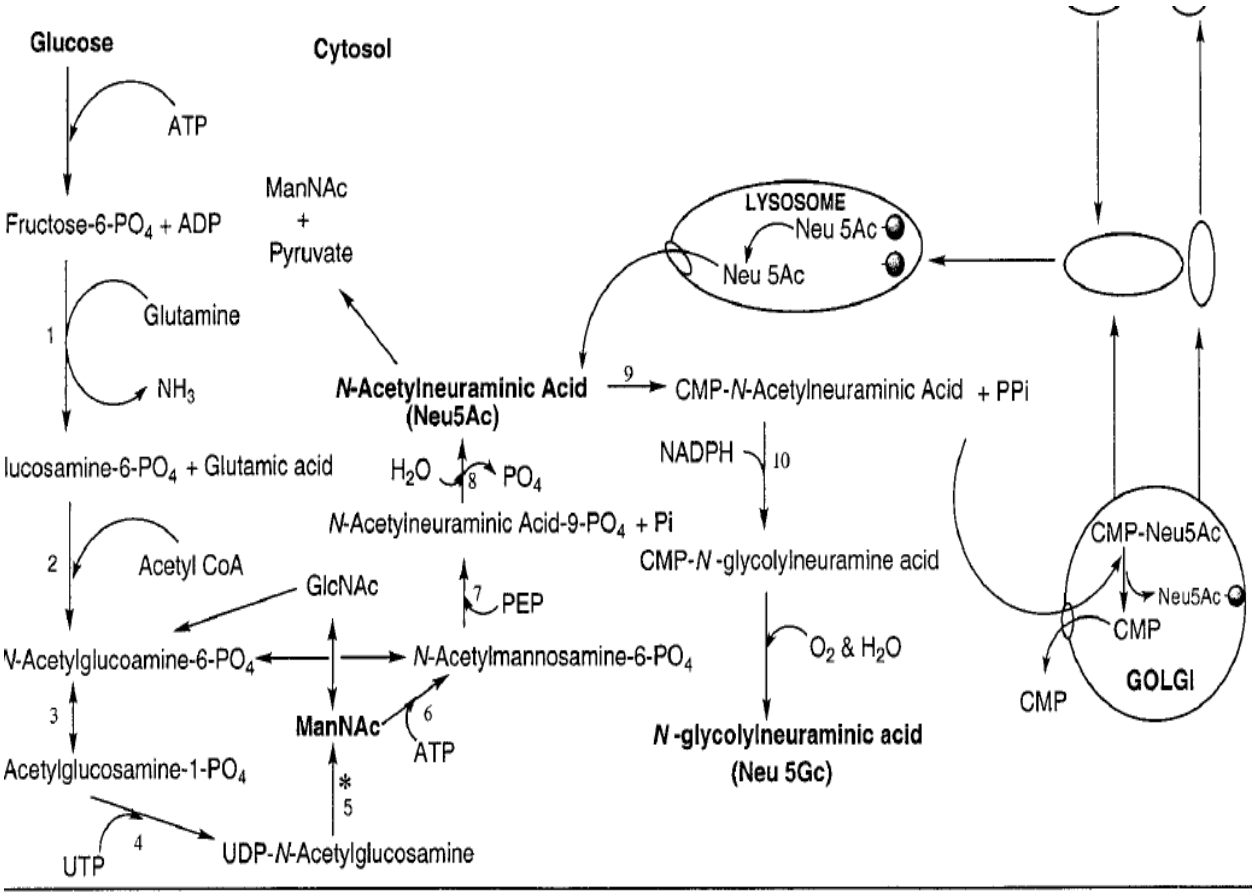


Figure 2.1: The Pathway for Synthesis of Sialic Acid. (Wang and Brand-Miller, 2003)

2.3.1 Synthesis: This is the production of sialic acid a 9-carbon compound from simple precursors as glucose, glutamine, acetyl-coA and phosphoenulpyruvate. This involves a multi-step process and five major enzymes are involved.

Sialic acid synthesis in animals takes place primarily in the cytosol. The synthesis proceed with the conversion of free glucose to uridine-5'-diphosphate–N-acetyl-D-glucosamine by the action of a kinase enzyme, before the dual activity enzyme UDP-GlcNAc 2-epimerase/ManNAc-6-kinase converts the substrate uridine 5′-diphosphate-*N*-acetyl-d-glucosamine (UDPGlcNAc) to *N*-acetyl-d-mannosamine (ManNAc) with removal of the UDP moiety and epimerization of the carbohydrate. The kinase function of the same enzyme phosphorylates the sugar to produce *N*-acetyl-d-mannose 6-phosphate (ManNAc-6-P). Then the condensation reaction between phosphoenolpyruvate (PEP) and ManNAc-6-Pinitiated by NeuAc-9-P-synthetase results in the phosphorylated sialic acid precursor *N*-acetylneuraminic

acid 9-phopshate (Neu5Ac-9-P). This precursor is dephosphorylated by Neu5Ac-9-P phosphatase to produce the key sialic acid *N*-acetylneuraminic acid (2-ketoacetamido-3,5-dideoxy-dglycero-d-galactononulosonic acid; Neu5Ac) (Varki and Schauer, 2009).

2.3.2 Transportation and Activation: At this point, the synthesized sialic acid (Neu5Ac) in the cytosol is transported to the nucleus for activation. This process is the same in both eukaryotic and prokaryotic bacteria (Li and Chen, 2012.), it occurs in the nucleus in eukaryotes but in the cytoplasm in prokaryotes (Varki and Schauer, 2009). In the nucleus, the enzyme CMP-NeuAc synthetase transfers a cytidine-5'-monophsophate to the sialic acid to activate it. The donor compound here is cytidine-5'-triphosphate. The product here is transported back into the cytoplasm specifically to the Golgi bodies where they wait to be used by the transferase enzyme.

CMP-Neu5Ac

Neu5Ac+ CTP

2.3.3 Modification: Usually the modification of sialic acid takes place in the Golgi, either before or after the sialic acid has been transferred to an acceptor conjugate by sialyltransferase, except for *N*-glycolylneuraminic acid (Neu5Gc), the hydroxylated form of Neu5Ac. In non-human vertebrates, including other primates, the first precursor sialic acid (Neu5Ac) is converted to (Neu5Gc) by substituting acetyl group with glycol group in the cytosol by the action of cytidine monophosphate-N-acetylneuraminic acid hydroxylase which is a cytoplasmic enzyme (Varki and Schauer, 2009).

2.3.4 Detachment and Recycling/Degradation: Sialic acids bonded both extracellularly and intracellularly to non- reducing ends of glycolipids and glycoprotiens are removed from their

conjugates by the action of an enzyme called sialidase or neuraminidase. Mainly, four types of this exoglycosidases have been identified.

1. The lysosomal sialidase (Neu1)
2. Cytosolic sialidase (Neu2)
3. The plasma membrane sialidase (Neu 3)
4. The lysosomal and mitochondrial membrane-associated sialidase (Neu4) (*Natori.,et al 2013 and* Li and Chen, 2012).

The removed and free sialic acids either undergo recycling i.e another cycle of sialyl conjugate production by been activated in the nucleus by CMP-Neu5Ac synthase or they are been broken down by sialic lyase into pyruvate and *acylmannosamine.*

2.4 Functions of Sialic Acid

Sialic acid a 9-carbon neuraminic acid is typically found on all cell surfaces and on the outermost chains of most secreted glycolipids and glycoproteins that have been implicated in modulating various normal and pathological processes in both eukaryotes and bacteria alike. The presence of carboxylic carbon at C1 that gives it negative charge and hydrophilic nature allows sialic acid to perform many structural and modulatory roles (Varki, 2008).The various functions of sialic acids in health and diseases will be discussed under the following headings:

2.4.1 Physiology: It is true that sialic acids are found on the surfaces of all living cells in vertebrates, the negative charge on these sialic acids help to give the cells charge repulsion and preventing the cells from unwarranted interaction with itself that can lead to agglutination and destruction of these cells, a typical example is the erythrocytes. In a study

carried out by Nigam et al., (2006), cells aggregation is prevented by sialic acids on cells surfaces.

The presence of sialic acids at the terminal end of glycolipids, glycoproteins and gangliosides prevent them from destruction and also the function depends on these.

2.4.2 Fertilization: Evidence clearly shows that sialic acids on the surfaces of cells especially the spermatozoa and the female egg plays a critical role during sperms egg interaction, sperm and other fluids interaction during fertilization. There are receptors on these reproductive cells that use sialic acid for recognition and therefore enhance agglutination between these cells which is key to fertilization (Varki, 2008).

2.4.3 Immunity: Sialic acid is involved in regulating the alternative pathway of the complement system. The activation is via a process that relies on the H-factor recognizing sialic acid as "self". The complement system is made up of a large number of distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses that help to fight infection and invading pathogens. The complement system has the ability to distinguish "self" from "non-self" through a range of specialized cell-surface *interaction anchored on the possession of sialic acids on their surfaces. The system has many regulations, one of which is the H factor. This is a* protein present in plasma that binds C3b of the complement system, Factor H binds preferentially and with higher affinity to C3b in vertebrate cells as it has an affinity for the sialic acid residues present on these cells surfaces.

Also it has been established that a member of immunoglobulin (Ig) proteins that specifically recognizes and binds to sialic acids (Siglecs) are expressed on the surfaces of immune cells. Recognition of sialic acid by this group of immunoglobulins (Siglecs) could play a role in the regulation of the innate immune system (Crocker and Varki, 2001). The immunoglobulin (Ig) like lectins (siglecs) have various functions such as helping in the internalization of sialylated

pathogens by the macrophages, attenuation of inflammation, restraining cellular activation, attenuation of damage-associated molecular pattern-mediated inflammation along with inhibition of natural killer (NK) cell activation (Khatua *et al*., 2013).

2.4.4 Sialic Acid in Diseases Pathology: Alteration in sialic acid level expression has been seen in several pathological diseases state. Due to *their location and ubiquitous distribution and* the pivotal role sialic acid plays, any disturbances to its metabolism can lead to unhealthy medical condition. The level of sialic acid is seen to be altered in several diseases such as cardiovascular diseases, cancer, diabetes, and liver diseases, also the level of sialic acid can be used to predict disease risk and monitor prognosis during therapy.

2.5 Diabetes Mellitus

This is a group of carbohydrate disorder that is characterized by high glucose level in the serum (hyperglycemia), presence of sugar in urine, (glycosuria) that is caused by lack of insulin or insulin insensitivity or both. Diabetes mellitus is one of the most important chronic diseases that worries both developed and developing countries of the world. Diabetes mellitus always give rise to risk of several microvascular and macrovascular complications (Fowler, 2008). Microvascular diseases such as diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy. While the macrovascular complications are stroke, ischemic heart diseases, peripherial artery diseases, and cardiovascular diseases (Chineye and Young, 2011). All these complications resulting from diabetes can very well reduce the quality of life and life expectancy.

The recent estimate of diabetes by the International Diabetes Federation (IDF), says that about 382 million people of the world population are living with diabetes and the figure is expected to rise to 592 million by the year 2035 (IDF, 2015).

2.5.1 Prevalence and Significance of Diabetes Mellitus: Globally, it was estimated that there were 382 million people living with diabetes in 2015. While in Africa about 20 million

people are living with the disease (IDF, 2015). The rate varies substantially with country and regions of the world because of diverse environmental factors and life style. In 2010 it was estimated that in Nigeria, about 4.7 million people were living with diabetes and this toll is expected to rise to 5. 3 million in the year 2030 (Shaw *et al.*, 2010). The data also shows that Nigeria has the highest number of people living with diabetes in Africa, with a prevalence that varies from 1% in the rural settlement to 7% in the urban areas

In a more recent report of a coordinated national survey of non-communicable diseases in Nigeria the national prevalence of diabetes was 2.2%. The result also shows an inverse in prevalence with increase in age (Chinenye and Young, 2011).

Table 2.1: Patterns of Diabetes in Nigeria

Parameters	Type 1	Type 2	Other types	GDM
Total Number (n)%	25(3.0)%	78(94)%	10(1.2)%	15(1.8)%
Positive family history of DM	3(12)%	408(523)%	Nil	8(53.3)%
Polyuria	25(100)%	713(91.4)%	10(100)%	11(73)%
Polydipsia	25(100)%	720(92.3)%	10(100)%	11(73) %
Lassitude	25(100) %	655(84) %	10(100) %	13(86)%
Polyphagia	12(48) %	720(92.3) %	-	-
Weight loss	25(100) %	590(75.6) %	10(100) %	7(46.7) %
Blurring vision	13(52) %	491(62.9) %	10(100) %	4(26.7) %
Coma	8(32) %	39(5.1) %	-	-
Peripheral neuropathy	3(12) %	439(56.3) %	-	7(46.7)%
Erectile dysfunction	-	283(36.3) %	-	-
Hypertension	-			
*Antedated	-	106(13.6) %	-	-
*Simultaneously	-	197(25.3) %	-	5(33.3) %
Retinopathy				
*Background	-	57(7.3) %	-	-
*Proliferative	-	-	-	-
Nephropathy	-	72(9.3) %	-	-
Dietary (mono) therapy	-	7(9.0) %	-	-
Insulin therapy	25(100) %	176(22.6) %	10(100) %	15(100) %
Oral hypoglycemic agent (OHA)	-	593(76.0) %		

(Chinenye and Young, 2011)

2.5.2 Classification of Diabetes: Generally there are three types of diabetes

1. Type I diabetes mellitus also known as insulin dependent diabetes mellitus (IDDM) or juvenile onset diabetes.
2. Type II diabetes mellitus also known as non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes.
3. Gestational diabetes.

2.5.2.1 Type I diabetes mellitus (IDDM): Type 1 diabetes is responsible for about 10% of all cases of diabetes in the world (IDF, 2015). This disease is usually caused by an auto-immune reaction where the body's defense system attacks the cells that produce insulin. It is a chronic autoimmune condition in which destruction or damaging of the beta-cells in the islets of Langerhans in the pancreas results in lack of insulin and subsequent hyperglycemia. This is because insulin that is responsible for controlling blood glucose are been produced by the same beta cells. The pathologies of this disease condition are characterized by the following;

a. Presence of immuno-competent and accessory cells in infiltrated pancreatic cells.

b. Presence of islet cell specific autoanti-bodies.

c. Inconsistent of T_{reg} cells (Figure 2.2).

d. High expression of monokines and interleukins and

e. Frequent occurrence of other organ specific auto-immune diseases in affected individuals or family members (Ozougwu *et al.,* 2013).

The complete destruction of the beta-cells follows up with the onset of the disease conditions, though it has been proven that there is a gap between the initiations of autoimmunity when specific autoantibodies of the beta-cells are seen in the serum to when the condition begins manifestation. This condition is also with altered function of pancreatic α-cells which result to excessive secretion of glucagon (Ozougwu *et al*., 2013). This will result to early

development of diabetic ketoacidosis i.e accumulation of ketone bodies in the urine; there is also the presence of hypertriglyceridemia in the absence of insulin. The combined effect of hyperglycemia, dislipideamia and ketoacidosis result to damages to body tissues and cells such as loss of sight, lower limb amputation, kidney failure, and cardiovascular diseases (Ozougwu *et al*., 2013).

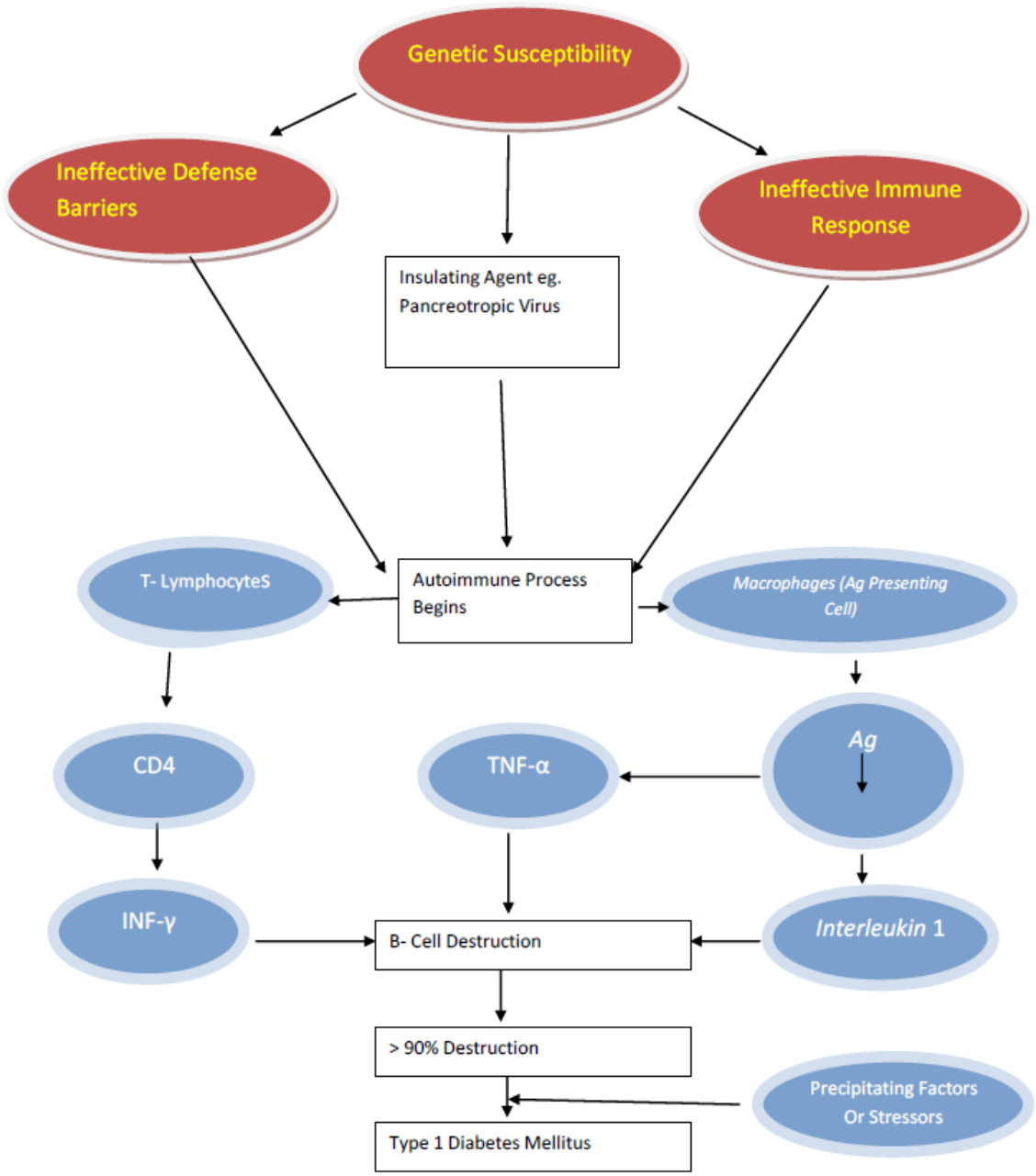


Figure 2.2: Pathogenecity of type I Diabetes Mellitus (Ozeougwu *et al*., 2013)

2.5.2.2 Type II diabetes mellitus: This is the most common form of diabetes and it accounts for at least 90% of all cases of diabetes mellitus in the world, (IDF, 2015). This disease can result from the combination of genetic factors that affect or alter insulin secretion and insulin resistance and host of other environmental and life style factors which we can control such as, poor dieting, lack of exercise, that may result to obesity, stress, as well as aging, (Kaku, 2010). Obesity that results from poor lifestyle and at times genetic is also thought to be responsible for developing type II diabetes. The rate and prevalence of this disease condition have been on the increase lately in parallel to obesity, this may be due to the sedentary life style of this recent generation, (Chinyelu and Ebelechukwu, 2014). Though, the etiology of this disease is not so clear but it is a multifactorial disease resulting from the combined effect of several genes and several environmental factors. This multifactorial model hold sway because it has been noticed that those genetically predisposed will hardly fully or overtly develop the disease until when exposed to the other environmental factors (Chinyelu and Ebelechukwu, 2014).

Impaired insulin secretion**:** This is as a result of pancreatic β-cell dysfunction or inability to produce enough insulin. It is characterized by decreased glucose responsiveness. This condition is noticed before the onset of type II diabetes. Poor glucose-responsive early-phase insulin secretion and a decrease in additional insulin secretion after meals cause postprandial hyperglycemia, this condition result to glucose intolerance (GIT). This condition of impaired insulin secretion is a progressive condition that may results to glucose toxicity and lipo-toxicity. It can also cause a fall in pancreatic beta-cell mass (Kaku, 2010). This progressive deterioration of the beta-cells result to long term loss of control of blood glucose and subsequent rise in blood glucose seen in type II diabetes.

Insulin resistance *(*IR*):* This is a condition where by insulin secreted and circulating in the body does not exert sufficient action proportional to their concentration in circulation (Kaku,

2010). This condition results from inherited and acquired factors. The inherited causes are mutations of the following in the body, insulin receptor, glucose transporter, and signaling proteins, while the acquired causes are lack of physical exercise, poor dieting, medications and hyperglycemia i.e. (glucose toxicity), increased dislipidemia, and aging. The resistant of insulin in different organs in the body is a common feature of type II diabetes though this condition shows before the onset of overt type II diabetes. Genetic and environmental factors are said to be responsible for impaired insulin responsiveness. Obesity is known to be one of the major risk factors for developing this condition.

Induction of insulin resistance: The liver is the only organ in the body that metabolises fructose, when we eat moderate amount of fructose, it is used to generate energy and the excess may be converted to glycogen and stored in the liver. But when taken in excess, it seems to indirectly reduce insulin levels in the serum and obesity (Basciano *et al*., 2005). It has been reported that rats fed with 66 % fructose for two weeks had insulin receptor messenger ribonucleic acids (mRNA) in the muscle and liver significantly lowered, while triacylglycerol (TG) increased compared to rats fed with standard feed while the body weight remained the same (Basciano *et al*., 2005). Therefore, it evidently shows that high fructose consumption induce lipogenesis. It has also been shown that high carbohydrate feeding especially fructose can activate an insulin dependent cholesterol synthesis pathway and lipogenic pathway in insulin defective rats, evidently showing that lipogenesis can occur independent of insulin (Basciano *et al.,* 2005). High fructose feeding is able to induce insulin resistance by increasing triacylglycerol production while diminishing its clearance (Parks and Hellerstein, 2000).

Fructose Metabolism**:** Fructose unlike glucose is majorly metabolized in the liver, after a fructose meal, the free fructose is transported via the GLUT5 into the liver. The breakdown of fructose in the liver by-passes the two first highly regulated steps in the usual glycolytic

pathway i.e. the conversion of glucose to glucose-6-phosphate by hexokinase enzyme and the phosphorylation of fructose-1-phosphate by phosphofructokinase. These enzymes can be regulated by their product (feedback inhibition). The free fructose enters glycolysis through the fructokinase entrance that converts fructose to fructose-1-phosphate. This enzyme is not regulated by its product therefore allows great inflow of fructose into glycolysis. The fructose-1-phosphate is converted to dihydroxyacetone-phosphate and D-glyceraldehyde by the action of the aldolase, while the D-glyceraldehyde is phosphorylated and continued downstream in the glycolysis pathway to form pyruvate. The pyruvate formed is channeled into the tricarboxylic acid (TCA) cycle by been converted to acetyl-CoA in the mitochondrion. The acetyl-CoA combines with oxaloacetate to form citrate. The citrate in the presence of abundant energy is converted to CoA in the cytosol. The availability of CoA and glycerol signals for the activation of lipogenesis (Khitan and Kim, 2013).

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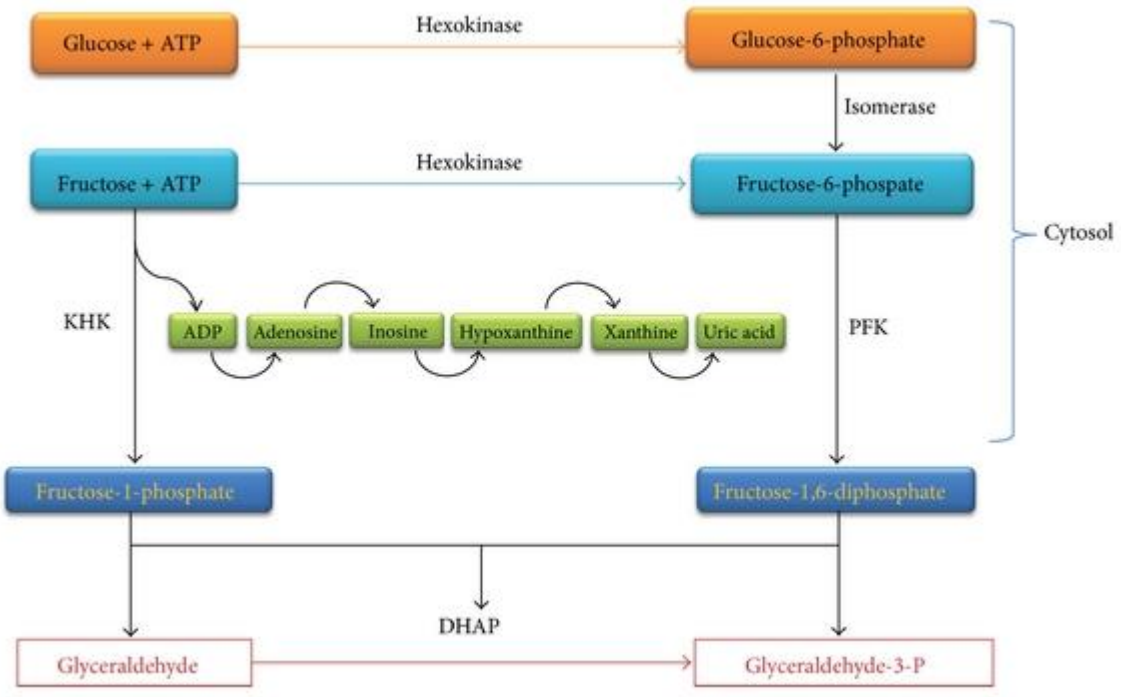

Figure2. 3: The committance of free fructose into glycolysis. (Khitan and Kim, 2013).

2.6 Diabetes Related Complications

The complications that can result from diabetes are classified into two;

a. Microvascular complications

b. Macrovascular complications

2.6.1 Microvascular Complications: These are diabetic complications involving small vessels such as the capillaries. Prolonged and chronic high serum glucose plays the central role in the initiation of this condition. The three major microvascular complications are.

2.6.1.1 *Diabetic Neuropathy*: It is believed that one-half of people living with diabetes have these conditions (Cade, 2008). The American Diabetes Association (ADA) defined diabetic neuropathy as "the presence of symptoms of peripheral nerve dysfunction in people with diabetes after exclusion of other causes". Peripheral neuropathy manifest as sensory, focal and autonomic neuropathies such as cardiovascular autonomic dysfunction. Peripheral neuropathy in diabetes is usually experienced at the lower extremity in the form of sensation that can lead to ulceration (common foot ulceration) and if not controlled can lead to amputation. Characteristic of this condition are axonal loss, basement membrane thickening, lost of pericytes and decreased blood flow (Cade, 2008).

2.6.1.2 *Diabetic Nephropathy:* This is one of the major causes of renal failure in the world; it is determined by high level of protein in the urine. The condition starts with microalbumin i.e. urinating more than 30-299mg/24 hours of albumin, then progress to overt proteinuria or diabetic nephropathy (Fowler, 2008). Apart from protein in the urine other features of the condition are glomerular basement thickening and glomerular hyper filtration.

2.6.1.3 *Diabetic Retinopathy:* This is the highest occurring form of diabetes related complications. This condition is either proliferative or background, it affects the peripheral retina and the macular of the eye leading to loss of visuality. The features of both the non-proliferative and proliferative form of diabetic retinopathy ranges from hemorrhages in the

retina, microaneurysms in the retina, retina edema and new capillary formation and lost of pericytes (Cade, 2008).

2.6.2 Macrovascular Complications of Diabetes: These are diabetic complications peculiar to large vessels such as arteries and vein. Arteriosclerosis is the main pathological mechanism for developing complications in these vessels (Fowler, 2008). The various complications are

2.6.2.1 *Cardiovascular Diseases* (*CVD*): This condition is the leading cause of mortality in people with diabetes in the world. Report has shown a strong relationship between arteriosclerosis and developing CVD. The formation of plaque on the coronary arteries interferes with circulation of blood.

2.6.2.2 *Cerebrovascular Diseases (Stroke):* This is the second leading cause of death among diabetics apart from CVD. The effect of atherosclerotic plaque on the arteries affects circulation in the cerebrovascular environment there by increasing the risk of developing stroke.

2.6.2.3 *Peripheral Artery Diseases (PAD):* This condition is characterized by occlusion of the lower-extremity arteries which results to pains insensitivity and claudication (Cade, 2008). This can result to loss of erectile function and foot ulceration.

2.7 Mechanism for Vascular Complication in Diabetes

The major cause of the complications is anchored on poor glucose management. When there is prolonged presence of high glucose level in the blood, it results to reacting unenzymatically with proteins in the serum to form a product that exhibit a wide range of negative influence on the surrounding cells. The products of the sugar and protein reactions are called advanced glycation end products (AGES). The presence of AGES initiates the following; induction and inhibition of growth inhibition and programmed cell death of the pericytes, thickening of collagen and endothelium, induction of overproduction of endothelial growth factors such as vascular endothelial growth factors, insulin-like growth factors 1,

basic fibroblast growth factor and hepatocytes growth factor. The induction of neovasculation, microaneurysms, hypoxia and increase vascular inflammation (Cade, 2008). Advanced glycation end products can also bind to their receptors and lead to endothelial cell dysfunction. Aldose reductase also plays a role in diabetic related complications as this enzyme in the polyol pathway converts glucose to sorbitol. The accumulation of this product in the presence of hyperglycemia leads to increased osmotic pressure and oxidative stress. This condition can damage endothelial cells (Cade, 2008).

In macrovascular complication cases, the damage to the vascular endothelial cells by hyperglycemia inhibits nitric oxide (NO) producing enzymes. This reduces vasodilation and results to over generation of reactive oxygen species (ROS).

Though the mechanism for developing complication in diabetes is multifactorial, but the major receiver of these injuries is the endothelium cells (Cade, 2008) i.e. the cells that form the lining of blood vessels.

2.8 Sialic Acid and Diabetes

One of the major characteristics of diabetes mellitus is hyperglycemia, in the presence of these prolong excess sugar in the serum, it is believed that the sugar undergoes non-enzymatic reaction with protein molecules to form glycation products. Also these sugars undergo oxidation. These conditions favour the initiation of inflammation and subsequent mobilization of cytokines. This is in accordance to a report by Krishnamurthy *et al.,* (2011) inflammation plays a major role in the pathogenesis of diabetes mellitus and its complications. Crook *et al.,* (2001) also proposed that cytokine-induced acute-phase response is an integral part of the pathophysiology of diabetes mellitus. These mobilized cytokines activates the synthesis of acute phase reactants from the liver that have sialic acids attached at their terminals, these has been proposed as the major pathophysiological mechanism responsible for increased sialic acids observed in diabetes mellitus. This claim corresponds

with the report of Khan and Rao (2014), describe diabetes mellitus as a disease of disordered innate immune response. The report also suggest that the various complications of diabetes can be considered as exacerbated response of this disordered immune response therefore Sialic acid can be used as a marker in identifying the various complications of diabetes mellitus.

Several authors have reported on the altered level of sialic acids in type I diabetes with or without complications, according to a report by Eraslan *et al.,* (2013), the level of serum sialic acid is increased in diabetic patients compared to their counterpart control group. The report further state that there is also an increased level of serum sialic acids in the patients with diabetes accompanied with complication (retinopathy), and that the level of increased serum sialic acids is proportional to the level of the severity of the complication (retinopathy). In a more recent report by Ghosh *et al*., (2016), shows that sialic acids is elevated in serum of diabetic patients with or without complications but did not report on the variation in sialic acids with complications and without complications. Furthermore on the report of the level of sialic acids in type I diabetes. Divija *et al.,* (2013) reveal a similar trend of increased sialic acid in the serum of diabetes with nephropathy. A similar work done but on total plasma sialic acid by Crook *et al.,* (2001) revealed a similar trend of increased sialic acid level in diabetic patients with micro and macrovascular complications. The report also shows a relationship between severity of complication and increment in the level of the sialic acid levels. Similarly, a report by Khurshid and Munir, (2008) on the level of total serum sialic acid in diabetic with retinopathy and without retinopathy compared to control, revealed an increased level of sialic in the diabetic groups compared to the control group. The same report also shows a greater increased level of the sialic acid in the group with retinopathy than the group without complication. Furthermore, a recent report by Divija *et al.,* (2014) demonstrated the relationship between sialic acids and glycemic status in diabetic

nephropathy patients, which reveals that there is an increase in serum sialic acid level in the diabetic patients compared to the negative control group. While the same result shows a strong positive correlation between sialic acid level and glycemic status.

Interestingly, more report have been made on sialic acid level in type II diabetes with or without complications, in this report by Mahendran *et al.,* (2013) on the level of sialic acid in patients with type II diabetes shows an increased level of the side chains glycan in the diabetic patients compared to their control group. Also, Prajnak *et al.,* (2013) shows that serum sialic acid level is seen to be elevated in diabetes type II with or without complications compared to the control group. From the report, it was also seen that the level of the serum sialic acid level is higher in the group with nephropathy compared to the group without complication. Furthermore, Khalili *et al.,* (2014), shows an increased sialic acid in the patients with type II diabetes compared to the normal control patients, and that the increased sialic acid is independently associated with other risk factors of diabetes. In a more recent report by Varma *et al.,* (2016), the level of serum total sialic acid in diabetic patients with or without nephropathy shows an elevated level of sialic acid in the both diabetic group compared to the control. The same report shows a higher level of the sialic acid in the group with nephropathy. Similarly, Krishnamurthy *et al.,* (2011), reveal that the level of serum sialic acid level in type II diabetes shows an increased level of sialic acid in the diabetic group compared to the control group. However, the report by Prakash and Sudha (2013) the relationship between sialic acid level and nitric oxide in diabetes type II patients shows an increased level of sialic acid level while a decreased level of the nitric oxide compared to the control group. Furthermore, another report by Ikhlas *et al.,* (2013) the level of serum total sialic acids amongst three groups of specified population shows that the lowest levels of total sialic acid was seen in the non-diabetic or control group, while a higher, though intermediate level in type II diabetic group without metabolic syndrome but the highest level of the total

serum sialic acids was seen in the type II diabetic mellitus group with metabolic syndrome. The same report also shows that the serum total sialic acid increased significantly as the components of the metabolic syndrome increases. Lastly, a report by Merat *et al.*, (2003), show that serum sialic acid level is significantly higher in the diabetic patient group as compared to the values in the age and sex-matched in the control group. The same report also showed an increased serum sialic acid in the diabetic group with vascular complications as significantly higher than that for diabetic group without retinopathy.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Reagents

Streptozocin was purchased from Sigma- Aldrich, Germany, Rat insulin ELISA kit was purchased from Wkea Med Supplies Corporation, China. Thiobarbituric acid was purchased from Kem Light Laboratories, India. Sodium Arsenate was purchased from Fox Chemicals, Germany, and Potassium Periodate was purchased from Simagchem Corporation China.

3.2 Experimental Animals

A total of 107 six weeks old white albino rats were purchased from Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The animals were housed in medium size cage in temperature and humidity controlled room, with a 12 hour light/dark cycle. The animals were fed with standard rat diet *ad libitum.* Their body weight was measured; blood was collected from their tail vein for measuring blood glucose level using a standard glucometer.

3.3 Grouping and Induction of Diabetes

The animals were grouped into three groups, with the normal control group and the type II diabetic group having 37 animals each but the type I diabetic group having 32 animals.

1. Normal Control Group (NCG):
2. Type I Diabetic Group (T1DG)
3. Type II Diabetic Group (TIIDG)

The animals of the NCG were fed with normal diets and no streptozotocin, the type 1 diabetic group (T1DG) were fed with normal rat diet and allowed to acclimatize for one week. The group was induced with diabetes by giving the animals a single intraperitoneal injection of 60 mg/kg body weight of streptozotocin dissolved in citrate buffer of pH 4.5 (Yan *et al*., 2012). The type II diabetic group (TIIDG) were induced with type II diabetes by subjecting the

animals to two weeks feeding of 10 % fructose solution *ad libitum* to induce insulin resistance, followed by giving them intraperitoneally low dose of 40 mg/kg body weight of streptozotocin dissolved in citrate buffer of pH 4.5 (Wilson and Islam, 2012).

For the confirmation of type II diabetes, five animals each were removed from the normal control group (NCG) and the type II diabetic group (TIIDG), the animals were fasted overnight and blood collected by cardiac puncture for measuring fasting blood glucose level and fasting insulin level in order to calculate for homeostasis model assessment for the groups so as to ascertain the level of insulin resistance and β- cells damage in the two groups. As indicated on Table 3.1. FBG > 200 mg/dl, Homa-IR > 5 and Homa-β < 200 were considered diabetic (Ibrahim and Islam, 2014).

After the confirmation of diabetes, for the type I group, every three weeks intervals, 5 animals were randomly removed and also from the normal control group and fasted over night. Blood collected from these animals via cardiac puncture and organs (liver, kidney pancreas, skeletal muscle and brain) harvested, for glucose and sialic acid assay. Whereas for the type II group, every two weeks intervals, 8 animals were randomly removed from the group alongside the normal control group and fasted overnight. Blood collected from these animals via cardiac puncture and organs (liver, kidney pancreas, skeletal muscle and brain) harvested, for glucose and sialic acid assay as described in Figure 3.1 and Table 3.1. During the period of the experiment, weekly fasting blood glucose level was also checked for both the diabetic groups and the normal control group.

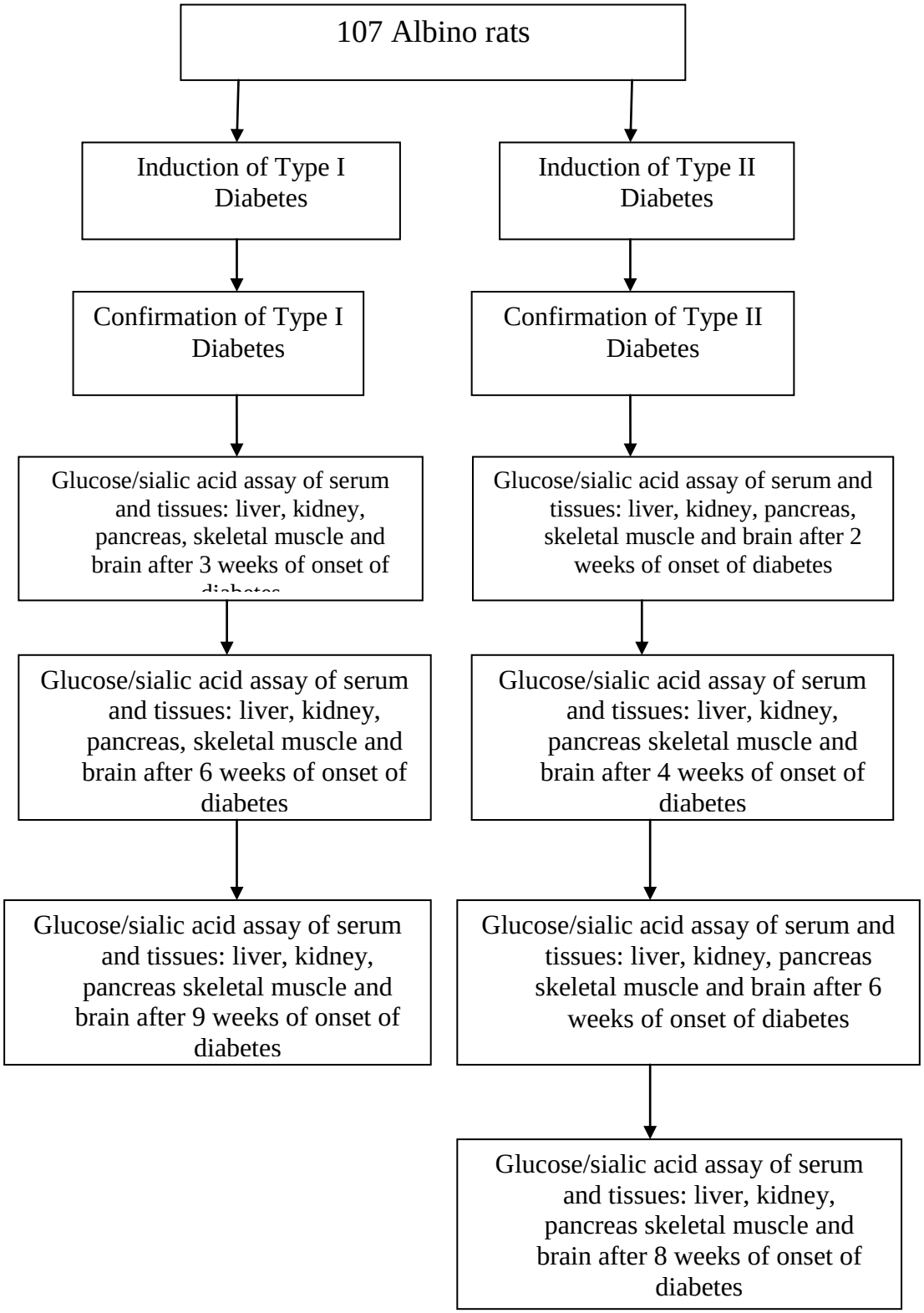

Figure 3.1: Flow chart showing the experimental design from induction to assay of sialic acids and glucose at intervals of three weeks for the TIDG and two weeks for the TIIDG.

Table 3.1The time line for the induction, confirmation and monitoring of fasting blood glucose and sialic acids during the experiment.

GROUPS	**Induction of Insulin Resistance**	**STZ Injection.**	**Confirmation of induction**	**2 weeks after confirmation of induction**	**4 weeks after confirmation of induction**	**6 weeks after confirmation of induction**	**8 weeks after confirmation of induction**
			1 week				
NCG				8 animals FBG, FSA, TSA assay.	8 animals FBG, FSA, TSA assay	8 animals FBG, FSA, TSA assay	8 animals FBG, FSA, TSA assay
TIDG		60 mg/kg b.w	FBG>300m g/dl	3week 8 animals FBG, FSA, TSA assay		6 week 8 animals FBG, FSA, TSA assay	9 week 8 animals FBG, FSA, TSA assay
TIIDG	10% fructose (2 weeks)	40 mg/kg b.w	Homa-IR>5, FBG>200mg/dl, Homa-β<200.	8 animals FBG, FSA, TSA assay	8 animals FBG, FSA, TSA assay	8 animals FBG, FSA, TSA assay	8 animals 4FBG, FSA, TSA assay

KEY: STZ: streptozocin, FBG: fasting blood glucose, TSA: total sialic acid, FSA: free serum sialic acid, HOMA-IR: haemostasis model assessment of insulin resistance HOMA-β: haemostasis model assessment of beta- cell function.

3.4 Weekly Fasting Blood Glucose

During the whole period of the experiment, fasting blood glucose of all the animals in the three groups was monitored weekly. Every week the animals were fasted over night and

blood was collected from the tail of the animals to check for fasting blood glucose using glucometer so as to ensure the consistency of the diabetic condition in the diabetic groups and normal condition in the normal control group. An Accu-check Glucometer was used, we slide the glucose test strips into the glucometer and punctured the animal on the tail, and then fresh blood was allowed to drop on the well of the glucose test strip already inserted in the glucometer. After which the reading of glucose level in the dropped serum is taken as it appears on the screen of the glucometer.

3.5 Collection of Serum and Tissue

After every three weeks of onset of diabetes for the type I diabetic group, and every two weeks for the type II diabetic group, five (5) and eight (8) animals respectively were collected from each group alongside their normal control group, fasted overnight and sacrificed using chloroform anesthesia. Their blood was collected by cardiac puncture, immediately preserved at low temperature (-4^0C). The cold blood was centrifuged at 3000 rpm for 30 min. and the serum was collected for assay, (Ibrahim and Islam, 2014). The other tissues (liver, kidney, pancreas, skeletal muscle and brain) were collected, washed in saline with normal saline and preserved in their respective buffer at temperature of-4^0C for further analysis. This was done for the period of eight weeks for the type I and nine weeks for the type II.

The Homeostasis Model Assessment: The homeostasis model assessment (HOMA-IR) was determined for each animal by multiplying the fasting blood glucose in mg/dL by the level of fasting blood insulin in micro unit per litre (mU/L) and dividing the sum by 22.5. While the level of β-cell damage (HOMA-β) was determined by multiplying the fasting blood insulin in mU/L by 20 and dividing the result by the sum of the fasting blood glucose minus 3.5 (Song *et al*., 2007).

FORMULA FOR HOMA-IR

$$\text{HOMA-IR} = \frac{\text{Glucose x Insulin (mU/L)}}{22.5}$$

$$\text{HOMA-}\beta = \frac{20 \text{ x Insulin (mU/L)}}{\text{Glucose} - 3.5}$$

3.6 Rat Insulin Assay (Elisa Kit) (Wkea Med Supplies Corp).

Principle: The ELISA kit assay rat insulin levels in serum by use of antibody, antigen interaction. The ELISA kit uses an antibody specific for rat insulin coated on a 96 wells plate. Standards and samples are pipetted into the wells and insulin present in a sample bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti Rat Insulin antibody is added. After washing away unbound biotinylated antibody, horseradish peroxidase (HRP) conjugated streptavidin is pipetted to the wells. The wells are again washed, substrate solution is added to the wells and color develops in proportion to the amount of Insulin bound. The Stop Solution (sulphuric acid) changes the color from blue to yellow. The intensity of the colour is measured at a wavelength of 450 nm. The concentration of the insulin in the sample is measured by comparing the optical densities of the sample to that on the standard curve.

ASSAY PROCEDURE

To the wells where added 40 µl each of standard and sample solutions into the standard and sample wells respectively. The wells were covered and incubated for 90 min. at room temperature with gentle shaking.

After which the cover was removed, discarded the solution and washed the plate 3 times with wash buffer working solution.

During each time of the washing we allowed the wash buffer working solution to stay in the wells for 1-2 min. at the end of the washing, the plate were blotted onto paper towels.

To all the wells were added 100 µl of Biotin-labeled detection antibody working solution and incubated the plate at 37°C for 60 min.

Washing of plate 3 times with wash buffer working solution was repeated as done before.

To all the wells were added 100 µl of Streptavidin-HRP working solution and incubated the plate at 37°C for 45min.

This time the plate was washed 5 times with wash buffer working solution, and each time we let wash buffer solution stay in the wells for 1-2 min. then the wash buffer solution was discarded and the plate blotted onto paper towels.

To each well was added 100µl of substrate solution and incubated the plate at 37°C in dark for 30min.

Lastly, 100 µl of the stop solution (sulphuric acid) were added into each well. This was immediately followed by a colour change from blue to yellow. The optical density of each well was determined within 15 min. at 450 nm using a microplate reader.

3.7Sialic Acid Assay

Principle: This is based on the oxidation of sialic acid to formylpyruvic acid by periodate which form a red colour on reacting with thiobarbituric acid. The colour formed is measured at 549 nm. The colour concentration is proportional to the concentration of sialic acid in the solution.

Tissue Homogenization: One gram each, of the tissues were homogenized using a mortar and pestle, and dissolved in a 2 ml solution of 0.1 molar sulphuric acid and incubated at 80^{o}C in a water bath for one hour for hydrolysis of the bonded sialic acid. At the end of the

incubation, the tube was centrifuged at 1000 rpm for fifteen min. and the supernatant collected for sialic acid assay

Procedure: A 500 µl of sample containing sialic acid (tissue homogenate and serum) was added to 250 µl of 25 mM periodate solution. The tubes were shaken and incubated at 37^{o}C for 30 min. using a water bath. Two percent (2%) arsenite solution (200 µl) was added and the tubes shaken until a yellow-brown color disappears to remove the activity of excess periodate. Seventy percent (70%) thiobarbituric acid solution, 2 ml, was added, the tubes shaken, capped with a glass bead, and then heated in a vigorously boiling water bath for 7.5 min. The tubes were then removed and placed in cold water for 5 minutes. During cooling the red color fades and the solution becomes cloudy. Two and half milli litres (2.5 ml) of *n*-butanol in acid was added. Then the tube was shaken and centrifuged at 3000 rmp for 5 min. to facilitate the separation. The upper phase (butanol layer) or organic solvent was red and the colour was more intense than in water. The optical density of the organic phase was determined at 549 nm in a spectrophotometer (Warren, 1959).

3.8 Statistical Analysis

The data were analysed using, statistical tool such as, excel and SPSS version 20. All data are presented as mean ± standard error of the mean. Pearson Correlation analysis was conducted to determine the level of correlation among the organs and the serum.

CHAPTER FOUR

4.0 RESULTS

4.1Type I Diabetes Results

4.1.1: Weekly Fasting Blood Glucose Level of Type I Diabetes and the Normal Control Groups During 9-Week Experimental Period

The weekly fasting blood glucose (FBG) of the streptozotocin- induced type I diabetes and the normal control group during the period of experiment, shows that the glucose level for the diabetic group was significantly ($p<0.05$) higher than that of the normal control group which was maintained at above 300 mg/dL (Figure 4.1). This condition was maintained throughout the experimental period of nine weeks.

4.1.2: Free Serum Sialic Acid (FSA) Level for the Period of Nine (9) Weeks among Type I Diabetes and Control

The level of free serum sialic acid level (FSA) for the type I diabetic and the control group for the period of nine weeks, shows that FSA for the type I diabetic group experienced a drastic but not significant ($P>0.05$) fall below the control at the early stage of diabetes from week 3 to week 6 which was at 0.50±0.08 mg/ml and 0.39±0.09 mg/ml respectively while the control had 0.58±0.09 mg/ml and 0.56±0.08 mg/ml. However, the FSA experienced an insignificant rise from the sixth week (0.39±0.09 mg/dl) to the ninth week (0.69±0.10 mg/dl) (Figure 4.2).

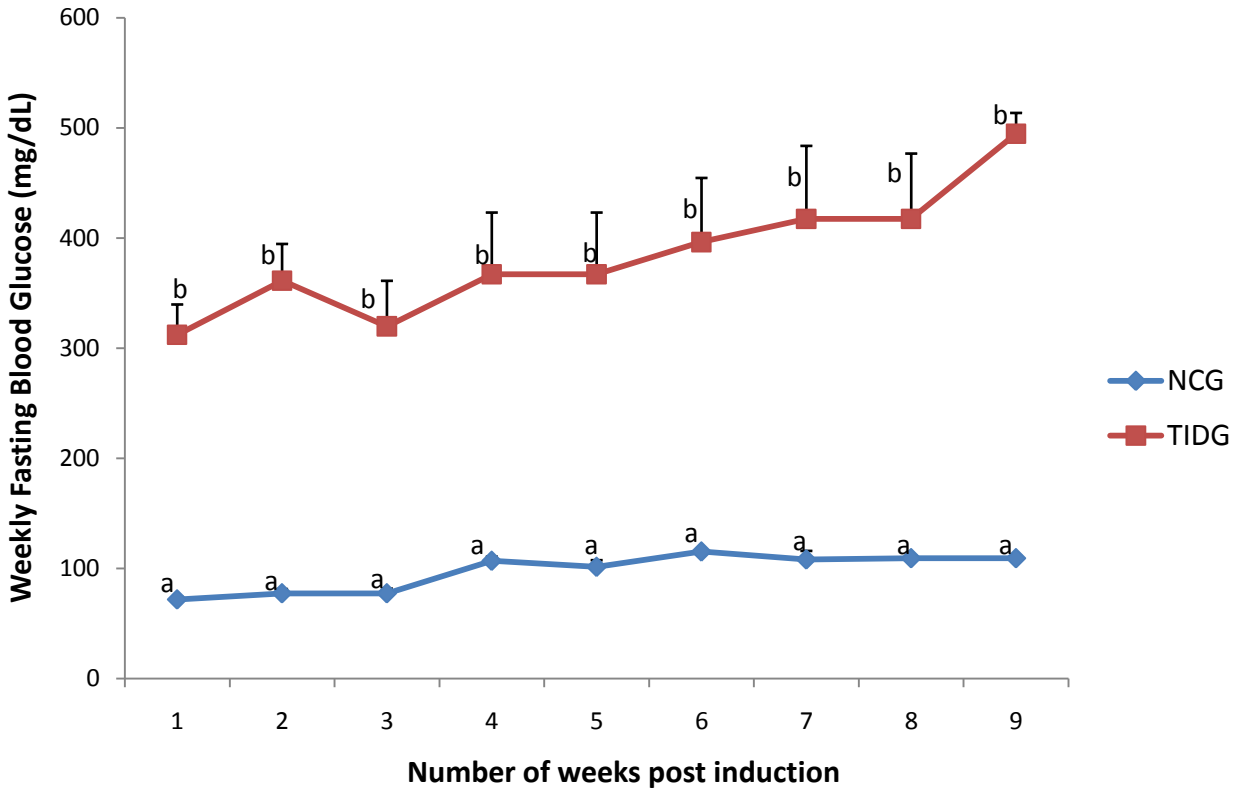

Figure 4.1: Weekly Fasting Blood Glucose Level of Type I Diabetes and the Normal Control Groups during 9-Week Experimental Period. [a-b] values of fasting blood glucose with different letters for a given week are significantly different from each other ($P<0.05$)

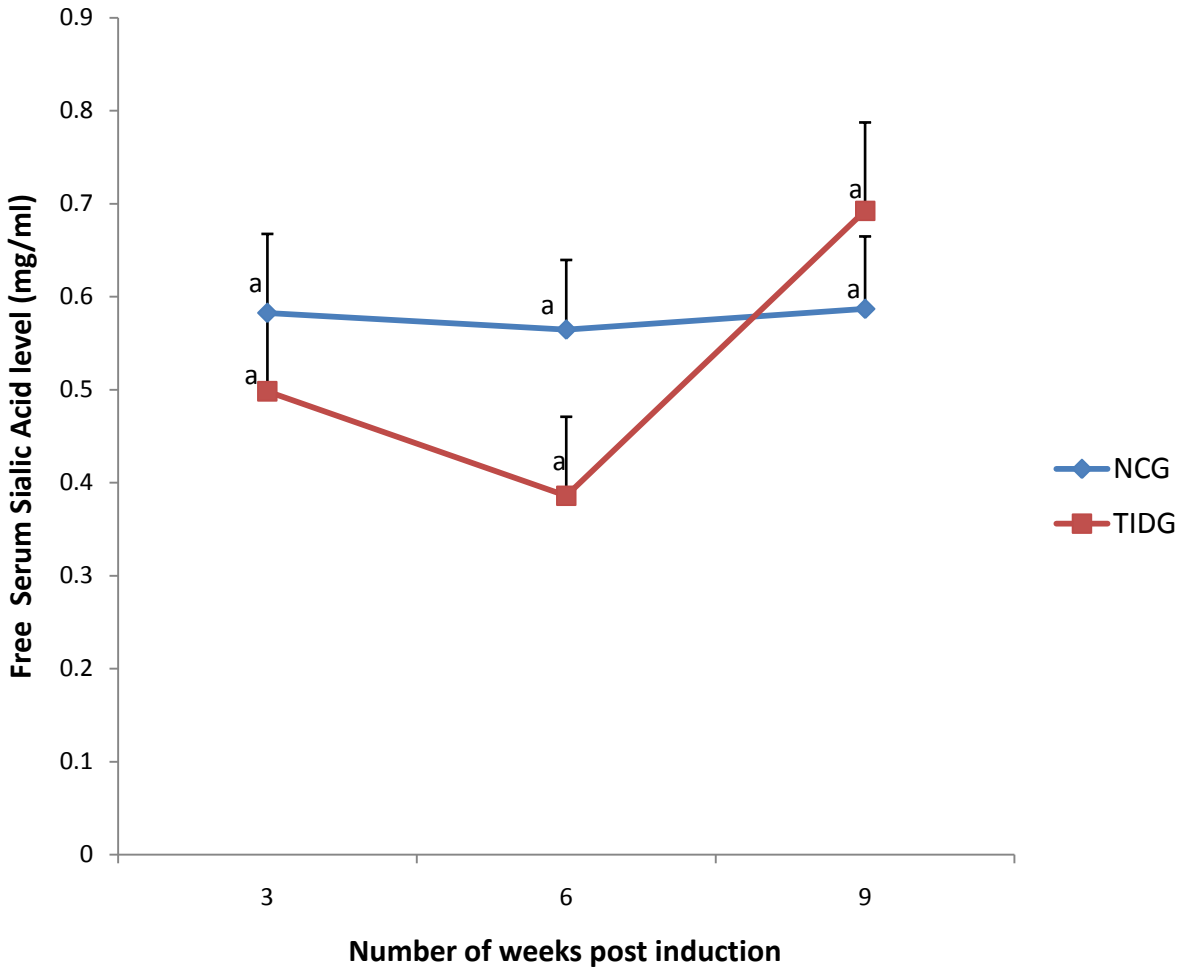

Figure 4.2: Free Serum Sialic Acid Level for the Period Of Nine Weeks among Type I Diabetes and Control. [a-b] values of free serum sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

4.1.3: Changes in the Level of Total Sialic Acid (TSA) in the Liver over a Period of Nine (9) Weeks among Type I Diabetes and the Control

From Figure 4.3 below, it is obvious that the TSA level for the TIDG was insignificantly higher ($P>0.05$) than the NCG at the early stage of diabetes at weeks 3 and 6. The TSA for the diabetic group were 0.16±0.05 mg/g and 0.25±0.07 mg/g respectively and the control had 0.13±0.05 mg/g and 0.14±0.04 mg/g respectively. However, as the diabetic condition progresses, the TSA level of the diabetic group declined.

4.1.4: Changes in the Level of Total Sialic Acid (TSA) in the Kidney over a Period of Nine (9) Weeks among Type I Diabetes and the Control

The changes in TSA level of the kidney in TIDG and NCG for the period of nine weeks shows a significant ($P<0.05$) rise in kidney TSA for the diabetic group at week 3, but at week 6 and 9, there was no significant difference ($P>0.05$) between the level of TSA in the kidney of the diabetic and the control group. The level of TSA for the NCG were at 0.43±0.01 mg/g, 0.62±0.08 mg/g and 0.50±0.09 mg/g for the 3rd, 6th and 9th weeks, respectively, whereas that of the TIDG were at 1.13±0.05 mg/g, 0.51±0.07 mg/g and 0.65±0.07 mg/g for the 3rd, 6th and 9th weeks.

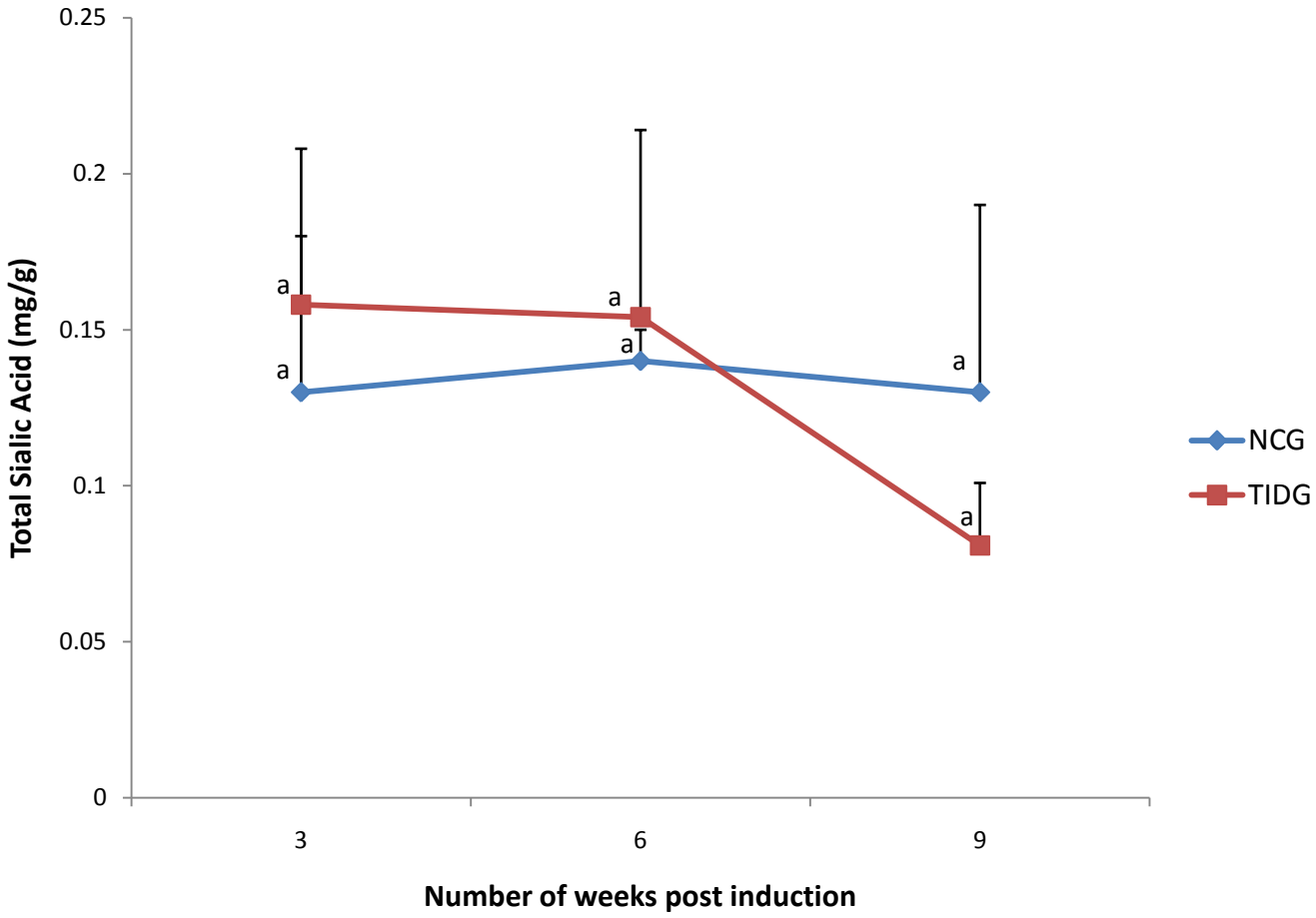

Figure 4.3: Changes in the Level of Total Sialic Acid in the Liver Over a Period of Nine Weeks among Type I Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

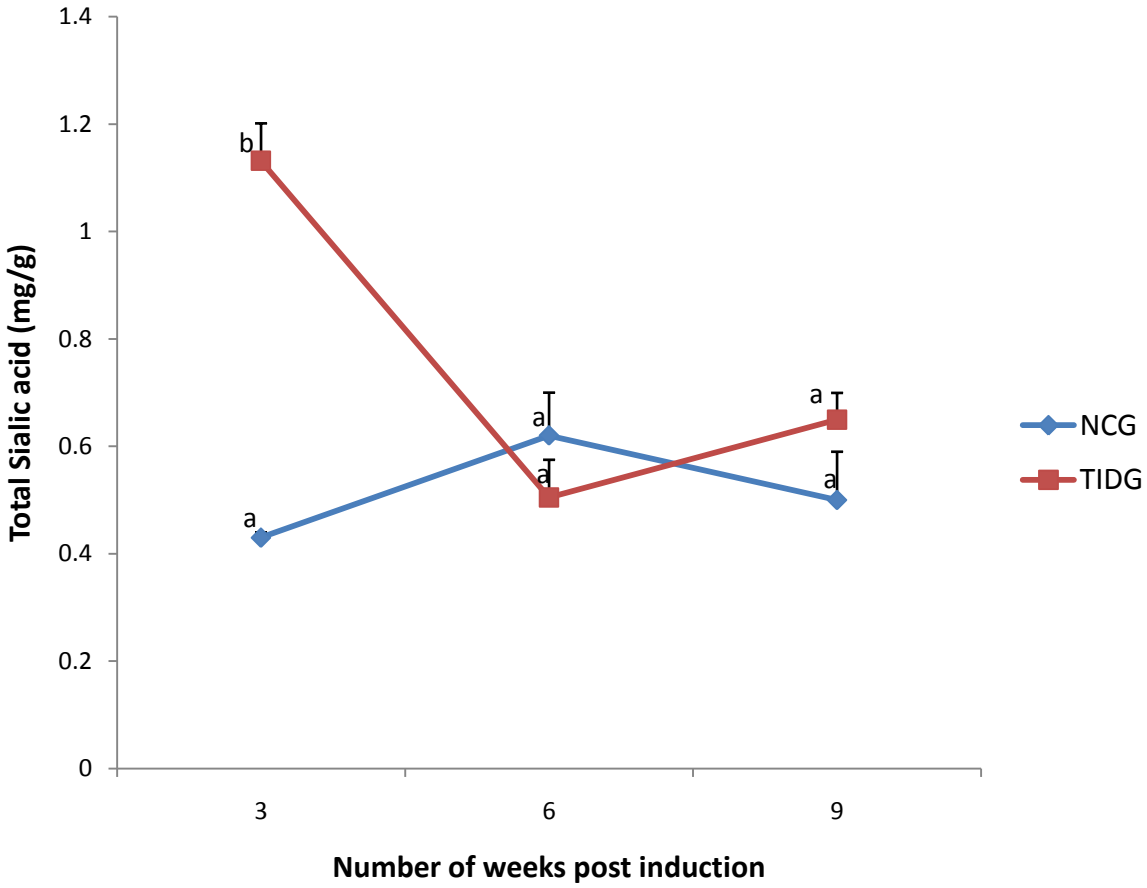

Figure 4.4: Changes in the Level Of Total Sialic Acid in the Kidney Over a Period of Nine Weeks among Type I Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

4.1.5: Changes in the Level of Total Sialic Acid (TSA) in the Pancreas over a Period of Nine (9) Weeks among Type I Diabetes and the Control

The changes in the TSA level in pancreas of TIDG and NCG during the period of nine weeks shows a significantly higher ($P<0.05$) TSA level in the pancreas of the TIDG compared to the NCG at week 3 and 9. However, a sharp decline in the level of TSA in the diabetic group compared to the control was observed during week 6 (Figure 4.5). The values were 1.27±0.12 mg/g, 0.43±0.0.17 mg/g and 0.61±0.25 mg/g for the 3rd, 6th and 9th week, while the TSA of the control were 0.12±0.03 mg/g, 0.29±0.15 mg/g and 0.29±0.03 mg/g for 3rd, 6th and 9th week.

4.1.6: Changes in the Level of Total Sialic Acid (TSA) in the Skeletal Muscle over a Period of Nine (9) Weeks among Type I Diabetes and the Control

The changes in level of TSA for skeletal muscle tissues in the TIDG and NCG for the period of nine weeks, shows a consistent rise in skeletal muscle TSA in the TIDG with progression of the diabetic condition. Though at the initial 3 weeks, the TSA level was significantly decreased ($P<0.05$) in the TIDG compared to the NCG. However, at the 6th and 9th week the TSA in the TIDG was significantly higher ($P<0.05$) compared to the NCG. The values were 0.27±0.03 mg/m, 0.91±0.19 mg/m and 1.65±0.19 mg/m respectively for weeks 3 6 and 9 in the TIDG and 0.48±0.08 mg/m, 0.48±0.13 mg/m and 0.28±0.09 mg/m respectively for weeks 3, 6 and 9 in the NCG (Figure 4.6).

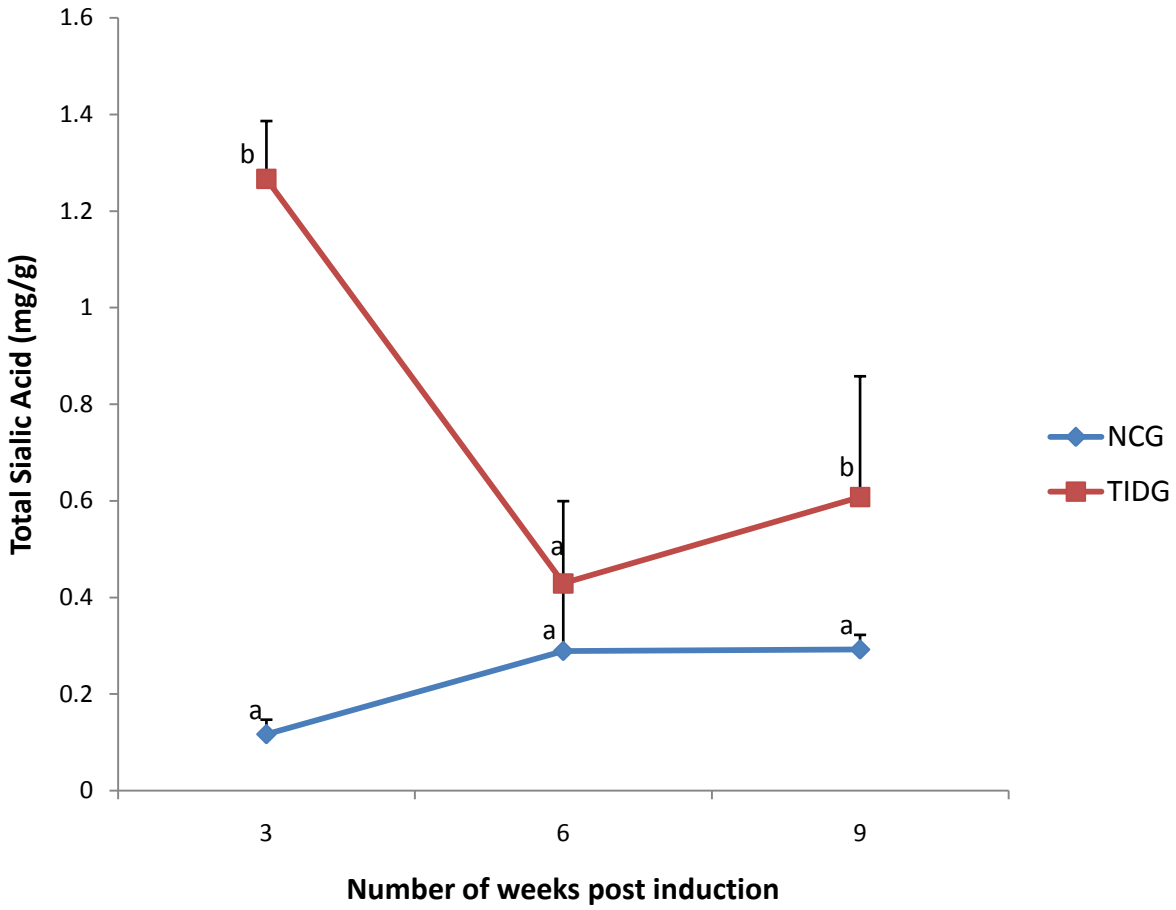

Figure 4.5: Changes in the Level of Total Sialic Acid in the Pancreas Over a Period Of Nine Weeks among Type I Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

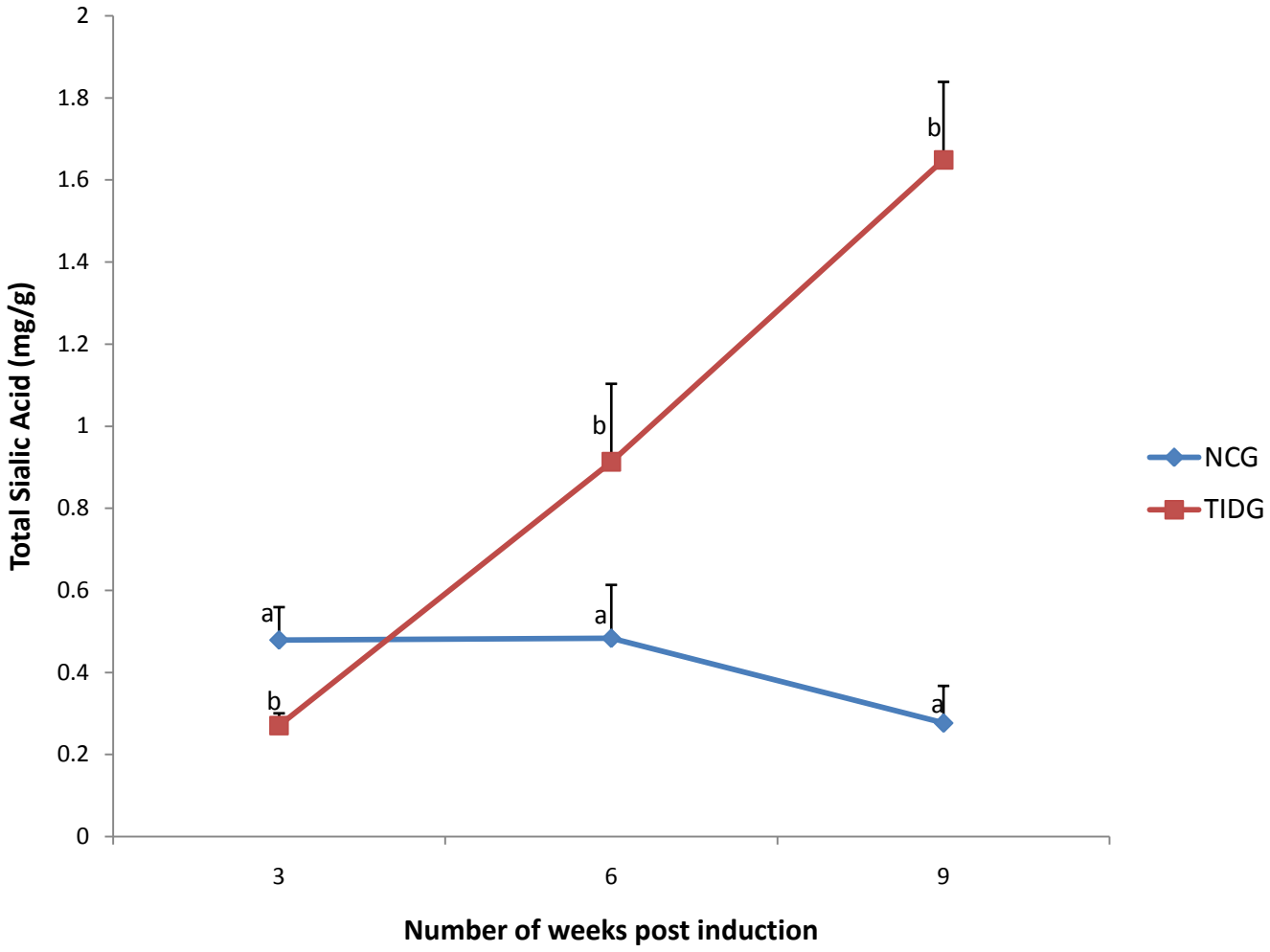

Figure 4.6: Changes in the Level of Total Sialic Acid in the Skeletal Muscle Over a Period Of Nine Weeks among Type I Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

4.1.7: Changes in the Level of Total Sialic Acid (TSA) in the Brain over a Period of Nine (9) Weeks among Type I Diabetes and the Control

The level of total sialic acid (TSA) in the brain tissue for the TIDG and NCG for the period of nine weeks, shows that the TSA in the brain of the TIDG was significantly ($P<0.05$) higher. At week 3, TSA was 1.02±0.09 mg/g and 0.5±0.09 mg/g for the TIDG and the NCG respectively. At week 6, the TSA of the TIDG and NCG were elevated to 4.92±0.02 mg/g and 2.58±0.38 mg/g respectively. During the week nine period, the TSA for the TIDG decreased from 4.92±0.02 mg/g to 3.91±0.45 mg/g (Figure 4.7).

4.1.8: The Level of Correlation between Sialic Acid Level in Serum and Tissues; Brain, Liver, Kidney, Skeletal Muscle and Pancreas in Type I Diabetes during Weeks 3, 6 and 9 after Induction

The correlation between the level of sialic acid in the serum and the tissues in TIDG during the third week of the experiment shows a positive correlation between the serum and the kidney and the skeletal muscle, but a negative correlation for the liver, the pancreas and the brain (Table 4.1). During the week 6, the result (Table 4. 1) shows a positive correlation between the serum and all the tissues: liver, brain, kidney, pancreas and the skeletal muscle. However, in the 9^{th} week of the experiment, there was a positive correlation between the serum and the liver, but a negative correlation for the brain, the kidney, the pancreas and the skeletal muscle as seen in Table 4.1.

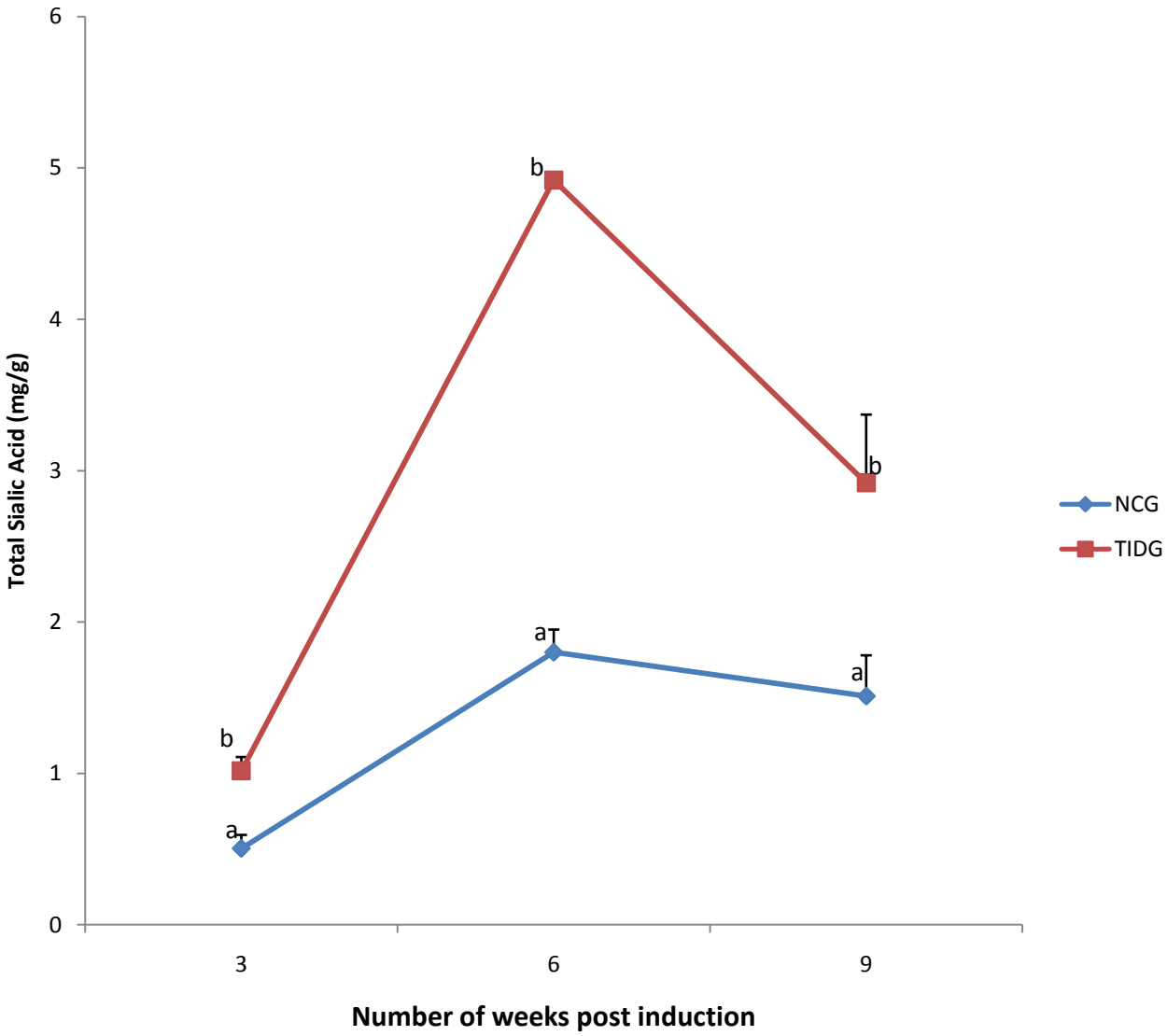

Figure 4.7: Changes in the Level Of Total Sialic Acid in the Brain Over a Period of Nine Weeks among Type I Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

Table 4.1: The Level of Correlation between Sialic Acid in Serum and Tissues; Liver, Kidney, Pancreas Skeletal Muscle and Brain, in Type I Diabetes during week (3), (6) and after Induction.

WEEK 3		LIVER	KIDNEY	PANCREAS	SKELETAL MUSCLE	BRAIN
SERUM	Pearson Correlation	-0.393	0.420	-0.021	0.753	-0.261
	Sig.	0.607	0.482	0.973	0.247	0.672
WEEK 6						
SERUM	Pearson Correlation	0.300	0.054	0.168	0.363	0.013
	Sig.	0.700	0.946	0.832	0.637	0.987
WEEK 9						
SERUM	Pearson Correlation	0.641	-0.198	-0.207	-0.660	-0.638
	Sig.	0.359	0.802	0.793	0.340	0.362

4.2 Type II Diabetes Results

4.2.1: Weekly Fasting Blood Glucose Level from Week 1-8 in Type II Diabetes and Normal Control

The fasting blood glucose (FBG) level for the TIIDG and NCG for the experimental period of eight weeks showed that the TIIDG have FBG significantly higher ($P<0.05$) than the control at FGB >200 mg/dL , while the control has FBG<100 mg/dL. These conditions were maintained in the both group during the experimental period (Figure4.8).

4.2.2: Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and β-Cell Damage (HOMA-β) for Type II Diabetes and Normal Control Group

The homeostasis model assessment for insulin resistance (HOMA-IR) of the TIIDG and NCG revealed that the control group has an insulin resistance level of 1.82±0.25, while the insulin resistance for the diabetic group was 8.74±2.19. Whereas the β-cell damage (HOMA- β) of the type II diabetic and the normal control group revealed that the control group has a HOMA-β level of 555.12±131.39. While that for the diabetic group was 15.23±3.59.

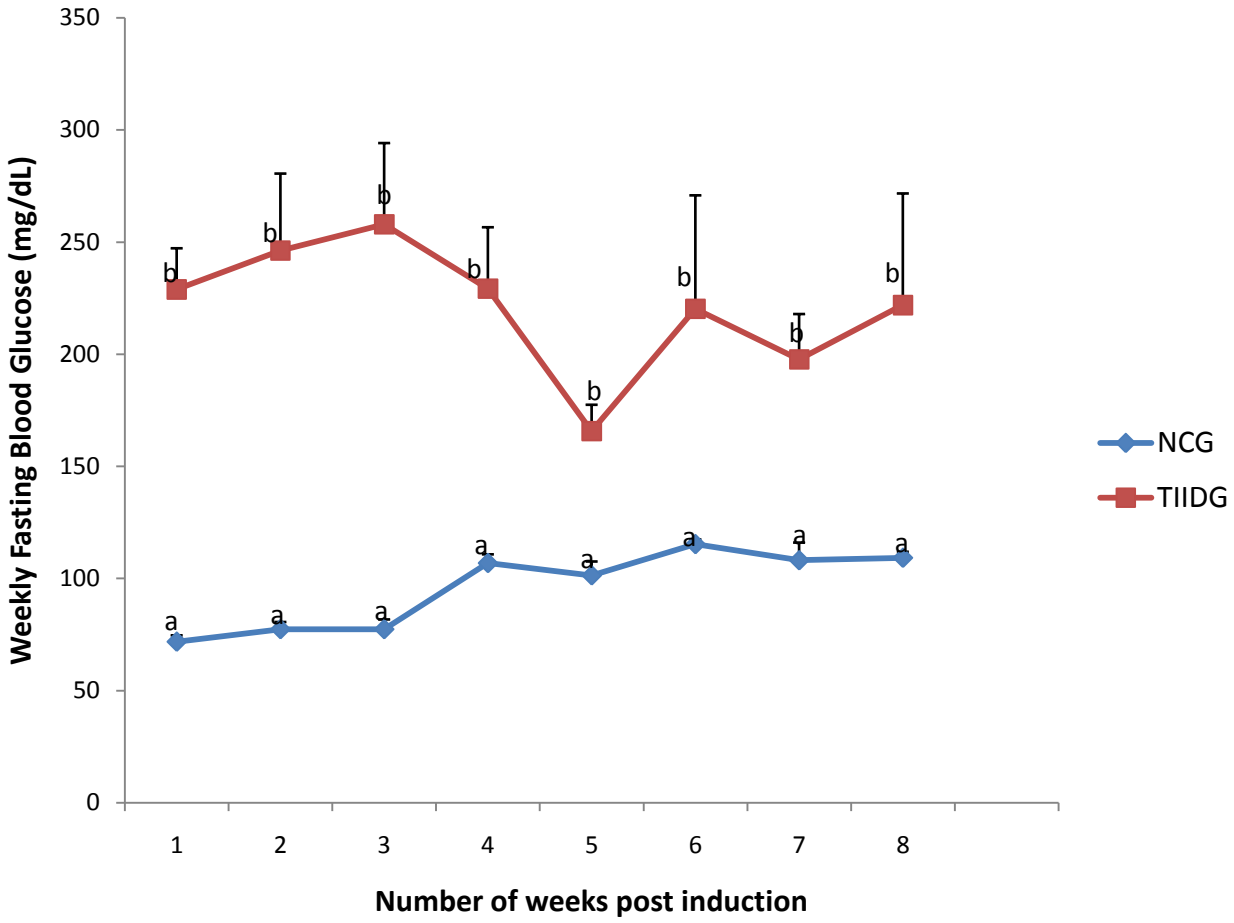

Figure 4.8: Weekly Fasting Blood Glucose Level from Week 1-8 in Type II Diabetes and Normal Control. [a-b] values of fasting blood glucose with different letters for a given week are significantly different from each other ($P<0.05$)

Table 4.2: Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and β-Cell Damage (HOMA- β) for Type II Diabetes and Normal Control Group.

Homeostasis Model Assessment	NCG	TIIDG
HOMA-IR	1.82±0.25	8.74±2.19
HOMA-β	555.12±131.39	15.22±3.59

4.2.3: Free Serum Sialic Acid (FSA) of Type II Diabetes and Control over the Period of Eight Weeks

Figure 4.9 shows that the FSA of the TIIDG fluctuated during the period while the FSA of the NCG was maintained. At the 2nd week, both the TIIDG and NCG had almost the same FSA level. However, the FSA for the TIIDG made a sharp fall during the 4th week to 0.36±0.04 mg/ml that made the FSA of the NCG significantly higher ($P<0.05$). Thereafter, the FSA of the TIIDG group began to rise from the 6th week to the 8thweek. The FSA of the TIIDG during the week 6 and 8 were 0.48±0.09 mg/ml and 0.56±0.05 mg/ml, respectively.

4.2.4: Changes in the Level of Total Sialic Acid (TSA) the Liver over a Period of Eight (8) Weeks among Type II Diabetes and the Control

The changes in the level of total sialic acid (TSA) in the liver tissues of the TIIDG and NCG during the period of eight weeks (Figure 4.10) revealed that the TSA in the liver of the TIIDG rats had a gradual but insignificant ($P>0.05$) rise during the period. The TSA values were 0.07±0.02 mg/g, 0.03±0.06 mg/g, 0.12±0.06 mg/g and 0.17±0.04 mg/g for the 2nd, 4th, 6th and 8th week respectively, while that of the NCG are 0.14±0.05 mg/g, 0.05±0.01 mg/g, 0.14±0.04 mg/g and 0.13±0.06 mg/g for the 2nd, 4th, 6th and 8th week, respectively.

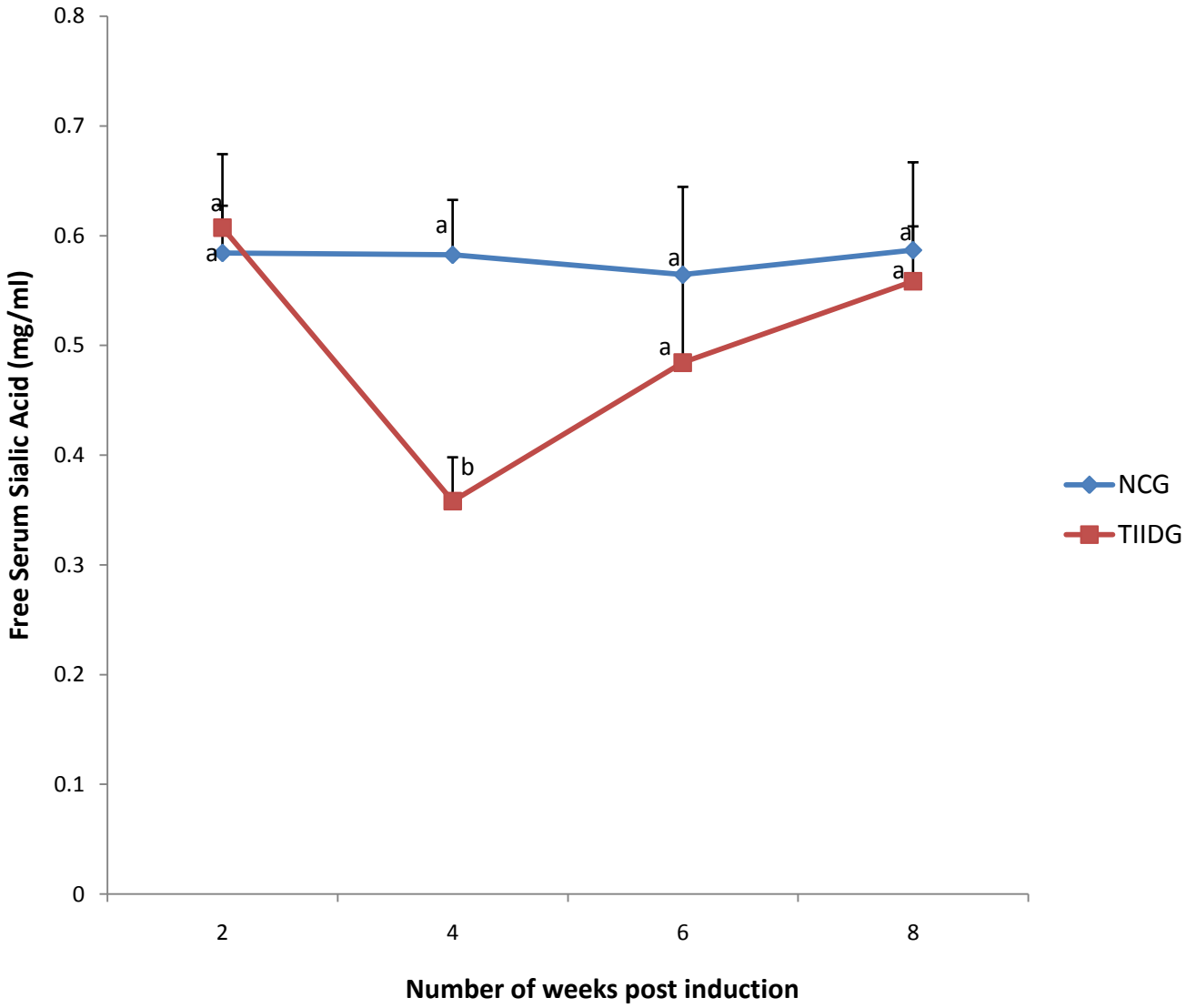

Figure 4.9: Free Serum Sialic Acid of Type II Diabetes And Control Over the Period of Eight Weeks. [a-b] values of free sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

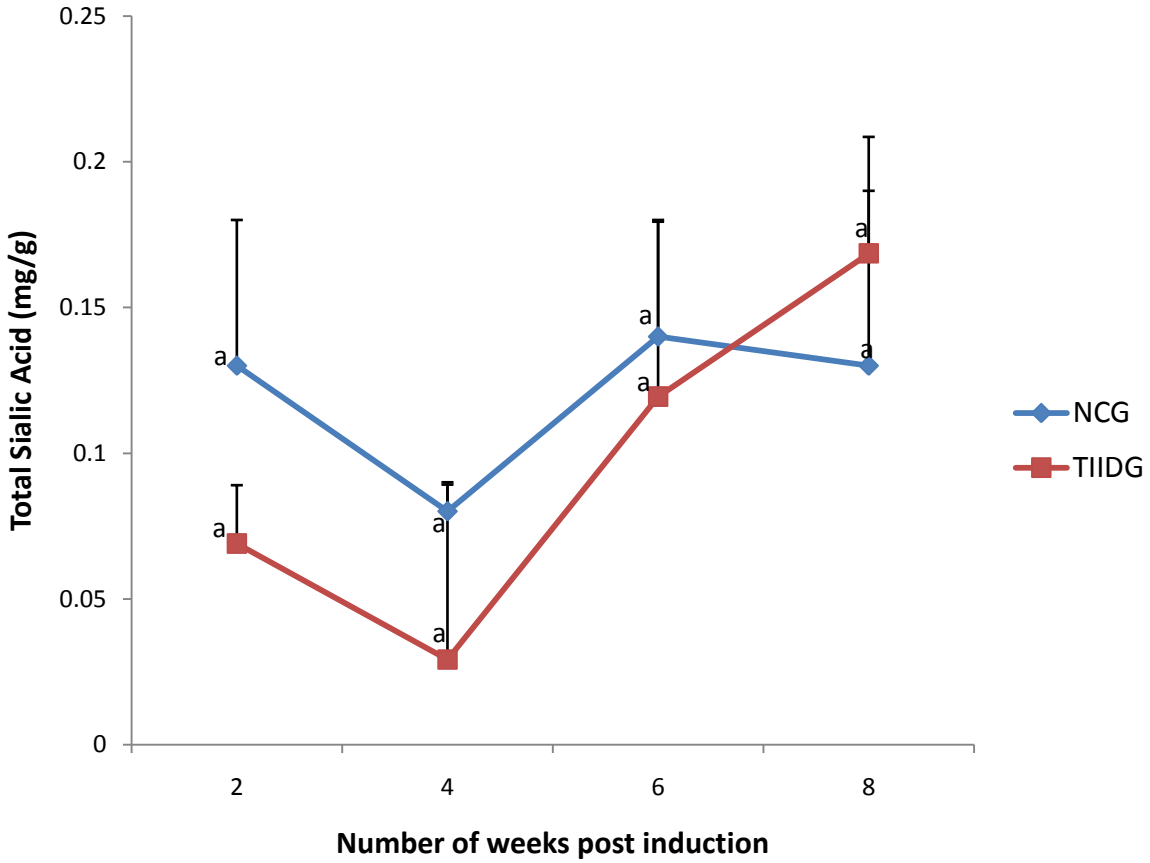

Figure 4.10: Changes in the Level of Total Sialic Acid in ,the Liver over a Period of Eight Weeks among Type II Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

4.2.5: Changes in the Level of Total Sialic Acid (TSA) in the Kidney over a Period of Eight (8) Weeks among Type II Diabetes and the Control

The changes in the total sialic acid (TSA) level in the kidney of TIIDG and NCG during the period of eight weeks shows an increased level of TSA in the kidney of TIIDG during the period. At the initial $2^{nd}$and 4^{th} week, the level of TSA in the kidney of both the TIIDG and NCG were insignificantly different. However, during week 6 and week 8, the TSA in the diabetic group was significantly ($P<0.05$) increased (Figure 4.11). The values were 1.01±0.27 mg/g, 0.78±.023 mg/g, 1.39±0.34 mg/g and 1.26±0.22 mg/g respectively for weeks 2, 4, 6 and 8 in the TIIDG and 0.98±0.26 mg/g, 0.71±0.01 mg/g, 0.62±0.08 mg/g and 0.47±0.09 mg/g respectively for weeks 2, 4, 6 and 8 in the NCG.

4.2.6: Changes in the Level of Total Sialic Acid (TSA) in the Pancreas over a Period of Eight (8) Weeks among Type II Diabetes and the Control

The level of TSA in the pancreas of TIIDG and NCG for the period of eight weeks, demonstrated that the TSA in the pancreas of the TIIDG was significantly higher ($P<0.05$) compared to the NCG at the initial four weeks, but the same decrease with progression of the disease. The values were 1.20±0.28 mg/g, 1.16±0.32 mg/g, 0.47±0.09 mg/g and 0.43±0.10 mg/g respectively for weeks 2, 4, 6 and 8 in the TIIDG and 0.12±0.03 mg/g, 0.09±0.06 mg/g, 0.29±0.15 mg/g and 0.29±0.03 mg/g respectively for weeks 2, 4, 6 and 8 in the NCG (Figure 4.12).

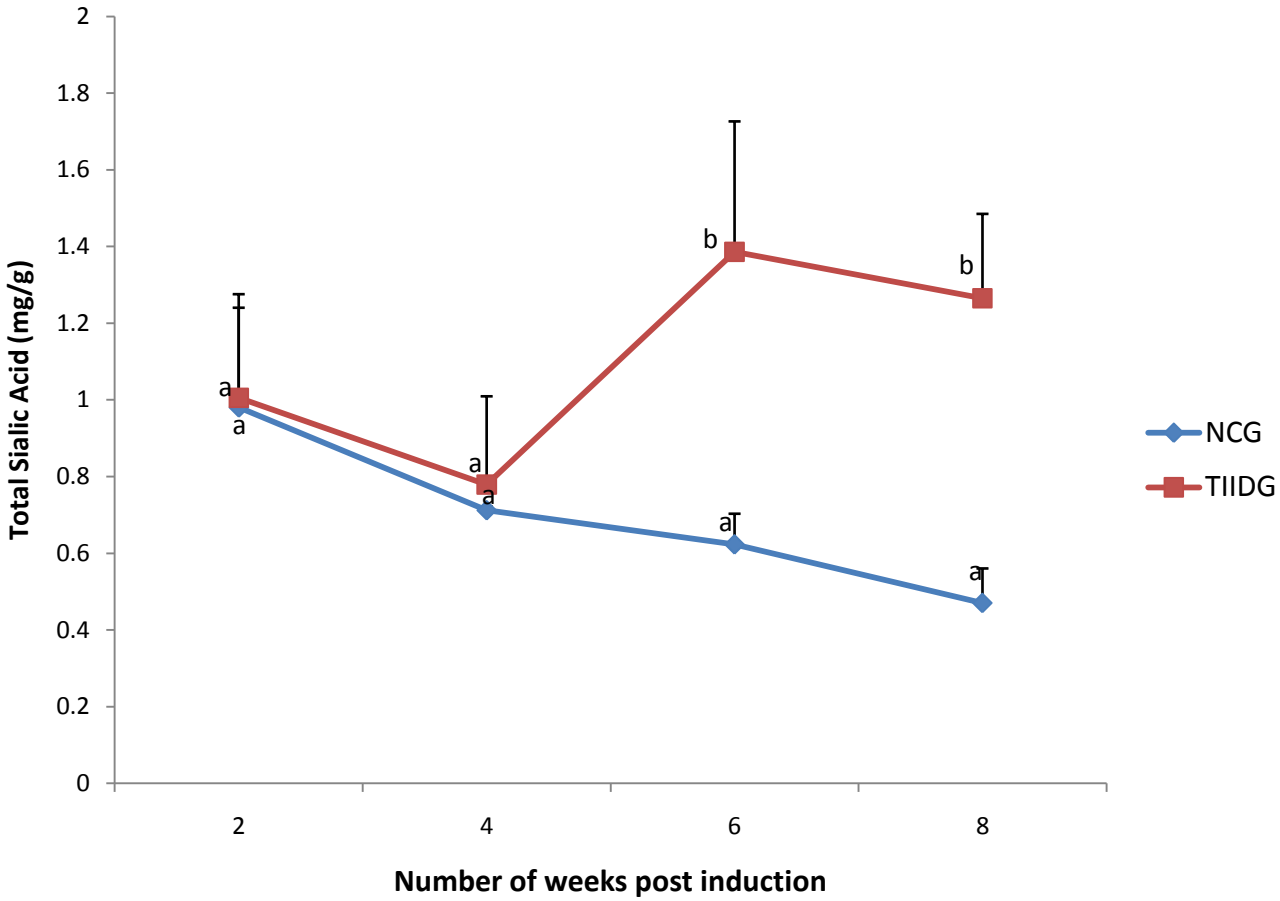

Figure 4.11: Changes in the Level of Total Sialic Acid in the Kidney over a Period of Eight Weeks among Type II Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

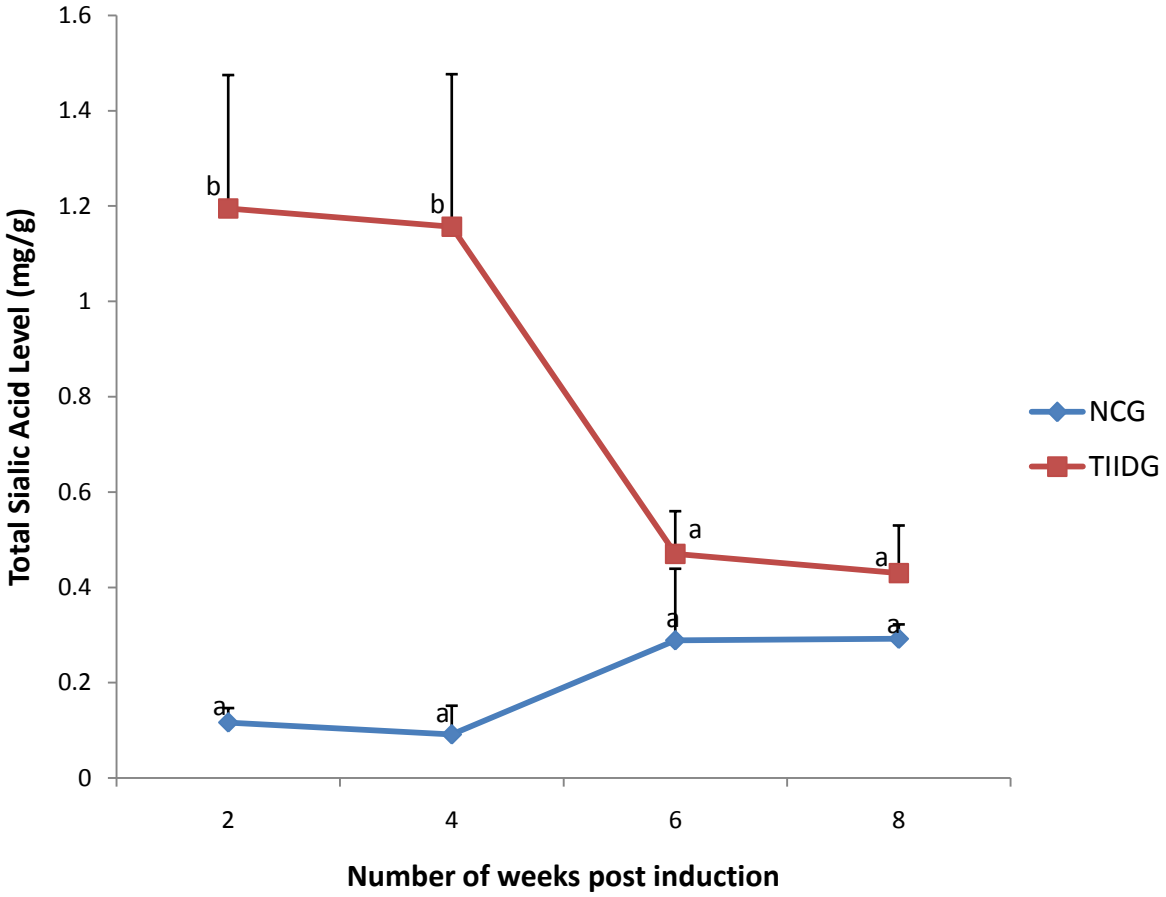

Figure 4.12: Changes in the Level of Total Sialic Acid in the Pancreas over a Period of Eight Weeks among Type II Diabetes and the Control. , . [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

4.2.7 Changes in the Level of Total Sialic Acid (TSA) in the Skeletal Muscles over a Period of Eight (8) Weeks among Type II Diabetes and the Control

The changes in the TSA level of skeletal muscle in TIIDG and NCG for the period of eight weeks, shows an increase in the level of TSA in the skeletal muscle for TIIDG. From Figure 4.13 at the beginning, the TSA shows a significant ($P<0.05$) decrease in the TIIDG. However, during the 6^{th} and 8^{th} week, there was a significant ($P<0.05$) increase in the level of skeletal muscle TSA in the TIIDG compared to the NCG. The values were 0.29±0.08 mg/g, 0.51±0.27 mg/g, 0.78±0.13 mg/g and 0.9±0.09 mg/g respectively for weeks 2, 4, 6 and 8 in the TIIDG and 0.48±0.08 mg/g, 0.49±0.27 mg/g, 0.48±0.13 mg/g and 0.28±0.09 mg/g respectively for weeks 2, 4, 6 and 8 in the NCG.

4.2.8: Changes in the Level of Total Sialic Acid (TSA) of the Brain over a Period of Eight (8) Weeks among Type II Diabetes and the Control

The changes in the level of TSA in the brain tissues of TIIDG was significantly ($P<0.05$) increased compared to the NCG. The values were 1.11±0.27 mg/g, 4.00±0.20 mg/g, 4.75±0.19 mg/g and 5.01±0.02 mg/g respectively for weeks 2, 4, 6 and 8 in the TIIDG and 0.50±0.09 mg/g, 1.08±0.15 mg/g, 1.8±0.58 mg/g and 1.51±0.27 mg/g respectively for weeks 2, 4, 6 and 8 in the NCG (Figure 4.14).

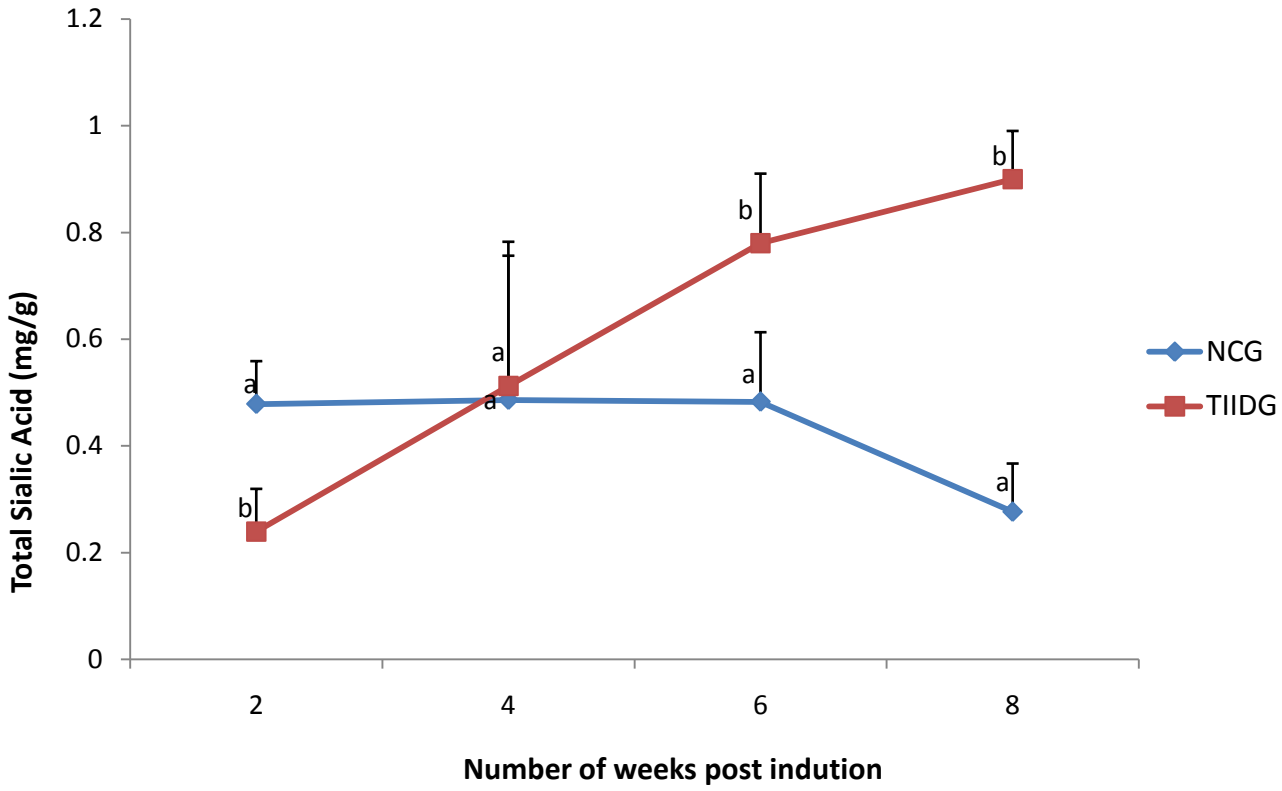

Figure 4.13: Changes in the Level of Total Sialic Acid Level in the Skeletal Muscles over a Period of Eight Weeks among Type II Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

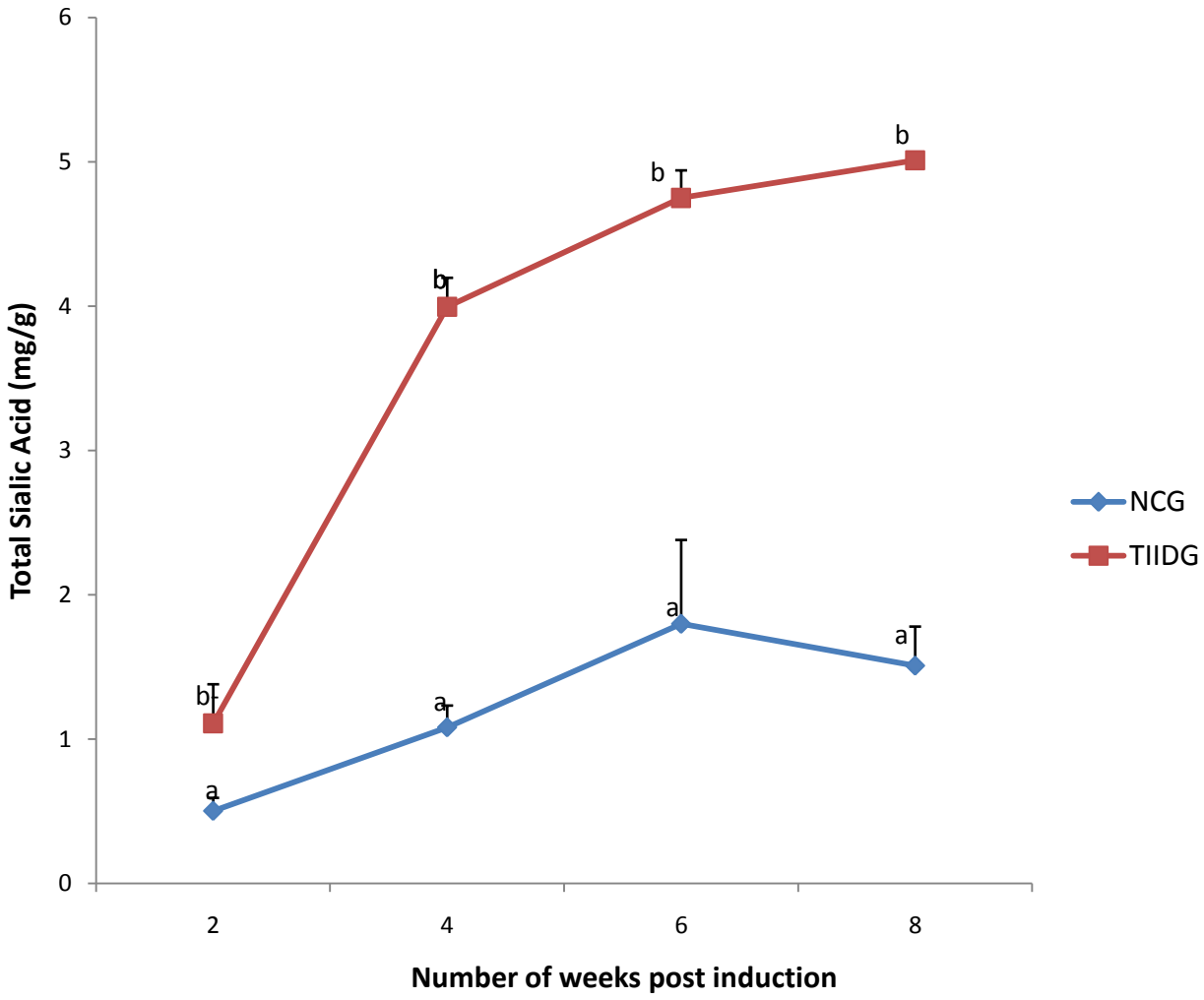

Figure 4.14: Changes in the Level of Total Sialic Acid Level of the Brain over a Period of Eight Weeks among Type II Diabetes and the Control. [a-b] values of total sialic acids with different letters for a given week are significantly different from each other ($P<0.05$)

4.2.10: Correlation between Sialic Acid Level in Serum and Tissues; Liver, Kidney, pancreas, Skeletal Muscle and Brain in Type II Diabetes During Weeks 2, 4, 6 and 8 after Induction

The correlation between the level of sialic acid in the serum and the tissues during the second week of the experiment shows a positive correlation between the serum and the liver, but shows a negative correlation for the brain, the kidney, the pancreas and the skeletal muscle (Table 4.3). Whereas, the correlation during week 4 reveal a level of positive correlation between the serum and the liver, skeletal muscle and the brain but shows a negative correlation for the kidney and the pancreas as seen in (Table 4.3). The result for the correlation during week 6 (Table 4.3) demonstrated that there is a level of positive correlation between the serum and the liver, kidney, skeletal muscle and the brain but shows a negative correlation for the pancreas. However, during the eight week of the experiment there was a positive correlation between the serum and the liver and the brain, but a negative correlation for the kidney, the pancreas and the skeletal muscle (Table 4.3).

Table 4.3: Correlation between Sialic Acid Level in Serum and Tissues; Liver, Kidney, Pancreas, Skeletal Muscle and Brain in Type II Diabetes during Weeks (2), (4), (6) and (8) after Induction

WEEK 2		LIVER	KIDNEY	PANCREAS	SKELETAL MUSCLE	BRAIN
SERUM	Pearson Correlation	0.380	-0.772	-0.198	-0.422	-0.041
	Sig.	0.528	0.228	0.802	0.479	0..947
WEEK 4						
SERUM	Pearson Correlation	0.064	-0.526	-0.066	0.342	0.284
	Sig.	0.880	0.225	0.888	0.453	0.495
WEEK 6						
SERUM	Pearson Correlation	0.312	0.896	-0.970	0.998	0.858
	Sig.	0.798	0.293	0.155	0.043	0.344
WEEK 8						
SERUM	Pearson Correlation	0.503	-0.705	-0.598	-0.834	0.271
	Sig.	0.664	0.295	0.402	0.166	0.729

CHAPTER FIVE

5.0 DISCUSSION

In recent time, diabetes mellitus has been identified as one of the leading cause of mortality in both developed and developing countries of the world. In the desire to fight this disease a lot of recent development, especially the development of a competent and cost-effective means of mimicking this condition in animal models was made. Among the methods, chemically-induced method offers the most rapid and cost-effective option for diabetes in animal modeling. Streptozotocin, an antibiotic has been extensively used to study both the pathology of diabetes mellitus conditions and related complications. Treatment of our animal with 60/mg/kg b.w of streptozotocin induced a hyperglycemia condition modeling a type I form of diabetes mellitus. The use of STZ alone either at low or high dose cannot induce insulin resistance, which necessitates the need to find alternatives that will help to induce the actual pathologies of type II diabetes. The use of high fat diet has been in use but the recent development of combining the effect of fructose feeding *ad libitum* and latter injection of low dose STZ excellently expresses the pathophysiological conditions associated with type II diabetes (Wilson and Islam, 2012). Figure 4.8 and Table 4.2 show the level of weekly fasting blood glucose, insulin resistance and β-cell function in our type II diabetes model. The FBG>200 confirming the presence of hyperglyceamia, insulin resistance IR>5 confirming substantial level of insulin resistance in the animals and HOMA-β<200 revealing that the β-cells of the pancreas were partially damaged and therefore functionality reduced. This result is also in tandem with the work of Ibrahim and Islam (2014).

Sialic acid, a nine carbon carbohydrate, and a derivative of neuramunic acid is reported to be implicated in the pathology of diabetes and its related complications. Sialic acid, a terminal carbohydrate component of oligosaccharide chains of glycoprotiens, glycolipids and gangliosides, plays a vital role in cell to cell interaction and also in immunity. The compound has been reported severally to be involved in the pathology of several human diseases including diabetes and its complication. Some reporters argue that sialic acid can be adopted as a biomarker for diabetes and related complications as it is reported that sialic acid level is always altered in diabetic patients with or without complications (Khalili *et al*., 2014, Mahendran *et al*., 2013, Divija *et al*., 2013, Prajnak *et al*., 2013, Nayak and Bhaktha 2005, Crook *et al*., 1993,). The action of sialidases can liberate sialic acids from their place of bond into circulation. Also, physiological disturbances such as injury and inflammation to the cells carrying sialic acids can cause them to be removed into circulation (Prajnak *et al*., 2013).

Free serum sialic acid has been reported to increase with diabetes with or without complication. However, the FSA levels in type I and type II diabetes shows a pattern of initial fall while it begins to increase, though, insignificantly from weeks 6, 8, and 9. At the 9^{th} week, there was a rise in the levels of FSA in the TIDG and this rise in sialic acid in the diabetic group observed in this study is in concordance with the work of Divija *et al.*, (2013), that the development and severity of the complications associated with diabetes is dependent on the duration and management of the condition. It has been reported that increased sialic acid level correlates with severity of organ damage in diabetes (Crook *et al.,* 1993). The rise in free serum sialic acid observed during the week 9 of type I diabetes may be due to the no management of the diabetic condition and also the duration, as it has been speculated earlier by Divija *et al*., (2013) and Crook *et al*., (1993) that sialic acid increases with severity and duration of diabetic condition.

Hyperglycemia is one of the major characteristics of diabetes, the prolong presence of glucose in the serum allows glucose to non-enzymatically react with primary amines of proteins forming glycated compounds such as glycated heamoglobin. The advanced glycation end products (AGES) formed, activate their receptors which initiate the activation of the innate immunity and subsequent mobilization of inflammatory cytokines such as interleukine (IL-6) and TNFα. The presence of these cytokines activates the mobilization of acute phase proteins from the liver (John and Pickup, 2004). Sialic acid exists as conjugate to most of these cytokines and the acute phase reactants. Also, vascular endothelium is associated with high level of sialic acids and therefore tissue injury caused by the action of AGES to the endothelium lead to shedding of their sialic acids into circulation while increasing their peameability (Divija *et al*., 2013). All these lead to increased sialic acid in circulation. This explains the gradual rise in serum sialic acid observed in the type I and type II diabetic groups. For the type II diabetic group, it is clear why the rise for the period of 8 weeks of the experimental period did not exceed that of the control, because of the less severity of the diabetic condition experienced in this group and the duration of the condition.

The initial fall in sialic acid level in the serum experienced in both the type I and type II diabetes as seen in figures 4.2 and 4.9 may be as a result of the body reaction to the presence of AGES thereby mobilizing high number of cytokines (TNFα and (IL-6) and acute phase reactants (C-reactive protein CRP, and alpha-(1)-acid glycoprotein AGP). These compounds carry sialic acids on their terminal end and therefore at the initial period of the diabetic condition, the body may be scouting for the free sialic acid in circulation for the synthesis of these compounds. This is coupled with the disturbance of glucose metabolism associated with diabetes. Diabetes is associated with altered insulin production and action. Therefore at the initial stage of diabetes,

the body may not have adjusted to the sudden drop in insulin supply and action, since the body tissues depend on insulin to transport glucose into the cytosol of cells. There may be shortage of glucose in the cells. In the cytosol of cells, glucose is the major precursor for sialic acid synthesis as it metabolism generate phosphoenulpyruvate (PEP), a good precursor for sialic acid synthesis.

Total sialic acid in tissues is comprised of bound and free sialic acids. The assay results (Figures 4.3 to 4.7 and Figures 4.10 to 4.14) show a significant increase in the brain TSA level of the diabetic animal during the initial first 2 and 3 weeks in the diabetic groups, though, the concentration increased further with the progression of the condition. This is in tandem with the report of Ibrahim *et al*., (2016), which demonstrated that total sialic acid is elevated in the tissues during insulin resistance and hyperglycemia.

From this result, it is obvious that among all the tissues, the brain has the highest concentration of sialic acid, both in the diabetic and control groups, though the concentration is increased in the diabetic groups. This is because the brain contains high amount of gangliosides that have sialic acid attached especially glycosphingolipids, which could account for approximately 80% of total glycome mass in the brain. Also, the brain contains the most complex glycoproteins such as the neural cells adhesion molecules that have polysialic acid attached. The polysialic acids molecules have about 90 units of sialic acids (Schnaar and Gerardy-Schahn, 2014) which account for the higher level of sialic acid concentration observed.

In all the tissues, (kidney, skeletal muscle and the pancreas) apart from the liver, there was a remarkable increase in sialic acid in the diabetic group compared to their control counterpart, this agrees with the report of Crook *et al.,* (2001) that shows an increased total sialic acid in diabetes. However, it contradicts in part the report of Ibrahim *et al.,* (2016) that demonstrated a fall in total

sialic acid in pancreas of insulin resistant and hyperglycemic animals. The rise in the sialic acid experienced in these tissues may be linked to the effect of hyperglycemia and advanced glycation end products and subsequent mobilization of acute phase proteins. Also coupled with the fact that during diabetes the body tissues result to gluconeogenesis to supply its energy need, this result to accumulation of phospoenulpyravate; a metabolite for sialic acid synthesis (Ibrahim *et al*., 2016), therefore there is an increased rate of sialic acids synthesis during diabetes.

Sialic acid may be reduced in the liver of the diabetic group because the liver is the sole organ responsible for the synthesis and mobilization of the acute phase reactants to other cells during inflammatory reaction. Most of these acute phase reactants mobilized from the liver have sialic acids attached on their terminal ends and therefore sialic acids synthesized in the liver may be channeled to the synthesis of these acute phase reactants. This may be responsible for the fall of sialic acid level in the liver of the diabetic group observed.

There is a degree of varying correlations between the level of sialic acid in the serum and that of the liver, kidney, pancreas, skeletal muscle, and the brain during type I diabetes. Though these correlation varied during the experiment. The varying correlations observed signify that there is a linear relationship between levels of sialic acids between the serum and the tissues. The positive correlations imply that as sialic acids increases in the following tissue, it also increases in the serum. Whereas the negative correlation observed explains the fact that as sialic acid concentration increases in the serum, it decreases in the respective tissues.

From our observation, we can deduce that free sialic acids circulating in the serum were liberated from their place of bond the tissues. From the type I result Figure 4.2, it was observed that during

the early stage of diabetes at week 3. There was weak negative correlation between the serum and the following tissues (liver, pancreas and the brain), but a strong positive correlation with skeletal muscle. This implies that these tissues (liver, pancreas and the brain), may be shedding little or nothing in to the serum while the synthesis of sialic acid was high in the skeletal muscle. This could be responsible for the fall in sialic acid concentration observed in the serum. However, during the 6^{th} week when there was net reduction in the level of sialic acid in the serum, there was no negative correlation between the serum and the tissues. At this time the tissue may not be shedding off it sialic acid into circulation. While at the 9^{th} week the number of tissues showing negative correlation increased (kidney, pancreas, skeletal muscle and the brain). The skeletal muscle and the brain showed a stronger level of negative correlation 0.660 and 0.638 respectively increasing their chances of shedding sialic acid, while the liver shows an increased sialic acid synthesis with higher positive correlation. This may be responsible for the increased level of sialic acid concentration in the serum. In the type II diabetes, the level of negative correlation between the serum and tissues is proportional to the level of sialic acids concentration in the serum. This was observed during the 2^{nd} week, when they was high level of sialic acid in serum at the same time there was high level of negative correlation between serum and tissues (kidney, pancreas, skeletal muscle and brain) with kidney showing a strong correlation. Only the liver shows low synthesis of sialic acid. Also, as the level of sialic acid decreased in the serum during the 4^{th} week, the number of tissues showing negative correlation reduced (kidney and pancreas). It has been observed that during diabetes there is an increased synthesis of sialic acids in these respective tissues. Injuries to endothelial cells in the respective tissues and the action of sialidase during diabetes liberate the sialic acids from the tissues into

circulation. Moreover, as the tissues are being turned over they liberate their sialic acids into circulation, (Ibrahim *et al*., 2016, Cade 2008, Crook *et al*., 2001).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECMENDATIONS

6.1 Summary

From this research, on the monitoring of the level of sialic acid in the serum and some tissues (liver, kidney, pancreas, skeletal muscle and brain) in streptozotocin-induced type I and type II diabetes in rats over a period of 9 weeks and 8 weeks respectively for types I and II. The following can be deduced as observed.

There was an initial fall in the level of free serum sialic acid during the early stage of diabetes. However, the level rises as the condition progressed, both in type I and type II diabetes.

The level of total sialic acid is increased in the kidney, pancreas, skeletal muscle and brain except the liver during both type I and type II diabetes.

The brain tissue has the highest concentration of total sialic acid among the other organ; liver, kidney, pancreas and skeletal muscle both in diabetic and non diabetic condition.

There is a level of negative and positive correlation between serum sialic acid level and the following organ; liver, kidney, brain, and the skeletal muscle and the correlation varies with the progression and concentration of serum sialic acid level. For the type I diabetes, during week 3, there was a weak negative correlation between the serum and the liver, pancreas and brain, but positive correlation between kidney and skeletal muscle, the skeletal muscle showing a strong correlation. While at week 6, there was a weak positive correlation with all the tissues and no negative correlations. However, at week 9 there was negative correlation with all the tissues except the liver, the negative correlation was a bit strong with skeletal muscle and the brain. Whereas in the type II diabetes, there was a weak positive correlation between the serum and the

liver during week 2 but strong negative correlation with kidney and weak ones with pancreas and skeletal muscle, and the brain. However, during the 4^{th} week the negative correlations were with the kidney and the pancreas, but weak positive correlations with the rest tissues. Whereas, the 6^{th} week, there was a strong negative correlation with pancreas alone but the other tissues show strong positive correlation except the liver. However, in the 8^{th} week, the tissues showing negative correlation increased kidney, pancreas, and skeletal muscle. While liver and brain showed weak positive correlation.

6.2 Conclusions

From this experiment, the source of increased level of sialic acids observed in the serum during types I and II diabetes may have come from the respective tissues (liver, kidney, pancreas, skeletal muscle and brain). There was a level of varying negative correlation of sialic acids concentration among the serum and the organs (liver, kidney, pancreas, skeletal muscle and brain) at various stages of the diabetic condition in both types I and II diabetes. The level of the negative correlation shows an increasing level of free serum sialic acids with decreasing total sialic in the respective tissues. This means that as the level of sialic acid is increasing in the serum, it is decreasing in the tissues. At this time, the tissues may be shedding their sialic acids into circulation; this could be responsible for the upsurge in sialic acid level observed in the serum. This, also, is applicable to the level of the positive correlation which shows inverse relationship with the level of sialic acid in the serum.

6.3 Recommendations

We recommend that more work should be done in this area especially during the early stage of diabetes, so as to fully establish the pathological condition leading to the initial fall in sialic acid observed in this study.

Also, studies be made on the enzymes involved in metabolism of sialic acids such as sialidases and sialytransferase so as to establish their role in the pathological situations.

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