THE RELATIONSHIP BETWEEN LEPTIN AND ANTHROPOMETRIC PARAMETERS IN TYPE 2 DIABETIC PATIENTS IN KANO, NIGERIA

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DECEMBER, 2015

DECLARATION

I declare that the work in the thesis entitled "The Relationship between Leptin and Anthropometric Parameters in Type 2 Diabetic Patients in Kano, Nigeria" has been carried out by me in the Department of Human Physiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any Institution.

Nafisatu Wali Yusuf _____ _____

Signature Date

CERTIFICATION

This thesis entitled THE RELATIONSHIP BETWEEN LEPTIN AND ANTHROPOMETRIC PARAMETERS IN TYPE 2 DIABETIC PATIENTS IN KANO, NIGERIA by Nafisatu Wali YUSUF meets the regulations governing the award of the degree of Doctor of Philosophy in Human Physiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this thesis to my late father Prof. Yusuf Wali and to all Type 2 Diabetic patients

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With gratitude to Allah for his protection and guidance, I wish to express my sincere gratitude to my supervisors, Prof M.A Mabrouk, Prof. A.G. Bakari and Dr A. Mohammed for their guidance, supervision and encouragement. I am grateful to Dr A. Mohammed for his cooperation as head of department and also to the entire staff of Human Physiology department.

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ABSTRACT

Obesity, a state of hyperleptinemia and leptin resistance, may play a role in the aeitiopathogenesis of type 2 diabetes mellitus. Leptin hormone which was originally recognized for its role as a satiety factor has now been implicated in a wide variety of multisystem biological functions, including the regulation of glucose homeostasis. However, there is paucity of information regarding leptin and its effect on the West African population. This study was therefore aimed at studying the relationship between leptin, obesity and insulin levels in type 2 diabetes mellitus. One hundred and eighty- three diabetics (183) and 96 control non-diabetics were studied. Pulse, blood pressure and anthropometric parameters of all the respondents were measured. Fasting blood sugar (FBS) was analyzed using the glucose oxidase method and lipid profile was determined using enzymatic method. Serum leptin and serum insulin were also analysed using enzyme linked immunoassay (ELISA) method. Homeostasis Model Assessment was used to determine insulin resistance. Type 2 diabetes mellitus patients and non- diabetic controls that were normotensive with no disease complications or associated chronic illness were used for the study. The mean serum leptin levels for the diabetics and non diabetics was observed to be 4.57 ± 0.17 ng/ml and 4.70 ± 0.25ng/ml respectively ($p > 0.05$) and the mean insulin level for the diabetic and non diabetic groups are 4.70 ± 0.25 μU/mL and 4.49 ± 0.01 μU/mL respectively ($p < 0.05$). A positive correlation was observed between leptin and body mass index (BMI) in both type 2 diabetic patients ($r = 0.36$, $p < 0.05$) and non-diabetics ($r = 0.48$, $p < 0.05$). A positive correlation was also found between leptin and waist circumference (WC) in both type 2 diabetic patients ($r = 0.32$, $p < 0.05$) and non-diabetics ($r = 0.39$, $p < 0.05$). Hip circumference (HC) also correlated positively with leptin in both the diabetics ($r = 0.33$, $p <$

0.05) and non-diabetics. The study also found a positive correlation between leptin and FBS in the non-diabetic group ($r = 0.21$, $p < 0.05$) but a negative correlation in the diabetic group($r = -0.18$, $p < 0.05$). A correlation was also found between leptin and HOMA-IR in both the diabetic ($r = -0.018$, $p < 0.05$) and non-diabetics ($r = 0.20$, $p < 0.05$). However there was no significant relationship between leptin and insulin in both type 2 diabetic patients ($r = 0.11$, $p > 0.05$) and non-diabetics ($r = -0.06$, $p < 0.05$). Age, BMI and WC emerged as the independent predictors of leptin in the diabetic group while BMI, WC and HC emerged as independent predictors of leptin in the non-diabetics.

TABLE OF CONTENT

	Page
Cover page ……	i
Title Page.....	iii
Declaration	iv
Certification.....	v
Dedication	vi
Acknowledgement.....	vii
Abstract	viii
Table of contents	x
List of tables----------	xv
List of figures----------	xvii
List of appendices----------	xix
Abbreviations----------	xx
1.0 INTRODUCTION	**1**
1.1 Statement of research problem	**3**
1.2 Rationale/ justification	**4**
1.3 Missing link	**5**
1.4 Aim and objectives	**5**
1.4.1 Aim	5
1.4.2 Objectives	5
1.5 Research hypothesis	**6**

2.0 LITERATURE REVIEW 7

2.1 Blood glucose homeostasis 7

2.1.1 Blood glucose regulation 7

2.1.2 Insulin hormone and blood glucose homeostasis 10

2.1.3 Mechanism of action of insulin 15

2.1.4 Role of insulin in metabolism 15

2.1.5 Insulin resistance 16

2.1.6 Role of catabolic hormone in blood glucose homeostasis 17

2.1.6.1 Glucagon 17

2.1.6.2 Cortisol 17

2.1.6.3 Epinephrine 18

2.2 Adipocyte hormone leptin 18

2.2.1 Mechanism of action of leptin 22

2.2.2 Role of Leptin in blood glucose homeostasis 26

2.3 Obesity and type 2 diabetes mellitus 28

2.3.1 Pathophysiology of obesity 29

2.3.2 Role of adipose tissue in obesity 31

2.4 Pathogenesis of type 2 diabetes mellitus 33

2.5 Relationship between leptin, insulin and obesity in type 2 diabetes mellitus ... 34

3.0 MATERIALS AND METHOD..... 37

3.1 Materials..... 37

3.1.1 Instrument for data collection 37

3.2 Method..... 38

3.2.1 Study area---------- 38

3.2.2 Study population 40

3.2.3 Study design 40

3.2.4 Sample size estimation 40

3.2.5 Sampling method 41

3.2.6 Inclusion and exclusion criteria 41

3.2.7 Procedure for data collection..... 42

3.2.8 Ethical considerations 42

3.2.9 Measurement of vital signs 42

3.2.10 Anthropometric measurements..... 43

3.2.11 Laboratory procedures 44

3.2.12 Data analysis 47

3.2.13 Limitation..... 49

4.0 RESULTS..... 50

4.1 Socio-demographic characteristics of the respondents ……. 50

4.1.1 Age and sex distribution …… 50

4.1.2 Educational status …….. 52

4.1.3 Respondents' occupations …… 52

4.1.4 Respondents' ethnicity ……. 52

4.1.5 Smoking and alcohol status of the respondents …….. 56

4.1.6 Physical exercise of the respondents ……... 56

4.2 Physiologic and biochemical parameter of respondents …….. 60

4.2.1 Weight, height, body mass index, waist circumference, hip circumference and waist hip ratio of the respondents ……. …… 60

4.2.2 Mean values of pulse rate, systolic, diastolic and mean arterial pressure of the respondents …… ……. 64

4.3 Mean serum leptin, insulin and glucose levels of the respondents ……. 68

4.3.1 Serum leptin levels of the respondents ……. 68

4.3.2 Serum insulin levels of the respondents …… 80

4.3.3 Serum lipid levels of the respondents …… 87

4.4 Relationship between serum leptin, insulin, lipids, blood glucose and anthropometric parameters of the respondents ……. 94

4.5 Relationship between leptin, obesity and type 2 diabetes mellitus ……. 114

5.0 Discussions ……. 118

5.1 Weight, height, body mass index, waist circumference, hip circumference and waist hip ratio of the respondents ……. 118

5.2 Serum leptin, insulin and glucose levels of respondents …….. 120

5.2.1 Serum leptin levels of respondents …… 120

5.2.2 Serum insulin levels of respondents ……. ……. 122

5.2.3 Serum lipids levels of respondents ……. 128

5.3 Relationship between leptin, insulin, fasting blood glucose and anthropometric parameters of respondents …… 130

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS 135

6.1 Summary 136

6.2 Conclusion 137

6.3 Recommendations 138

REFERENCES ……139

APPENDICES …… 163

LIST OF TABLES

Table 4.I: Age and sex distribution of the subjects 51

Table 4.2: Weight (kg), height (m) body mass index, hip circumference (cm) waist circumference (cm) and waist hip ratio of the respondents 61

Table 4.3: Weight (kg), height (m) body mass index, hip circumference (cm) waist circumference (cm) and waist hip ratio in Male and Female non-diabetics 62

Table 4.4: Weight (kg), height (m) body mass index, hip circumference (cm) Waist circumference (cm) and waist hip ratio in male and female type 2 diabetic patients 63

Table 4.5: Mean values for pulse rate (min), systolic blood pressure (mmHg), diastolic blood pressure (mmHg) and mean arterial pressure (mmHg) in the respondents 65

Table 4.6: Mean values for pulse rate (min), systolic blood pressure (mmhg), diastolic blood pressure (mmhg) and mean arterial pressure (mmhg) non-diabetics and type 2 diabetic patients 66

Table 4.7: Mean values for pulse rate (min), systolic blood pressure (mmHg), diastolic blood pressure (mmHg) and mean arterial pressure (mmHg) in male and female type 2 diabetic subjects 67

Table 4.8: Mean serum leptin, insulin and glucose levels of the subjects 71

Table 4.9: Relationship between lifestyle factors and serum leptin (ng/ml) 72

Table 4.10: Mean serum leptin, insulin and glucose levels in male respondents 73

Table 4.11: Mean serum leptin, insulin and glucose levels in female respondents 74

Table 4.12: Mean serum leptin, insulin and glucose levels in male and female type 2 diabetic patients 75

Table 4.13: Leptin levels by age group in respondents 76

Table 4.14: Leptin levels by age group in type 2 diabetic patients males and females 77

Table 4.15: Leptin levels by age group in non-diabetics males and females 78

Table 4.16: Leptin levels by reproductive age group in female respondents 79

Table 4.17: Relationship between lifestyle factors and serum insulin (µiu/ml) 83

Table 4.18: Serum fasting insulin levels by age group in non-diabetics and type 2 diabetic patients 84

Table 4.19: Serum Insulin levels by age group in non-diabetics males and females 85

Table 4.20: Insulin levels by age group in type 2 diabetic patients 86

Table 4.21: Mean serum lipids of the subjects 90

Table 4.22: Lipid profile in male non-diabetics and type 2 diabetic patients 91

Table 4.23: Lipid profile of female respondents 92

Table 4.24: Lipid profile of male and female type 2 diabetic patients 93

Table 4.25: Relationship between leptin, anthropometric parameters, lipid profile, insulin and FBS among the respondents 110

Table 4.26: Relationship between insulin, anthropometric, lipids profile, FBS and leptin 111

Table 4.27: Relationship between HOMA- IR, anthropometric and other biochemical parameters 112

Table 4.28: Relationship between FBS, anthropometric and other biochemical parameters 113

Table 4.29: Serum Leptin levels (ng/ml) of obese and non- obese type 2 diabetic subjects 115

Table 4.30: Serum leptin levels of obese non-diabetics and type 2 diabetic patients by sex 116

Table 4.31: Relationship between obesity and type 2 diabetes mellitus 117

LIST OF FIGURES

Figure 1.1: Structure of leptin 1

Figure 2.1: Release of insulin 14

Figure 2.1: Map of Kano city 39

Figure 4.1: Educational status of respondents 53

Figure 4.2: Occupation of respondents 54

Figure 4.3: Respondents' ethnicity 55

Figure 4.4: Smoking status of respondents 57

Figure 4.5: Alcohol consumption status of respondents 58

Figure 4.6: Exercise status of the Respondents 59

Figure 4.7: Relationship between serum leptin and BMI among diabetic patients 97

Figure 4.8: Relationship between serum leptin and BMI among non diabetic subjects 98

Figure 4.9: Relationship between serum leptin and waist circumference among diabetic patients 99

Figure 4.10: Relationship between serum leptin and waist circumference among non diabetic subjects 100

Figure 4.11: Relationship between serum leptin and waist hip ratio among diabetic patients 101

Figure 4.12: Relationship between leptin and waist hip ratio among non diabetic subjects 102

Figure 4.13: Relationship between serum leptin and insulin among the diabetic patients 103

Figure 4.14: Relationship between serum leptin and insulin among non diabetic Subjects 104

Figure 4.15: Relationship between serum leptin and fasting blood sugar among the diabetic patients 105

Figure 16: Relationship between serum leptin and fasting blood sugar among non diabetic subjects 106

Figure 17: Relationship between serum leptin and HOMA-IR among the diabetic patients 107

Figure 18: Relationship between serum leptin and HOMA-IR among non diabetic subjects 108

LIST OF APPENDICES

Appendix 1: Questionnaire 163

Appendix 2: Informed consent 167

Appendix 3: Standard curve for leptin estimation 170

Appendix 4: Standard curve for insulin estimation 171

Appendix 5: Ethical clearance 172

Appendix 6: Reagents purchase invoice 173

ABBREVIATIONS

AgRP	Agouti Related Protein
ATP	Adenosine Triphosphate
BMI	Body Mass Index
Ca	Calcium
CHD	Coronary Heart Disease
CNS	Central Nervous System
CRH	Corticotropin Releasing Hormone
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
ELISA	Enzyme Linked Immuno Sorbent Assay
ER	Endoplasmic Reticulum
FATP	Fatty Acid Transporter Protein
FBS	Fasting Blood Sugar
FFA	Free Fatty Acid
GH	Growth Hormone
GIP	Gastric Inhibitory Peptide
GLUT	Glucose Transporter
HC	Hip Circumference
HDL	High Density Lipoprotein
HOMA IR	Homeostasis Model Assessment of Insulin Resistance
HRP	Horseradish Peroxidase
HT	Height

IL	Interleukin
IP	Inositol Triphosphate
IR	Insulin Resistance
IRS	Insulin Receptor Substrate
JAK	Janus Kinase
JNK	c-Jun Amino- terminal Kinase
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
MAP	Mean Arterial Pressure
MAPK	Mitogen Activated Protein Kinase
MCH	Melanin
MCP	Macrophage Chemo Attractant Protein
MMHg	Millimeter of Mercury
MODY	Maturity Onset Diabetes of the Young
mRNA	messenger Ribonucleic Acid
MSH	Melanocyte Stimulating Hormone
Na+K+ ATPase	Sodium Potassium Adenosine Triphosphate
NEFAS	Non- Esterified Fatty Acid
NO	Nitric Oxide
NYP	Neuropeptide – Y
POMC	Pro- Opio-Melanocortine
PTP	Protein Thyroxine Phosphotase
SBP	Systolic Blood Pressure

SOCS	Suppressors of Cytokine Signaling
STAT	Signal Transducers and Activators of Transcription
TC	Total Cholesterol
TG	Triglyceride
TMB	Tetramethylbenzidine
TNF	Tumour Necrosis Factor
TRH	Thyrotropin Releasing Hormone
VLDL	Very Low Density Lipoprotein
WC	Waist Circumference
WHC	Waist Hip Ratio
WHO	World Health Organisation
WT	Weight

CHAPTER ONE

INTRODUCTION

Leptin is a 167-amino-acid protein transcribed from the obese (ob) gene of mice and human subjects (Zhang *et al*., 1997; Brennan *et al*., 2006). The hormone is expressed and secreted from the adipose tissue of mice and humans, and the quantity secreted is highly correlated with body fat mass and adipocyte size (Maffei *et al*., 1995; Considine *et al*., 1996). Obese people have been shown to have higher leptin levels than normal weight in people (Maffei, 1995).

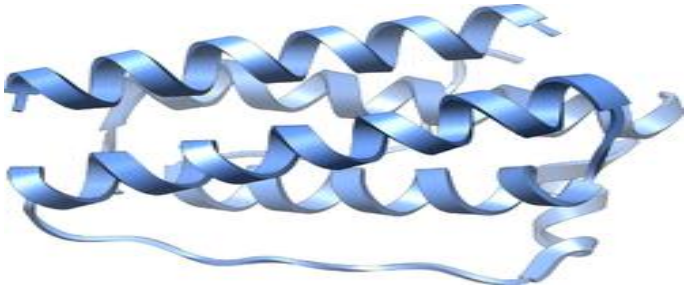


Figure 1.1: Structure of Leptin

Leptin hormone was initially thought to play a vital role only in the regulation of food intake, energy expenditure, and whole-body energy balance in rodents and humans (Janechova *et al*., 2001). Since the discovery that leptin replacement improves insulin sensitivity and diabetes in mice homozygous for mutations of the obesity gene (*ob* gene), it is now been implicated in the pathophysiology of obesity and is shown to play a significant role in insulin resistance related to obesity (Harris *et al*., 1996). Leptin is also shown to play a role in blood glucose homeostasis (Heather *et al*., 2012). It has also been reported to normalize blood glucose and insulin levels in *ob/ob* mice with type 2 diabetes mellitus (Pellymounter, *et al*., 1995). Leptin has roles in insulin secretion by the β cells of the pancreas, and in the regulation of insulin action and energy metabolism in adipose tissue and skeletal muscle (Houseknecht *et al*., 1998). Reports from several studies also

demonstrated that obese people with type 2 diabetes mellitus have high leptin levels (Mehmet *et al*., 2004; Mohti *et al*., 2005). Similarly, Ramazan *et al*., (2010) showed high leptin levels in obesity and metabolic syndrome, including type 2 diabetes mellitus.

Diabetes Mellitus results from insulin deficiency, or impaired insulin response resulting in excess glucose in the blood (Guyton and Hall, 2003a). Type 1 is an autoimmune disease that often starts early in life and involves near total destruction of the β cells over time. Eventually, little or no insulin is produced, leading to a complete dependence on exogenous insulin. On the other hand, type 2 diabetes mellitus is characterized by insulin resistance in insulin dependent tissues, which may lead to a compensatory increase in insulin production and release in the blood. Persistent high levels of insulin will result in β cell damage and hence little insulin formation (Bakari and Onyemelukwe, 2005) However both hyperinsulineamia and hypoinsulinemia have been reported among type 2 diabetes mellitus patients (Bakari and Onyemelukwe, 2002). Type 2 diabetes mellitus is the most common form of diabetes mellitus and account for approximately 90-95% of cases of diabetes. Type 2 diabetes mellitus patients are vulnerable to varied forms of both short and long term complications, which often lead to disability (Thévenod *et al*., 2008).

Obesity, especially visceral obesity is an established risk factor for type 2 diabetes mellitus (Haffner, 2006). Data emerging over the past several years reported a worldwide increase in the number of obese people (Must *et al*., 1999). The increasing prevalence of obesity has resulted in similar increase in the number of persons with type 2 diabetes Mellitus (Hu, 2011). There is currently a body of knowledge corroborating the fact that obesity is the major risk factor for insulin insensitivity in type 2 diabetes

mellitus (Khan and Flier, 2000; Petersen and Shulman, 2006). However, not all obese people have type 2 diabetes mellitus implying that other factors may also contribute to the pathology.

Anthropometry, including measurement of height (HT), weight (WT), body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) have served as simple, cheap and non-invasive markers of obesity (Adamu *et al*., 2013). Specifically, body mass index (BMI), a composite measure that compares weight and height, defines people as overweight (pre-obese) if their BMI is between 25 and 29.99 kg/m^2; and obese when it is up to 30 kg/m^2 (World Health Organization, 2014).

Physiologically, a combination of excessive food intake and inadequate physical activity explain most cases of obesity (Swinburn *et al*., 2004). However, the cause of obesity is multifactorial comprising of genetic, psychosocial and environmental factors (Nammi *et al*., 2004). However, the discovery of Leptin by Friedman and colleagues (1994) has lead to a breakthrough in the understanding of body weight regulation and the role of fat tissue as an endocrine organ (Marti *et al*., 1999).

1.1 Statement of the Research Problem

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2013)

International Diabetes Federation (2014) estimates about 387 million people living with diabetes worldwide with global diabetes prevalence of 8.3% and the number is projected to increase to 592 million by the year 2035. This global trend will occur because of population ageing and growth, obesity, unhealthy diets and a sedentary lifestyle. The number of adult population affected by diabetes in Africa is 22 million with prevalence of 5.1% and is projected to increase to 41.5 million by the year 2035. More importantly however, an estimated additional 13.5 million undiagnosed cases of diabetes mellitus and up to 480.9 thousand diabetes related deaths occurred in the African continent in the year 2014 (American Diabetes Association, 2014). Diabetes mellitus is one of the major threats to economic development confronting the African Region with total diabetes health expenditure of 4.5 billion USD in 2014 (International Diabetes Federation, 2014).

An increasing trend of diabetes mellitus and its complications had been observed in parts of Nigeria, from 0.5% in the 1960s to 1.4% and 1.7% in Kwara and Lagos states respectively in 1988; and to 1.6% in Kaduna state (Adadevoh, 1970, Ohwovoriole *et al*., 1988, Erasmus *et al*., 1989, Bakari *et al*., 1999). A study by Dahiru *et al*., (2008) reported a prevalence rate of 2% in Zaria, a city in North western Nigeria. A similar study by Nyenwe *et al*., (2003) from Port Harcourt, South-southern Nigeria, found a prevalence rate of 7.9%. In the same vein, Adeleke *et al*., (2010), observed a prevalence rate of 3.1 per 1000 in their review of childhood diabetes mellitus in Kano, Nigeria between 1999 and 2006.

1.2 Rationale/Justification

Diabetes Mellitus is a major cause of morbidity and mortality worldwide with high prevalence rate worldwide and in the developing countries including Nigeria (Mbanya *et*

al., 1996). Type 2 diabetic patients are commonly obese and may have insulin resistance and an increase in prevalence of obesity has resulted to increase in type 2 diabetic patients (Bakari *et al.*, 2007). Studies have shown that obese diabetics have higher levels of leptin (Misra *et al*., 2001; Adil, 2010) indicating that the problem may not be leptin deficiency but its resistance. However, there is paucity of information regarding leptin and its effect on the West African population. This study was therefore aimed at studying the relationship between leptin, obesity and insulin levels in type 2 diabetes mellitus patients. It is also hoped that the findings will be useful to physicians for improved management of type 2 diabetes mellitus.

1.3 Missing Link/Data

Data to show the relationship between plasma Leptin, Obesity and type 2 Diabetes Mellitus is scarce in the West African population more especially in Kano, North-western Nigeria where the study took place.

1.4 Aim and Objectives

1.4.1 Aim

To determine the relationship between plasma Leptin, Glucose, Insulin and Anthropometric parameters (waist circumference, hip circumference, waist to hip ratio, height, weight, BMI) in known type 2 diabetic patients.

1.4.2 Objectives

i. To assess the height, weight, waist circumference, hip circumference, waist-hip ratio and body mass index (BMI) of known type 2 diabetes mellitus patients and their matched non diabetic subjects.

ii. To estimate serum leptin, glucose, insulin and lipid levels of type 2 diabetic mellitus patients and non- diabetic subjects.

iii. To determine the relationship between serum leptin, obesity and type 2 diabetes mellitus.

iv. To determine the relationship between levels of serum insulin, glucose, leptin, lipid profile, insulin resistance and anthropometric measurements (height, weight, body mass index, waist circumference, hip circumference and waist-hip ratio).

1.5 Research Hypothesis

The level of serum leptin and obesity are related to type 2 diabetes mellitus.

CHAPTER TWO

LITERATURE REVIEW

2.1 BLOOD GLUCOSE HOMEOSTASIS

2.1.1 Blood Glucose Regulation

Glucose is the primary source of energy available for the various physiologic processes of the cell. Energy is required for muscle activity, secretion by the glands, maintenance of membrane potentials, synthesis of substances in the cells, absorption of foods from the gastrointestinal tract, and many other functions (Guyton and Hall, 2003a). It is found in the blood of animals and humans and maintained within a narrow range by two major pancreatic hormones insulin and glucagon in a process known as blood glucose homeostasis. Glucose is highest in the blood during the absorptive period and lowest during the post absorptive period when the stomach and intestines are empty (Ralph and DeFronzo, 2004).

Glucose is a hexose sugar which naturally exists in its monosaccharide form in some citrus fruits, and in the disaccharide form maltose, lactose and sucrose, and in the polysaccharide form starch. It is a simple monosaccharide composed of carbon, hydrogen and oxygen and has the general formula $C_6 H_{12} O_6$. After final digestion of monosaccharide (glucose, fructose and galactose), glucose and galactose are absorbed into the epithelial cells of the small intestine by Na+ dependent co-transport while fructose is absorbed by facilitated diffusion attached to a carrier protein. Glucose and galactose are absorbed across the apical membrane by secondary active transport mechanisms on the Na^+-glucose co-transporter (SGLT 1), against an electrochemical

gradient. The energy for this step does not come directly from adenosine triphosphate (ATP) but from the Na^+ gradient across the apical membrane which is created and maintained by the Na^+-K^+ ATPase on the basolateral membrane. They are both transported from the cell into the blood, across the basolateral membrane, by facilitated diffusion attached to a protein carrier glucose transporter 2 (GLUT 2) while fructose is transported across both apical and basolateral membranes by facilitated diffusion attached to glucose transporter 5 (GLUT 5) and glucose transporter 2 (GLUT 2) respectively (Linda, 2007). However, significant amount of fructose is converted into glucose on its way through the enterocyte and finally transported in the form of glucose (Guyton and Hall, 2003a).

Glucose is stored endogenously in the liver through the processes of gluconeogenesis and glycogenolysis when there is reduction in blood glucose levels. Production of glucose in the liver from non-sugar molecules such as amino acids, lactate, and glycerol is known as gluconeogenesis while glycogenolysis is the breakdown of glycogen to release glucose molecules. Although fat tissue is responsible for only a small amount of total body glucose disposal, it plays a very important role in the maintenance of total body glucose homeostasis (Groop, 1989).

After digestion and absorption of glucose, about one third is taken up by the liver and the rest by peripheral tissues, primarily skeletal muscle through an insulin dependent mechanism. The postprandial hyperglycemia stimulates insulin secretion from the pancreas and the rise in plasma insulin concentration stimulates glucose uptake in skeletal muscle leading to the disposal of ingested glucose (DeFronzo, 1997). Skeletal muscle utilizes both glucose and free fatty acid (FFA) as sources for energy production.

During the fasting state, muscle glucose uptake is low and the plasma FFA concentration is elevated because of increased lipolysis. Thus, under fasting conditions, FFA serves as the principal fuel source for energy production in skeletal muscle, while the brain exclusively utilizes glucose (Fernandez and Valdeolmillos, 1998).

The mean normal fasting blood glucose level in humans is about 4 mmol/L or 72 mg/dL, and ranges between 70 to 110mg/dl. The level fluctuates throughout the day. Glucose level is usually lowest in the morning, before the first meal of the day that is the fasting level, and rise after meals for an hour or two by a few mmol/L. The blood glucose level is maintained within a narrow standard level and the levels are dependent on the balance between glucose output that is, utilized for energy and input from dietary absorption and from the liver. Glucose storage is predominantly in the liver in form of glycogen. Reduction in blood levels of glucose leads to release of glucose from the liver through the processes of glycogenolysis and gluconeogenesis (DeFronzo and Ferrannini, 1987).

In the fasting state, glucose leaves the circulation at a constant rate. To keep pace with glucose disappearance, endogenous glucose production is necessary. Under normal circumstances, the sole source of endogenous glucose production is the liver. Renal gluconeogenesis contributes substantially to the systemic glucose pool only during periods of extreme starvation. Although most tissues have the ability to hydrolyze glycogen, only the liver and kidneys contain glucose-6-phosphatase, the enzyme necessary for the release of glucose into the circulation. After reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels. In the immediate post-feeding state, glucose removal into skeletal muscle and adipose tissue is driven mainly by insulin. At the same time, endogenous glucose

production is suppressed by the direct action of insulin, delivered via the portal vein, on the liver and the paracrine effect or direct communication within the pancreas between the α- and β-cells, which results in glucagon suppression (Stephens *et al*., 2004).

Glucose homeostasis reflects a precise balance between hepatic glucose production and peripheral glucose uptake and utilization. Insulin is the most important regulator of this metabolic equilibrium, but neural input, metabolic signals, and catabolic hormones like glucagon, growth hormone, cortisol and epinephrine result in integrated control of glucose supply and utilization. . Although several interacting factors are involved, hormone regulation is most important in keeping the plasma glucose within a normal range (Baron *et al*., 1987).

Two types of mutually antagonistic metabolic hormones; catabolic and anabolic are involved in keeping blood glucose within the normal range. Catabolic hormones such as glucagon, growth hormone, cortisol and catecholamines increases blood glucose levels while insulin which is an anabolic hormone decreases blood glucose levels. In the bi-hormonal model of glucose homeostasis, insulin is the key regulatory hormone of glucose disappearance, and glucagon is a major regulator of glucose appearance (Baron *et al*., 1987).

2.1.2 Insulin and Blood Glucose Homeostasis

Insulin is a hormone that changed the lives of diabetic patients from that of rapid decline and death to that of nearly normal persons. It is a protein hormone discovered by Banting and Best in 1922 that has profound effect on carbohydrate, protein and fat metabolism (Guyton and Hall, 2003b).

Insulin is a peptide hormone composed of 51 amino acids and has a molecular weight of 5808 Da. It is produced in the islets of Langerhans in the pancreas. Insulin's structure and metabolic control effect varies slightly between species of animal. The Porcine insulin is closer to and differs from the human insulin with only one amino acid residue. Insulin has a plasma half-life of 3 to 5 minutes, and primarily degraded by the liver and kidneys (Faber *et al*., 1978).

Insulin is synthesized in the beta cells of pancreas in the form of preproinsulin which is the ultimate precursor. The gene is located on chromosome 11 (Bliss, 1982). Preproinsulin after synthesis is discharged into cisternal space of rough endoplasmic reticulum where it is cleaved into proinsulin by proteolytic enzymes. Proinsulin with a C (connecting) chain linking A and B chains is then transported by microvesicles to the Golgi apparatus where it is converted into insulin and then stored in the secretory vesicles of the beta cells (Hutton, 1994).

When Glucose enters the beta cells through the glucose transporter GLUT2, multiple high-energy ATP molecules are produced by oxidation. Dependent on ATP levels, and hence blood glucose levels, the ATP-controlled potassium channels (K^+) closed and the cell membrane depolarizes. On depolarization, voltage controlled calcium channels (Ca^{2+}) open and calcium flows into the cells. An increased calcium level causes activation of phospholipase C, which cleaves the membrane phospholipid phosphatidyl inositol 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol. Inositol 1,4,5-triphosphate (IP3) binds to receptor proteins in the membrane of endoplasmic reticulum (ER). This allows the release of Ca^{2+} from the ER through IP3 gated channels, and further raises the cell concentration of calcium which causes the release of

previously synthesized insulin, which has been stored in secretory vesicles (Despopoulos and Stafen, 2003).

Insulin release is mainly initiated by glucose, as a signal of high carbohydrate intake. An increase in the plasma concentration of glucose is the most important physiological regulator of insulin secretion. However protein diet and autonomic nervous stimulus also stimulates the release in a mechanism yet to be understood (Shashank *et al*., 2007).

Insulin secretion in response to glucose is biphasic. The response is characterized by an immediate, or first-phase, that begins within 1 minute and lasts for 5-10 minutes followed by a more gradual, prolonged second phase that terminates soon after the glucose stimulus is removed. These two phases likely reflect the different intracellular pools of insulin one which is at the plasma membrane surface and the other that is distant from the membrane. Studies have shown insulin secretion to have a circadian rhythm. Boden *et al*., 1996, in their study concluded that normal human subjects have a circadian rhythm of insulin secretion, which becomes more apparent with rising insulin sensitive receptors, and that circadian changes in insulin sensitive receptors, rising during the day and falling during the night, may be one explanation for the well-established observation that glucose tolerance and insulin responses to glucose meals are higher in the morning than at night. Other substances known to stimulate insulin release include amino acids from ingested proteins, acetylcholine released from vagus nerve endings (parasympathetic nervous system), gastrointestinal hormones released by enteroendocrine cells of intestinal mucosa and glucose-dependent insulinotropic peptide(GIP). Three amino acids (alanine, glycine and arginine) act similarly to glucose by altering the beta cell's membrane potential (Shashank *et al*., 2007). Insulin secretion is inhibited by hypoglycemia (reduced plasma glucose) and somatostatin, a hormone also

secreted by the pancreas (Tirone *et al*., 2003). Insulin level varies with age, sex, race and location of individuals.

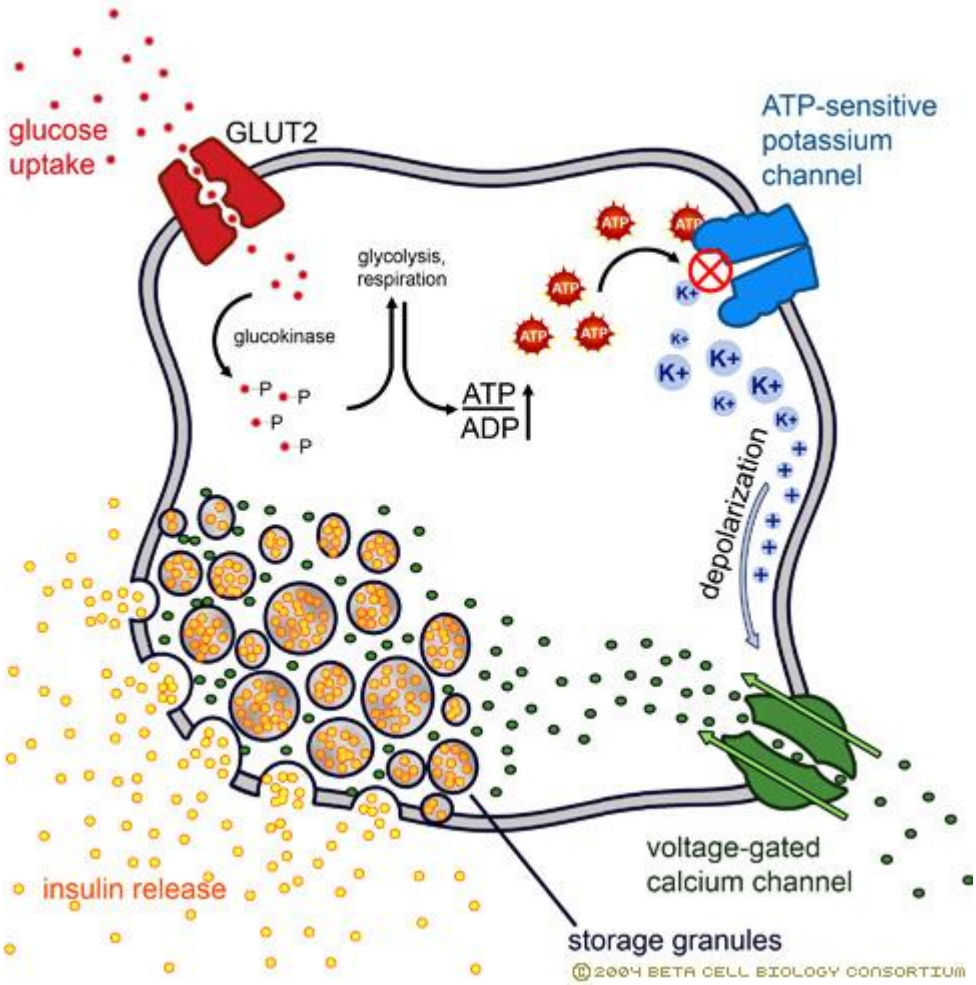


Figure 2.1: Release of insulin

Source: Beta cell biology consortium

2.1.3 Mechanism of Action of Insulin

The insulin receptor (IR) present on the cell membrane is an integral membrane protein that exists as a dimer of two identical subunits that spans the cell membrane. Each of the two subunits is made of one α-chain and one β-chain, connected together by a single disulfide bond. The α-chain lies on the exterior of the cell membrane, while the β-chain spans the cell membrane in a single segment. Although each α-subunit contains a binding site for insulin, binding of one insulin molecule decreases affinity for binding of a second molecule. This reduced binding for the second insulin molecule is termed negative cooperativity. The β-subunits traverse the membrane, having tyrosine kinase enzyme domains on the cytoplasmic side. Insulin binding to the α-chains transmits a signal through the transmembrane domain of the β-chains to activate the tyrosine kinase activity (Sasaoka *et al*., 1993).The protein kinase signal systems include: The pathways signaling through Phosphoinositol 3-kinase and phosphatidylinositol PI-3 kinase and protein kinase B/Akt), Mitogen-activated protein kinases (MAPKinases), Possible interaction via kinases not coupled to IRS proteins. Activation of the tyrosine kinase leads to phosphorylation of several cytoplasmic proteins that can carry the insulin signal into the interior of the cell.

2.1.4 The Role of Insulin in Metabolism

Insulin plays a vital role on carbohydrate, lipid and protein metabolism and on growth and development. The liver, adipose tissue and the muscle are the target organs for the anabolic action of insulin. In the liver, insulin promotes glycogen synthesis by stimulating glycogen synthase and inhibiting glycogen phosphorylase regulating the rate of glucose release from the liver. In contrast, insulin induces a rapid uptake of glucose in muscle and fat tissue by recruiting intracellular GLUT 4 transporters and, thus,

increasing their cell-surface expression. As a consequence, muscle converts glucose to glycogen. In adipose tissue, glucose is converted to fatty acids for storage as triglyceride. Insulin is a potent antilipolytic hormone, and even small increments in the plasma insulin concentration markedly inhibit lipolysis, leading to a decline in the plasma level of free fatty acid (Groop, 1989). The decline in plasma FFA concentration augments muscle glucose uptake and contributes to the inhibition of hepatic glucose production. Thus, changes in the plasma FFA concentration in response to increased plasma levels of insulin and glucose play an important role in the maintenance of normal glucose homeostasis (Raph *et al*., 2004).

On protein metabolism insulin also has effect on lowering blood glucose. Insulin stimulates tissue amino acid uptake, inhibit protein degradation and regulates gene expression. The net effect of insulin is lowering of blood glucose to normal levels Absolute or relative absence will therefore result in excess glucose in the blood a situation known as hyperglycemia (Campbell *et al*., 1993).

2.1.5 Insulin Resistance

Insulin resistance is a recognized central feature of metabolic syndrome and strongly associated with increased risk of type 2 diabetes mellitus and cardiovascular disease (Anwar *et al*., 2007). In insulin resistant people, their muscle, fat, and liver cells do not respond properly to insulin. As a result, their bodies need more insulin to help glucose enter cells. The pancreas tries to keep up with this increased demand for insulin by producing more. Eventually, the pancreas fails to keep up with the body's need for insulin. Excess glucose builds up in the bloodstream, setting the stage for diabetes. Many

people with insulin resistance have high levels of both glucose and insulin circulating in their blood at the same time (Osei *et al*., 1993).

2.1.6 Role of Catabolic Hormones on Glucose Blood Homeostasis

Catabolic hormones are hormones that raise blood glucose to maintain glucose within normal levels by enhancing gluconeogenesis and glycogenolysis. The effects of these hormones are opposite to that of insulin. The most important catabolic hormones are glucagon, cortisol and epinephrine (King, 2006).

2.1.6.1 Glucagon

Glucagon is a catabolic hormone released from α (alpha) cells of the pancreas. It is a peptide hormone with 29 amino acids derived from proglucagon (glicentin). Synthesis is stimulated by hypoglyceamia as a result of prolonged fasting, prolonged physical exercise and sympathetic impulse and its secretion is inhibited by glucose and somatostatin as well as by high plasma concentrations of free fatty acids. The action of glucagon is mainly to antagonize that of insulin. Glucagon maintains a normal blood glucose level between meals and during phases of increased glucose consumption to ensure a constant energy supply by increasing glycogenolysis in the liver but not in the muscle and by stimulating gluconeogenesis from lactate, amino acids and glycerol from lipolysis (King, 2006).

2.1.6.2 Cortisol

Cortisol is a steroid hormone synthesized by the adrenal cortex. It is involved in regulating protein and carbohydrate metabolism by promoting protein degradation and conversion of amino acids into glucose. As a result, the blood glucose level rises. Cortisol, under the normal basal concentration enhances stimulation of gluconeogenesis and lipolysis in the post- absorptive phase. Increase plasma concentration causes

increase protein catabolism, increased gluconeogenesis, decreased glucose uptake by muscle cells and adipose tissue cells and increased triacylglycerol breakdown with net result of increased plasma concentration of amino acids, glucose and fatty acids (Khani and Tayeb, 2001).

2.1.6.3 Epinephrine

Also known as adrenaline, it is secreted by the adrenal medulla in response to stimulation of the sympathetic nervous system at times of stress. It is a catabolic hormone which raises blood glucose by gluconeogenesis at the time of stress (King, 2006).

2.1.6.4 Growth hormone

Growth hormone (GH or HGH), also known as somatotropin or somatropin, is a peptide hormone that stimulates growth, cell reproduction and regeneration in humans and other animals. It is a type of mitogen which is specific only to certain kinds of cells. Growth hormone is a 191-amino acid, single-chain polypeptide that is synthesized, stored, and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland. GH is a stress hormone that raises the concentration of glucose and free fatty acids in the blood. The hormone also reduces glucose uptake by the liver and promotes gluconeogenesis thereby increasing blood glucose levels (King, 2006).

2.2 ADIPOCYTE HORMONE LEPTIN

The regulation of fat stores and blood glucose level is very critical for survival of humans. Apart from regulation of glucose by the major pancreatic hormones, central regulation of glucose is also vital. Food intake, energy expenditure and endogenous glucose production are regulated centrally by hypothalamic areas of the brain in response to various peripheral stimuli as suggested by various researches. The brain

(hypothalamic area) initiates responses in order to promote positive energy balance when there is reduction in energy stores and negative energy balance when there are excess energy stores to maintain glucose homeostasis (Friedman and Halaas, 1998, Carol *et al*., 2009). Leptin, a protein hormone, discovered in 1994 was found to be responsible for initiating these responses. The hormone has been shown to be involved in the regulation of the function of the pituitary by regulating food intake and energy balance (Janeckova, 2001; Akther *et al*., 2009). On the Hypothalamic- Pituitary- Thyroidal axis, leptin influences the thyroidal axis by regulating the expression of thyrotropin-releasing hormone (TRH) (Flier *et al*., 2000; Sanchez *et al*., 2004). Similarly, on the Pituitary-Hypothalamic-Adrenal axis, leptin causes a dose-dependent stimulation of CRH release in vitro (Malendowicz *et al*., 2007). Leptin has been shown to stimulate LH secretion in rodents both in vitro and in vivo, deficiency which causes infertility (Ogura *et al*., 2001). Leptin has also been shown to play a role in maintenance of blood glucose homeostasis (Heather *et al*., 2012).

The circulating hormone is released in proportion to body fat mass (Luke *et al*., 1998) and act in the brain to maintain energy balance (Collins *et al*., 1996; Morton, 2011). It is considered as one of the best biological markers reflecting total body fat in both animals and humans (Zhang *et al*., 2005).

Leptin an adipocyte hormone was first identified as the product of a gene designated OB (obese) in laboratory mice. Mice with two defective copies of this gene that is ob/ob genotype show the behavior and physiology of animals in a constant state of starvation (elevated serum cortisol levels, inability to stay warm, abnormal growth, reproduction and exhibition of unrestrained appetite). As a consequence of increased appetite they

become severely obese weighing as much as three times more than normal mice. These mice also have metabolic disturbances and insulin resistance similar to those of diabetic animals. When leptin was injected to these ob/ob mice, they lost weight and increased their locomotor activity and thermogenesis (Caro *et al*., 1996). Exogenous administration of leptin in this ob/ob mice also effectively reduces hyperphagia and obesity (Halaas *et al*., 1995). A second mouse gene, designated DB (diabetic), has also been found to have a role in appetite regulation. Mice with two defective copies (db/db) are also obese and diabetic. The DB gene encodes the leptin receptor**.** When the leptin receptor is defective, the signaling function of leptin is lost Exogenous administration of leptin to this mice that are deficient in the signaling form of the leptin receptor (db/db) do not respond to leptin (Chen *et al*., 1996).

The release of leptin into the circulation is pulsatile in nature and its concentration follows a circadian rhythm (Schea *et al*., 2005). It is affected by sleep patterns (Mullington *et al*., 2009) and display highest levels between midnight and early morning and lowest levels in the early to mid-afternoon (Bluher and Moutzorous, 2009). The pulsatile nature of leptin's secretion pattern is similar in obese and lean individuals (Shea *et al*., 2005) but the obese have higher pulse amplitudes (Licinio *et al*., 1997) and overall greater leptin concentrations than lean subjects due to their greater amount of body fat (Bluher and Mountzorous, 2007). Women have been reported to have higher leptin concentration in than men for the same age and BMI (Kennedy *et al.,* 1997). Similarly in another study by Luke *et al*., (2008) leptin concentrations in women may be higher in the luteal phase of the menstrual cycle (Bluher and Mantzorousl, 2004), whereas menopause is associated with a decline in circulating leptin (Kiess *et al*., 2008).These observations suggest that sex differences in leptin concentration are likely as a result of differences in

sex hormones, e.g., estrogen and testosterone, besides those in body fat mass and/or distribution (Chan *et al*., 2002). However, Niels *et al*., (1998) in their study, "Disruption of the Relationship between Fat Content and Leptin Levels with Aging in Humans", concluded that unlike in young people, there was no correlation between body adiposity and leptin levels in the elderly. However in morbidly obese individuals, leptin concentration does not correlate with fat body mass (Garcia-Lorde *et al*., 2001).

Leptin is a protein hormone with 167 amino acid that is mainly expressed in white adipose tissue (Zhang *et al*., 1994). The hormone is seen in small quantities in skeletal muscle (Wang *et al*., 1997), gastric epithelium (Bado *et al*., 1998), placenta (Masuzaki *et al*., 1998) and mammary gland (Margetti *et al*., 2002). The proportion of this hormone in the blood is directly proportional to the amount of adipose tissue (Maffei *et al*., 1995). After synthesis, the hormone circulates in the blood bound to some proteins (Hale *et al*., 1996; Houseknecht *et al*., 1996), crosses the blood brain barrier into the central nervous system acting at the level of hypothalamus by binding to its receptors (Golden *et al*., 1997). Leptin production by white adipose tissue is stimulated by insulin and cortisol and attenuated by β-adrenergic agonists, cAMP, and thiazolidinediones. There is no cut off values for leptin because the adipocyte hormone varies with age, sex, race and body mass index of individuals. However, Pilon, 2011 suggests that in healthy individuals, the baseline leptin levels are between 1 ng/ml and 5ng/ml in men and 7ng/ml and 13ng/ml in women. Chow and Phoon (2003), in their study "Measurement Of Serum Leptin Concentrations In University Undergraduates by Competitive Elisa" reveals correlations with Body Mass Index and Sex, found leptin levels between 0.2 ng/ml and 71.5 ng/ml being the lowest and highest values respectively. They also observed that mean serum leptin concentrations were more than twice as high in females as in males of

corresponding BMI categories, especially in the healthy-weight category where females exhibited levels that were 5.7 times higher.

2.2.1 Mechanism of action of Leptin

Leptin receptors (ObR) are present in the central nervous system (hypothalamus), liver, muscle and adipose tissue (Fei *et al*., 1997) and pancreas (Kieffer, *et al*.,. 1997). There are six leptin receptor isoforms identified: ObRa, ObRb, ObRc, ObRd, ObRe, and ObRf (Lee *et al*., 1996). These receptor isoforms have homologous domains, but, due to alternative mRNA splicing, each receptor has a unique sequence and length. The short isoforms ObRa and ObRc are thought to have important roles in transporting leptin across the blood-brain barrier (Bjorbaek *et al*., 1998a) but not much is known about biology of the other short membrane-bound isoforms also present in the brain(Hileman *et al*.,., 2002). The long isoform ObRb is ubiquitously expressed the central nervous system (hypothalamus) and is primarily responsible for leptin signaling and for the regulation of energy balance and neuroendocrine functions (Tartaglia *et al*., 1997). The receptor is particularly important in the hypothalamus (Lee *et al*., 1996; Ghilard *et al*., 1996). Absence of ObRb has been demonstrated to cause morbid obesity in db/db mice (Chen *et al*., 1996). Following leptin binding to Ob-Rb, Janus kinase 2 (JAK2) is activated and both proteins are subsequently phosphorylated. As a result, numerous other pathways are activated. Pathways activated by JAKs include insulin receptor substrate (IRS) proteins (Ihle, 1995), phosphoinositide 3-kinase (PI 3-kinase), which is a common target downstream of IRS proteins (Myers and White, 1996), the STAT (signal transducers and activators of transcription) and MAPK (mitogen activated protein kinase) pathways(Ihle, 1995).

Binding of leptin to LRb in the CNS, results in autophosphorylation of JAK1 and 2, and phosphorylation and activation of STAT3. These result in regulation of expression of the neuropeptides important in signal transmission (Leshan, *et al*., 2006). In the pancreatic β cell, leptin acts via phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), c-Jun amino-terminal kinases (JNK) and nitric oxide (NO) pathways (Lee *et al*,. 2011).

Leptin controls specific neuronal groups within the arcuate (ARC) nucleus in the hypothalamus which controls food intake and metabolism (Schwartz and Porte, 2005). The blood level of NPY rises during starvation, and is elevated in both ob/ob and db/db mice. Leptin and insulin suppress feeding by inhibiting the Orexigenic neurons or the appetite stimulating neurons (Cone *et al*., 2005). With disruption of the inhibitory leptin signals due to gene mutations, the neurons become overactive, which contributes to hyperphagia and obesity in the ob/ob and db/db mice and fa/fa Zucker rat. These neurons therefore become overactive when levels of insulin and leptin falls (Williams *et al*., 2001).

The anorexigenic or the appetite-suppressing neurons also present in the arcuate nucleus and produce melanocyte-stimulating hormone (MSH) from its polypeptide precursor pro-opiomelanocortin (POMC). Release of MSH causes the next neuron in the circuit to send the signal to the brain, to stop eating. Leptin stimulates the release of MSH and therefore enhances decrease in appetite (Cone *et al*., 2005). High proportions of the POMC/CART and NPY/AgRP cells in the hypothalamus express ObRb and are therefore targets for leptin action (Hakansson *et al*., 1996; Cheung *et al*., 1997).

Studies have shown that ablation of the arcuate nucleus and LRb disrupts the negative feedback action of leptin and recapitulates the obese phenotype of db/db mice respectively and expression of LRb in the arcuate nucleus reverses obesity in Koletsky rats lacking all membrane-bound forms of LRb (Cohen *et al*., 2001; Morton *et al*., 2003). Ablation of NPY or MCH in db/db mice partially reverses hyperphagia, thermoregulatory defect, obesity, and hormonal abnormalities (Erickson *et al*., 1996; Segal-lieberma, 2003).

Leptin signaling is subject to negative feedback regulation and associated hyperinsulineamia (Oswal , 2009). Myers *et al*., (2010) in their study observed that reduced leptin signaling is related to increased levels or activity of SOCS3 (suppressor of cytokine signaling 3), PTP1B (protein tyrosine phosphatase 1B) and/or TCPTP (T-cell protein tyrosine phosphatase). Both proteins work as negative regulators of leptin-induced activation of the JAK/STAT endoplasmic pathway (Morris and Rui, 2009). SOCS3 acts by inhibit the phosphorylation and activation of JAK2 and ObRb tyrosine residue Tyr^{985} (Myers *et al*., 2008) and administration of leptin have been shown to increase STAT3 expression and greater weight loss in SOCS3 knockout mice (Moris *et al*., 2004). Similarly, PTP1B knockout mice have increased leptin sensitivity and reduced fatness (Oswal, 2009). Hybridization analysis showed that SOCS-3 mRNA levels were strongly elevated in the hypothalamus of A^y/a mice, a genetic model of leptin-resistant murine obesity (Bjorbaek *et al*., 1998b).

Reduced leptin signaling has also been observed to present at the peripheral tissues (Lam, *et al*., 2006). In the pancreatic β cell it has been proposed that leptin resistance

could contribute to diabetes mellitus in the obese since the function is altered (Seufert, 2004; Moriaka *et al*., 2007).

Although leptin is a regulator of food intake, energy balance and weight as documented, Obese people are shown to have more leptin concentration than lean individuals and are resistant or tolerant to the effect of leptin (Moon *et al*., 2011). Recent studies have shown most obese individuals to have high leptin levels (Considine *et al*., 1996; Dagogo *et al*., 1996; Dua *et al*., 1996; Have *et al*., 1996). This apparent leptin resistance defect, is as a result of defect in leptin signaling pathways. Defects in leptin transport across the blood brain barrier, defects in signal transduction involving reduced LRb expression or induction of feedback inhibitors resulting in leptin resistance (Morris and Rui 2009; Zhang, *et al*.,. 2008). Disruption of neuronal interaction has also been documented to have a reduced leptin action (Munzberg, 2010). Resistance could also occur as a result of failure of leptin to activate the leptin receptor as observed in obese rodent models with mutations of the leptin receptor gene (db/db mice and fa/fa Zucker rats) and produces complete leptin resistance.

Endoplasmic reticulum stress has recently been shown to play a role in the development of leptin resistance. Obese diabetic animals and humans have been shown to have higher levels of reticulum stress in the liver, adipose tissue, and pancreatic β-cells (Ozcan *et al*., 2004; Ozcan *et al*., 2009). Studies in obese mice have shown that increased endoplasmic reticulum stress inhibits leptin receptor signaling in the hypothalamus, and chemical chaperones that decrease endoplasmic reticulum stress have been shown to increase leptin sensitivity. Thus, the negative effect of endoplasmic reticulum stress on leptin sensitivity may also contribute to insulin resistance.

Production of an antagonist of melanocortin receptors, can also lead to leptin resistance both centrally and peripherally suggesting defect in leptin receptor (Halaas *et al*., 1997). An additional possibility is that the leptin signal to the hypothalamus can be overcome by the hedonic (pleasure) qualities of highly palatable foods. This idea is supported by a study in rats with diet-induced obesity in which central leptin administration reduced the intake of a standard rodent diet but not that of a more palatable high-energy diet. This is an important consideration to the extent that consumption of a highly palatable, high-fat diet contributes to obesity in humans (Widdowson, 1997).

Mutations involving leptin protein or leptin receptor is very rare and only two mutations have been documented so far. These are mutation in the coding sequence of the leptin gene in two members of a family in the United Kingdom and a mutation of the leptin-receptor gene in members of a family in France (Karine *et al*., 1998).

2.2.2 Role of leptin in blood glucose homeostasis

Blood glucose homeostasis which is the regulation of glucose in the blood within a normal range is critical for survival. Leptin, an adipocyte hormone known to play a role in the regulation of food intake, energy expenditure and body weight (Fruhbeck 2006) has also been demonstrated to have a vital role in blood glucose homeostasis (Pelleymounter *et al*., 1995; Muzzin *et al*., 1996; Schwartz *et al*., 1996; Farooqi *et al*., 1999; Yu *et al*., 2008) through both central and peripheral mechanisms. Studies have shown that animal models with defects in leptin (ob/ob mice) or in leptin receptors (db/db mice) develop insulin resistance, hyperinsulineamia and impaired glucose homeostasis (Dubuc 1976; Genuth, *et al*., 1971). Administration of leptin to the ob/ob mice reduces plasma glucose and hyperinsulineamia independent of any reduction in

body weight (Pelleymounter, *et al*., 1995; Schwartz, *et al*., 1996). In people with congenital or acquired lipodystrophy, leptin administration improves plasma glucose and insulin levels (Shimomura, *et al*., 1999). Central Leptin action on glucose homeostasis is dependent on signal transduction via phosphatidylinositol-3-kinase (PI3K) activity (Hill *et al*., 2009). German *et al*., (2010) concluded that leptin may exert its anti diabetic effect peripherally by enhancing insulin signaling in the liver. However Gelling *et al*., (2004) in their study demonstrated that insulin signaling in the brain plays a major role in maintaining peripheral glucose homeostasis.

The brain continuously transduces input from neural, hormonal, and nutrient-related signals into responses that maintain both energy and glucose homeostasis by adjustments to output systems that control food intake, energy expenditure, hepatic insulin sensitivity, and glucose uptake (Gregory and Michael, 2011). Leptin regulation of glucose homeostasis at the peripheral level has also been demonstrated. Leptin has been shown to work together with insulin in maintaining normal glucose homeostasis. Researchers who genetically engineered mice to lack both leptin and insulin receptors in their POMC neurons but both receptors remained intact in all other cell types and tissues found out that when they removed both receptors from these particular neurons the mice displayed systemic insulin resistance and became severely diabetic but not obese. They also found out that deleting only the insulin receptor has no noticeable impact on body weight or blood sugar regulation. The findings of this study however suggests that leptin and insulin when acting on these neurons in the brain are both necessary and can compensate for each other if there's a shortage of one.

A study by Ceddia and William, 1998, on incubated soleous muscle of white albino rats, shows leptin to have an insulin-like effect stimulating glucose uptake, glycogen synthesis, and lactate formation and also seems to potentiate the effect of insulin on glucose incorporation into glycogen in the soleus muscle. It has also been shown that leptin enhances systemic insulin sensitivity and whole body glucose utilization in the rat muscle (Chritiane *et al*., 2010). Several other studies have shown reversal of hyperglycemia with leptin replacement by improving post prandial hyperglycemia. Similarly it has been demonstrated that leptin can improve glucose metabolism independent of its effects on energy homeostasis (German *et al*., 2010; Hedbacker *et al*., 2010). However, Fürnsinn *et al*., 1998, in their findings showed no physiological role of direct insulin-mimetic or insulin-desensitizing effects of leptin on skeletal muscle tissue.

2.3 OBESITY AND TYPE 2 DIABETES MELLITUS

Obesity, especially visceral obesity is a life-threatening condition that is significantly associated with increase in chances of developing type 2 diabetes as well as cardiovascular disease (Pearson *et al*., 2012) and cancers of the colon (Katherine *et al*., 2006) , breast (Rose *et al*., 2002), prostate (Kristal *et al*., 2007), and endometrium (Schmandt *et al*., 2011) .

Obesity is defined as an excessive proportion of body fat relative to lean body mass of sufficient magnitude to produce adverse health consequences (Zamboni *et al*., 2005). It is a leading preventable cause of death worldwide and authorities view it as one of the most serious public health problem of this century. There is increasing prevalence of obesity globally contrary to what used to be regarded as disease of the industrialized countries. It is estimated that over one billion persons worldwide are overweight and

more than 300 million of whom are clinically obese (World Health Organisatio, 2005).The upsurge in obesity in Africa and other developing countries may not be unconnected with urbanization, adaptation of western life style and demographic transition (Ojofetimi *et al*., 2007). Studies done in Ghana, Republic of Benin and in the west African sub region found prevalence of obesity to be 18% in Ghana (Amoah, 2003), 13.6% in Republic of Benin (Sodjinou *et al*., 2008), and 10% in West African sub region (Abubakari *et al*., 2008). Similarly, Wahab *et al*., 2011, in their study, "Prevalence and determinants of obesity a cross-sectional study of an adult Northern Nigerian population", found a prevalence of 21% with higher prevalence in females than males.

Complications of obesity are either directly caused by obesity itself such as osteoarthritis, obstructive sleep apnea, social stigmatization; or indirectly related through mechanisms sharing a common cause such as a poor diet or a sedentary lifestyle resulting in diabetes, cardiovascular disease, non-alcoholic fatty liver disease among others (Bray, 1985).

2.3.1 Pathophysiology of Obesity

An adequate diet must meet the body's energy requirements and provide a minimum of carbohydrates, proteins and fats. Minerals, vitamins, and sufficient quantities of water are also essential. To ensure a normal passage time, especially through the colon, the diet must also provide a sufficient amount of roughage (indigestible plant fibers). Fat cells, residing within widely distributed adipose tissue depots, are adapted to store excess energy efficiently as triglyceride and, when needed, to release stored energy as free fatty acids for use at other sites (Ghildyal *et al*., 2009).This physiologic system, orchestrated

through endocrine and neural pathways, permits humans to survive starvation for as long as several months. However, in the presence of nutritional abundance and a sedentary lifestyle, and influenced importantly by genetic endowment, this system increases adipose energy stores and produces adverse health consequences. Therefore, excessive adiposity (obesity) is caused by energy intake in excess of energy output (Ghildyal *et al*., 2009).

The causes of obesity are complex. Although genes play an important role in determining food intake and energy metabolism, lifestyle and environmental factors may play a dominant role in Pathophysiology of obesity. The rapid increase in the prevalence of obesity in the past two to three decades emphasizes the important role of lifestyle and environmental factors, because genetic changes could not have occurred so rapidly (Bonnie and David, 2010).

Leptin, an adipocyte stimulating hormone is a major regulator of appetite by acting predominantly on the hypothalamus (Mortan *et al*., 2006). Other factors like neural afferent, metabolites like glucose and various hormones like insulin cortisol, ghrelin, cholecystokinin also regulate appetite (Wynne *et al*., 2005). These signals act by influencing expression and release of various hypothalamic peptides; α Melanocyte Stimulating Hormone (α-MSH), Neuropeptide Y(NPY), Agouti-Related Peptide and Melanin Concentrating Hormone (MCH) (Wynne *et al*., 2005). Normally, efferent impulses are sent to reduce nutrient absorption and increase energy consumption when plasma leptin are high and to increase nutrient absorption and decrease energy consumption when plasma leptin levels are low (Spiegelman and Flier, 2001). Leptin stimulates the release of α MSH which inhibits the absorption of nutrients and increases

sympathetic nervous activity and energy consumption to reduce the body weight. On the other hand it inhibits the release of NYP. NYP stimulates appetite, increases the parasympathetic activity and reduces energy consumption. In the absence of leptin however, or leptin insensitivity, release of α MSH is inhibited and effect of inhibition of NPY is removed which will result in increase appetite and absorption of food nutrient resulting in obesity. However studies have shown the role of insulin working together with leptin in regulating energy intake and metabolism.

2.3.2 The role of adipose tissue in obesity

Adipose tissue is located throughout the body, some proving structural support while several others are located within the body cavity, surrounding the heart and other organs, associated with the intestinal mesentery, and in the retroperitoneum. The visceral fat which drains directly in to the portal circulation is more linked with type 2 diabetes mellitus (Shulman, 2002). Apart from leptin and adiponectin, the adipose tissue also releases cytokines and non- esterified fatty acids (NEFAs) and glycerol Boden and Shulman, 2000). Studies have shown that obesity associated with or without type 2 diabetes mellitus is an inflammatory state and is consistent with the production of tumor necrotic factor, O2` and other cytokines by adipose tissue. Chronic inflammation of white adipose tissue characterized by macrophage infiltration is thought to contribute to insulin resistance associated with obesity. In obesity, the production of many of these adipokines is increased and in obese animals and humans, bone-marrow-derived macrophages are recruited to the fat pad under the influence of proteins secreted by adipocytes, including macrophage chemo attractant protein-1 (MCP-1) (Goodyear, 1995). In addition to adipocyte derived factors, increased release of TNF-, IL-6, MCP-1 and additional products of macrophages that populate adipose tissue might also have a

role in the development of insulin resistance. TNF- and IL-6 act through classical receptor-mediated processes resulting in up regulation of potential mediators of inflammation that can lead to insulin resistance (Ghildyal *et al*., 2009). Given that the release of non esterified fatty acid also is correlated with adipocyte size and that an increase in the non- esterified fatty acid concentration in plasma is a common feature of insulin resistance, increased non- esterified fatty acid levels are also associated with the insulin resistance observed in obesity and type 2 diabetes mellitus. The passage of non-esterified fatty acids across the plasma membrane and into the cell, where they are thought to exert their effects, is mediated in a specific manner by fatty acid transport protein 1 (FATP1), a transmembrane protein that enhances the cellular uptake of non-esterified fatty acids. In fact, it appears that the release of non- esterified fatty acids may be the single most critical factor in modulating insulin sensitivity. Insulin resistance develops within hours of an acute increase in plasma non- esterified fatty acid levels in humans (Shehperd and Khan, 1999). Conversely, insulin-mediated glucose uptake and glucose tolerance improve with an acute decrease in non- esterified fatty acid levels. Other mechanisms also contribute to insulin resistance in obesity. In morbid obesity, the expression of various insulin signaling molecules is reduced in skeletal muscle (Oodyear, 1995). In all forms of obesity and diabetes, a major factor contributing to the impaired insulin-stimulated glucose transport in adipocytes is the downregulation of GLUT4. However, in skeletal muscle of obese and diabetic humans, GLUT4 expression is normal (Shehperd and Khan, 1999) and defective glucose transport appears to be due to impaired translocation, docking, or fusion of GLUT4-containing vesicles with the plasma membrane. Obesity has also been reported to be associated with dyslipideamia (higher triglycerides, decreased HDL levels, and increased small, dense LDL particles) (Goodyear, 1995).

2.4 PATHOGENESIS OF TYPE 2 DIABETES MELLITUS

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of diabetes mellitus exist and are caused by a complex interaction of genetics, environmental factors, and life-style choices. Factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, and increased hepatic glucose production. Type 2 diabetes mellitus is characterized by peripheral insulin resistance (insulin insensitivity), impaired regulation of hepatic glucose production, high insulin levels with subsequent insulin deficiency as a result of β cell exhaustion. The initial hyperinsulineamia is as result of compensatory mechanism due to the cells insensitivity to insulin. According to Goldstein and Scheen (2003) the core defect in type 2 diabetes mellitus is insulin insensitivity to the cells and insulin secretory defect and not only failure of β cell function as in type 1diabetes mellitus. It has been shown that insulin resistance precedes failure of β cell dysfunction and diabetes develops only with inadequate insulin supply. Both environmental and genetic factors are also believed to play important roles in pathogenesis of type 2 diabetes mellitus. Most type 2 diabetic patients are obese and reduction in weight is shown to improve their glucose control and insulin sensitivity, however most obese individuals are not diabetic suggesting that the onset of diabetes requires an interaction between obesity-induced insulin resistance and other factors that predispose to diabetes, such as impaired insulin secretion.

Obesity, especially the abdominal type is a major risk factor associated with type 2 diabetes mellitus **(**Mohan *et al*., 2004**).** Studies over the years have also shown that obesity is associated with insulin resistance in the peripheral tissues and insulin

resistance is a cardinal feature of type 2 diabetes (Goldstein and Scheen, 2003; Muoio *et al*, 2006). Studies have also shown that obese individuals have glucose intolerance which can be explained by the presence of major insulin resistance that cannot be fully compensated for by adequate insulin secretion. With further intolerance to glucose, fasting hyperglycemia and diabetes occur. Reduction in weight has shown improvement in glucose tolerance, insulin sensitivity and insulin production (Henry *et al*., 1985).

Several theories have been postulated in the pathogenesis of diabetes. Elevated non-esterified fatty acid concentration in obese, especially central obesity is one hypothesis postulated in the pathogenesis of type 2 diabetes mellitus. However, adipose tissue as an endocrine hypothesis is gaining the attention of many researchers. The adipose tissues secretes various adipocytokins, like leptin, TNF-alpha, resistin and adiponectin which are implicated in insulin resistance and possibly beta-cell dysfunction. Leptin hormone is the most important adipocyte involved in the pathogenesis of obesity related type 2 diabetes mellitus. Although the effect of leptin on insulin secretion is controversial due to conflicting results in the literature, several studies have shown leptin to inhibit insulin secretion. However, some studies showed no effect of leptin on insulin secretion. Kieffer and collaborators (1997) reported that leptin receptors are also expressed in the insulin producing beta cells within the pancreatic islets, suggesting that leptin might influence insulin secretion through a direct action on these cells (Emilsson *et al*.,. in 1997).

2.5 RELATIONSHIP BETWEEN LEPTIN, INSULIN AND OBESITY IN TYPE 2 DIABETES MELLITUS

A mutation of the leptin gene in ob/ob mouse causes leptin deficiency. This resulted in hyperphagia, decreased energy expenditure, severe obesity, and insulin-resistant diabetes mellitus (Zhang *et al*., 1994). Leptin, when injected to leptin deficient ob/ob mice,

which have a mutation in the ob gene and do not make leptin, the obesity is corrected and an increase in energy expenditure, a decrease in food intake, and a decrease in plasma glucose, insulin, and cortisol levels (Pellymounter *et al*., 1993; Stephens *et al*., 1995; Halaas *et al*., 1995) occur. Insulin and leptin although synthesized from different sources, function as afferent signals to the hypothalamus in an endocrine feedback loop that regulates body adiposity. They exact overlapping effect on the arcuate nuclease which is the center for regulating body weight and glucose homeostasis (Niswender and Schwartz, 2003).

Studies over the years have shown that obesity is associated with insulin resistance in the peripheral tissues and insulin resistance is a cardinal feature of type 2 diabetes (Muoio *et al*, 2006). Leptin resistance which is also associated with obesity is observed to reduce the hypothalamic response to insulin, impairing peripheral glucose homeostasis contributing to the development of type 2 diabetes mellitus (Christiane *et al*., 2010).
Obesity is accompanied by elevated leptin levels, and besides fat mass and gender, insulin secretion and degree of insulin resistance seem to contribute significantly to leptin levels (Wauters *et al*., 2003).When obesity and diabetes coexist in the same individual, the severity of insulin resistance is only slightly greater than that in either the normal-weight diabetic or non-diabetic obese groups and the magnitude of the defects in glucose oxidation and non-oxidative glucose disposal are similar in all obese and diabetic groups. Although hyper-insulinemic and hypo-inuslinemic obese diabetic subjects were equally insulin resistant, the severity of glucose intolerance is worse in the hypoinsulinemic group, and this was related entirely to the presence of severe insulin deficiency (Defronzo, 1998).

Fasting or restriction of energy has been shown to reduce the plasma leptin concentration Dubuc *et al*., (1998) which indicate that low plasma leptin may be associated with low plasma glucose and adipocytes glucose may also have a role in leptin regulation (Peter, 1998).

Type 2 diabetes mellitus has the strongest link with obesity compared to the other public health problems. Previous studies have shown an association between obesity and the development of type 2 diabetes mellitus (kong *et al*., 2008). Increase in body fat alters the body's response to insulin, potentially leading to insulin resistance and hence type 2 diabetes mellitus. Although obesity is widely documented to be associated with type 2 diabetes mellitus, central obesity has been shown to be more associated than increase in total body adiposity as shown in a study, "Visceral and Central Abdominal Fat and Anthropometry in Relation to Diabetes in Asian Indians" (Mohan *et al*., 2004).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

The materials for the study included the subjects, a watch with second hand for measuring pulse and mercury sphygmomanometer for measuring blood pressure. A bathroom scale was used for measuring weight of the subjects while a standiometer was used to measure their height. A measuring tape was used to measure their abdominal (waist) and hip circumferences. Leptin was estimated from the serum by Elisa method using Abnova LEP (Human) ELISA Kit. Catalog number KA0025, 96 assays,version: 08 and Abnova Insulin (Human) ELISA kit catalog number KA0921, 96 assay, version: 04 was used to estimate serum insulin concentration. Spectrophotometer (Multiskan EX Labsystems) was used to measure the absorbance of the samples. Serum glucose was estimated using glucose oxidase method kit. Serum Total Cholesterol (TC) and HDL were estimated using Randox cholesreol (chol) enzymatic endpoint method kit. Triglyceride was estimated using glycerol-3-phosphate peroxidase p- aminophenazone kit.

3.1.1 Instrument of data collection

Data was collected using semi- structured interviewer administered questionnaire.

The questionnaire consisted of sections A and B. Section A elicited information about the subject personal data while section B elicited information about the subjects life style and medical history. Physiologic, anthropometric measurements and blood parameters were also recorded on the questionnaire (Appendix 1).

3.2 METHOD

3.2.1 Study area

The study was conducted at Murtala Muhammed Specialist Hospital (MMSH), Kano State and the city is situated at 11° 59' 47" North, 8° 31' 0" East. The hospital is located in the heart of Kano Municipal Local Government area (L.G.A). MMSH has the largest turnover of patients in Kano State with bed capacity of about 1,100 beds. An average of 230 patients attends the diabetic clinic of the facility weekly. The hospital is attended by patients from all the Northwestern states of Nigeria and the neighboring Niger Republic. Majority of the people attending the hospital are the Hausa and Fulani; and other ethnic groups like Yoruba, Igbo, Kanuri, Igala and Egbira that are resident in the area (Abba, 1999).

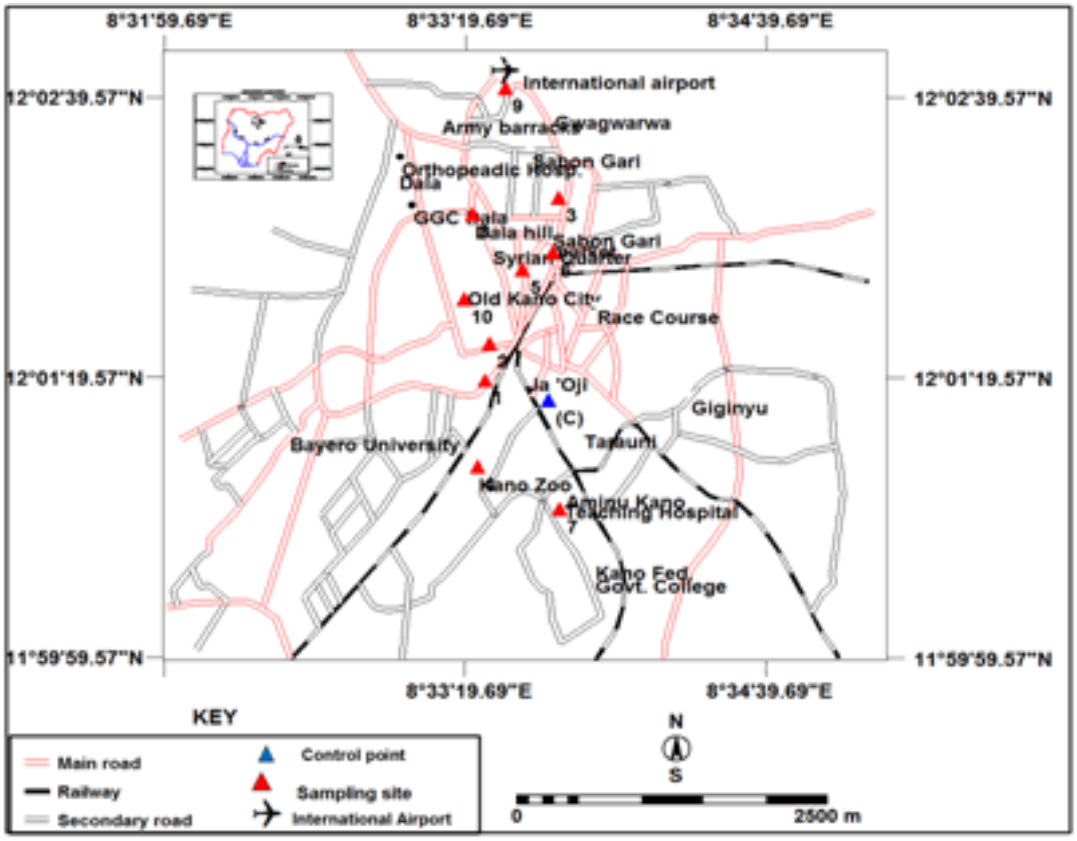


Fig 3.1: Map of Kano city

Source: Nigeria map cities guide system

3.2.2 Study population

The study population comprised of type 2 diabetic male and female patients 30 years and above attending diabetic clinic at the study hospital. Appropriate controls matched for age, height and weight were selected randomly from healthy non diabetics subjects in the city.

3.2.3 Study design

A descriptive cross – sectional design was used for the study. Anthropometric parameters, serum fasting blood sugar, insulin, leptin and triglycerides (T), total-cholesterol (TC), low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol (HDL-c) of the subjects were measured and compared with those non-diabetic controls.

3.2.4 Sample size estimation

The minimum sample size for the study was estimated using the formula for calculating minimum sample size for descriptive studies i.e. $n = Z^2pq/d^2$.

Where n = minimum sample size, Z is the standard normal deviate at 95% confidence interval i.e. 1.96 and p is the prevalence rate obtained from a past study. The q represents the complimentary probability of p i.e. q = (1-p); and d represents the precision (margin error) at 95% confidence limit i.e 0.05 (Lwanga and Lemeshow 1991).

Using 13.1% prevalence of obesity in Zaria, a suburban northern Nigerian city (Bakari *et al*., 2007); and a complementary probability q (1-0.13), the sample size 'n'= $(1.96)^2$ (0.13) (0.87)/ $(0.05)^2$ = 173.79 = 174.

The sample size was increased by 10% to cover for non response and incomplete responses. Thus, the sample size for the study was arrived at 191. Because the leptin and insulin kits contained 96 pits each, 2 kits ie 192 specimens were adjudged appropriate for the study. However, 9 pits were used as standards (6 pits), low and high quality controls (2 pits) and buffer (1pit) for estimating leptin concentration. In the same vein, 6 pits were used as standards in the estimation of insulin. The final number of pits available as samples for the estimation of leptin and insulin hormones was 183 pits for 183 samples. On the other hand, 96 complete pits were used for the control samples for the estimation of the concentrations of the hormones.

3.2.5 Sampling method

Systematic sampling method was used to select the subjects. The study population was estimated to be 920 diabetic patients attending diabetic clinic in a month using the average weekly attendance for the clinic of 230 patients. The sampling interval was determined by dividing the study population by the sample size (920/191 = 4.88) which is approximately 5. With sampling interval of 5, one in every 5 subject was selected until the required sample size was obtained.

3.2.6 Inclusion and exclusion criteria

The entire type 2 diabetic patients attending diabetic clinic in Murtala Muhammed Specialist Hospital Kano were eligible for the study.

Chronically ill diabetic patients with significant complications, insulin dependence, evidence of secondary diabetes, current insulin therapy, previous history of ketosis, and clinical evidence of disease of the liver, kidney or thyroid were excluded. Asthmatic patients and patients on long term steroid were also excluded from the study.

3.2.7 Procedure for Data collection

Data from respondents was collected using the study instrument. Anthropometric measurement (weight, height; hip, abdominal and waist circumference) was collected by the same trained investigator (examiner) and an assistant (recorder).

3.2.8 Ethical considerations

Ethical clearance for the study was obtained from the Scientific and Ethical Committee of Aminu Kano Teaching Hospital before commencement of the study (Appendix 2). A written informed consent was obtained from each of the respondents before commencement of the interview.

3.2.9 Measurement of vital signs

3.2.9.1 Pulse rate

The pulse was measured using a watch that has a second hand. The subjects positioned with their wrist resting on the table. Using the examiners three middle fingers, the radial artery was located and compressed enough to feel the pulse. The pulsations were counted for a full minute noting irregularities in the rhythm, volume and condition of the artery.

3.2.9.2 Blood pressure

Mercury sphygmomanometer was used to measure the blood pressure of the subjects.

After palpating the brachial artery, the bladder of the cuff was centered on the brachial artery above the bend of the elbow making sure that the gauge was at zero.

After palpating the radial pulse, the cuff was smoothly inflated until the pulse was no longer felt and the number at that point was noted and the cuff deflated. The stethoscope

was then placed over the brachial artery and held in position with fingers only. The cuff was then inflated smoothly and quickly to the peak inflation level plus 30 mm Hg. The cuff was then deflated at the rate of 2 to 4 mm Hg per heartbeat until the first sound was noted (first Korotkoff Phase I). The deflation was continued, noting the Korotkoff phases until when all sounds disappeared (Korotkoff Phase V). The deflation was continued for another 10 mm Hg after sounds have disappeared. The first Korotkoff sound was read as systolic blood pressure while the last was read as diastolic blood pressure (Center for Disease Control, 2007).

3.2.10 Anthropometric measurement

The weight, height, abdominal (waist) and hip (buttocks) circumferences of the subjects was measured using standard scales.

3.2.10.1 Body weight

Bathroom weighting scale was used to measure the weights of all the subjects. The scale was placed on a hard flat surface and calibrated at the beginning and end of each examining day. The subjects were asked to stand erect in the center of the platform of the scale bare feet with light clothing. The weight of the subject was then recorded (European Health Risk Monitoring, 2002).

3.2.10.2 Height

The subject standing erect with his shoes off, his back to the vertical backboard of the Standiometer and both heels touching the base of the vertical board. The buttocks, scapulae, and head were positioned in contact with the vertical backboard with the arms hanging freely by the sides of the trunk with palms facing the thighs. The head was maintained in the Frankfort horizontal plane position while the examiner lowers the horizontal bar snugly to the crown of the head with sufficient pressure to compress the

hair. The measurement was read by the examiner and recorded to the nearest 0.1 cm (European Health Risk Monitoring, 2002).

3.2.10.3 Abdominal circumference

The abdominal (waist) circumference was measured as follows. The subject in a standing position was asked to hold up his gown. The examiner, sanding behind the subject and palpated the hip area for the right iliac crest. The examiner marked a horizontal line at the highest point of the iliac crest and then crosses the line to indicate the mid-axillary line of the body. The pants and underclothing of the subject was lowered slightly for the examiner to palpate directly on the hip area for the iliac crest. The examiner then stood on the subject's right side and places the measuring tape around the trunk in a horizontal plane at the level marked on the right side of the trunk. The measurement was made at minimal respiration to the nearest 0.1 cm (Center for Disease Control, 2007).

3.2.10.4 Hip circumference

The subject, while standing erect with feet together and weight evenly distributed on both feet was asked to hold up his gown. The examiner then squatting on the right side of the subject placed the measuring tape around the buttocks at the maximum extension of the buttocks. The zero end of the tape held under the measurement values. The examiner then takes the measurement from the right side of the subjects (European Health Risk Monitoring, 2002).

3.2.11 Laboratory procedures

3.2.11.1 Blood collection

About 7mls of blood was drawn into plain bottles from each subject in the morning after about 10 to 12 hours of fasting and after observing all universal precautions. After centrifuging, the samples were kept in a refrigerator at -20^0C.

3.2.11.2 Serum Leptin estimation

Principle of the assay

In the LEP (Human) ELISA Kit, Clinical Range, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human leptin antibody. After 60 minutes incubation and washing, polyclonal anti-human leptin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured leptin. Following another washing step, the remaining HRP conjugate is allowed to react with the Substrate Solution tetramethylbenzidine (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance read at 450nm is proportional to the concentration of leptin. A standard curve was constructed by plotting absorbance values against concentrations of standard, and concentration of unknown samples were determined using this standard curve.

Assay procedure

100 µl of 6 diluted Standards, Quality Controls low and high, Dilution Buffer (=Blank) and 183 samples from study group and 96 samples from control group was drawn and placed into appropriate wells and the plate was incubated at room temperature, shaking it at 300 rpm on an orbital microplate shaker for an hour and then washed 3 times with wash solution. After final wash, the plate was inverted and tapped strongly against paper towel. Then100 µl of Conjugate Solution was added into each well, and the plate was incubated again at room temperature for another hour shaking it on an orbital microplate shaker. It was washed 3 times again with wash solution and tapped strongly again against paper towel. 100 µl of Substrate Solution was added into each well and the plate was covered with aluminium foil to protect it from sunlight. The plate is again incubated for another 10 minutes after which 100 µl of stop solution was added. Absobance was

read at 450nm. A standard graph was plotted and leptin concentration was deduced from the graph using the trend line equation for the relationship that is y = 0.2718x - 0.3658 where y represents the optical density and x is the corresponding leptin concentration in ng/ml (Appendix 3).

3.2.11.3 Serum Insulin estimation

Principles of the study

The Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with enzyme (HRP)-conjugated anti-insulin antibody and anti-insulin antibody bound to micro-titration well. A simple washing step removes unbound enzyme labeled antibody. The bound HRP complex is detected by reaction with TMB substrate. The reaction is stopped by adding acid to give a colorimetric endpoint that is read using ELISA reader.

Assay procedure

Prior to assay, all reagents and samples were allowed to stand at room temperature. The desired number of coated strips was placed into the holder and 25µl 0f sera from183 diabetic patients 96 non-diabetic subjects and 6 insulin standards was placed into their appropriate pits.

100 µl of working Insulin Enzyme Conjugate was then added to all wells and mixed for 10 seconds and was incubated for an hour at 26^0C. After incubation, the liquid in the wells was discarded and wells were washed three times with 300 µl of 1X wash buffer and absorbent paper towels were used to blot the pits. 100 µl of TMB substrate was added to all wells and again incubated for 15 minutes. 50 µl of stop solution was then added to all wells and solution mixed by gently shaking the plate. Absorbance was read

at 450nm within 15 minutes. A standard graph was plotted and insulin concentration for each sample was deduced from the graph using the trend line equation for the relationship that is y = 0.2073x - 0.2949 where y represent optical density for insulin and x represents concentration of insulin for each optical density (Appendix 4).

Assessment of insulin resistance

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) first developed in 1985 by Matthews *et al., to* assess β-cell function (HOMA-β) and Insulin resistance (HOMA-IR) from basal glucose and insulin concentrations was used to determine insulin resistance in the subjects. This was achieved using the equation for IR which uses a fasting plasma sample in which glucose (fasting plasma glucose; FPG) and insulin (fasting plasma insulin; FPI) were measured, together with a constant. The product of FPG × FPI is an index of IR. HOMA-IR = (glucose in mmol/L × insulin μU/L) /22.5. The constant of 22.5 is a normalizing factor, that is normal FPI of 5 μU/mL × the normal FPG of 4.5 mmol/L typical of a normal healthy individual = 22.5 (Eslam, 2011).

3.2.11.4 Fasting blood sugar estimation

Plasma glucose was estimated using glucose oxidase method (Trinder, 1969).

Principle of the Assay

Glucose is oxidized to glucoronic acid and hydrogen peroxide by the enzyme glucose oxidase, the peroxide is broken down to water and nascent oxygen which forms coloured complex with 4- aminophenazone.

3.2.12 Data analysis

Collected data was double entered into the computer system and analyzed using the Minitab 12.0 computer statistical software.

All quantitative variables were summarized using mean and standard error while qualitative variables were summarized using frequencies and percentages.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Waist Hip ratio was estimated as the ratio of circumference of the waist to that of the hip (State of Alaska Measuring Height/Weight and Calculating BMI, 2011). BMI of less than 18 was adjudged as underweight, between 18 and 24.99 as normal, while 25 to 29.99 was adjudged as overweight whereas > 30 was considered as obese (World Health Organisation, 1995). Waist circumference of less than 94cm in males and less than 80cm in females was adjudged to be normal. Similarly, Waist hip ratio of less than 0.9cm in males and 0.85cm in females was adjudged to be normal (World Health Organisation, 1999). Those who engage in brisk/ stroll for up to 30 minutes for at least five days in a week are adjudge to have adequate exercise, those who are engaged in farming or are herds men have moderate exercise and those who do not engage in brisk walk/ stroll for up to 30 minutes and less than five days in a week are adjudge to have inadequate exercise (Egger, 2001).

BMI, abdominal circumference and waist to hip ratio were correlated with serum leptin, FBS and insulin levels for both the study and control groups, and the strength of association between corresponding variables was determined using correlation coefficient (r). In the same vein, serum leptin for the two (2) groups were correlated with insulin and plasma FBS, and correlation coefficient was estimated to determine the strength of association. Student‘t’- test was used to compare mean values of all quantitative parameters between the diabetic and non- diabetic groups. Chi-square test was used to determine association between categorical variables. A p value ≤ 0.05 was considered significant.

HOMA IR score was used to determine insulin resistance in study subjects, where subjects that scored 1 were considered 100% sensitive and those that scored more than 1 were considered to have insulin resistance (Eslam, 2011).

3.2.13 Limitations

Measurement of leptin receptor sensitivity was not possible due to lack of equipment and cost.

CHAPTER FOUR

RESULTS

4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE RESPONDENTS

4.1.1 Age and Sex distribution

There were 183 type-2 diabetics and 96 non-diabetic subjects. More than half (57.4%) of the type-2 diabetics were females while the males constituted 42.6%. The male to female ratio in the group was 1:1.36. On the other hand 56.2% of the subjects in the non-diabetic group were males and 43.7% were females, with a male to female ratio of 1.28:1.

The ages of the type-2 diabetic subjects range from 30 years to 81 years, with a mean and standard error (S.E) of 54.09 ± 0.93 years. The mean age ± SE of the subjects in this group by sex was 54.95 ± 1.52 years and 53.45 ± 1.18 years for the male and female sex respectively. Similarly, the subjects in the non-diabetic group were aged between 30 years and 85 years. Their mean age ± SE was 50.27 ± 1.42 years. The mean age ± SE of the male and female subjects in the group was 50.54 ± 1.91 years and 49.93 ± 2.15 years respectively. There is no significant statistical difference between the ages of the diabetic and the non-diabetic subjects as summarized in Table 4.1.

Table 4.1: Age and Sex distribution of the subjects

Age group	Male Type 2 Diabetics Mean ± SE	n	Male Non Diabetics Mean ± SE	n	't' test	P value	Female Type 2 Diabetics Mean ± SE	n	Female Non Diabetics	n	't' test	P value
30-37	34.89 ±0.84	9	35.00 ± 0.75	11	0.11	0.92^{ns}	34.80 ± 0.69	10	34.70 ± 0.68	10	0.09	0.93^{ns}
38-45	41.94 ± 0.50	17	40.55 ± 0.77	11	1.51	0.14^{ns}	41.75 ± 0.55	24	41.30 ± 0.89	10	0.47	0.64^{ns}
46-53	50.20 ± 0.40	10	49.93 ± 0.56	15	0.32	0.75^{ns}	49.78 ± 0.61	18	48.40 ± 0.69	5	1.67	0.11^{ns}
54-61	56.67 ± 0.48	12	58.50 ± 0.92	6	1.83	0.09_{ns}	57.26 ± 0.56	23	57.22 ± 0.64	9	0.05	0.96^{ns}
62-69	64.61 ± 0.46	13	62.50 ± 0.75	4	1.52	0.15^{ns}	65.10 ± 0.72	19	65.00 ± 0.00	1	0.05	0.06^{ns}
70-77	71.87 ± 0.29	15	73.00 ± 1.53	3	0.90	0.83^{ns}	70.29 ± 0.49	7	72.50 ± 1.12	6	2.06	0.06^{ns}
78-85	79.50 ± 0.00	2	82.00 ± 1.78	4	0.89	0.42^{ns}	80.00 ± 1.5	4	80.00 ± 0.00	1	-	-
Total		78		54				105		42		

4.1.2 Educational status

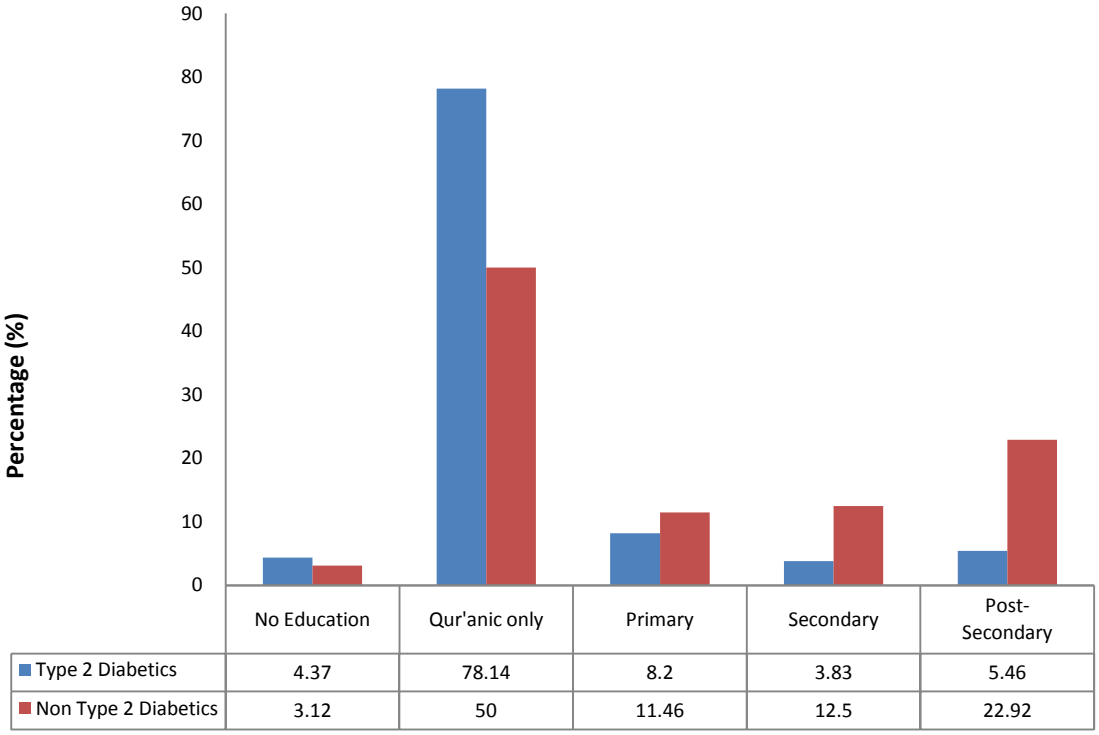
Majority of the type 2 diabetic subjects (82.5%) had no formal education while 46.9% of the non-diabetic subjects had formal education (Figure 4.1). There is association between type 2 diabetes mellitus and educational status ($X^2 = 31.93$, DF = 4, $P < 0.05$).

4.1.3 Respondents' Occupations

More than half of the diabetics (51.9%) were self employed, 10.4% were employed in public service whereas 36.1% were unemployed. Similarly, 51.0% and 14.6% of the non-diabetic subjects were self employed and in the public service respectively, and 28.1% were unemployed. There is no association between type 2 diabetes mellitus and respondents' occupation ($X^2 = 6.86$, DF = 4, $P > 0.05$) as shown in Figure 4.2

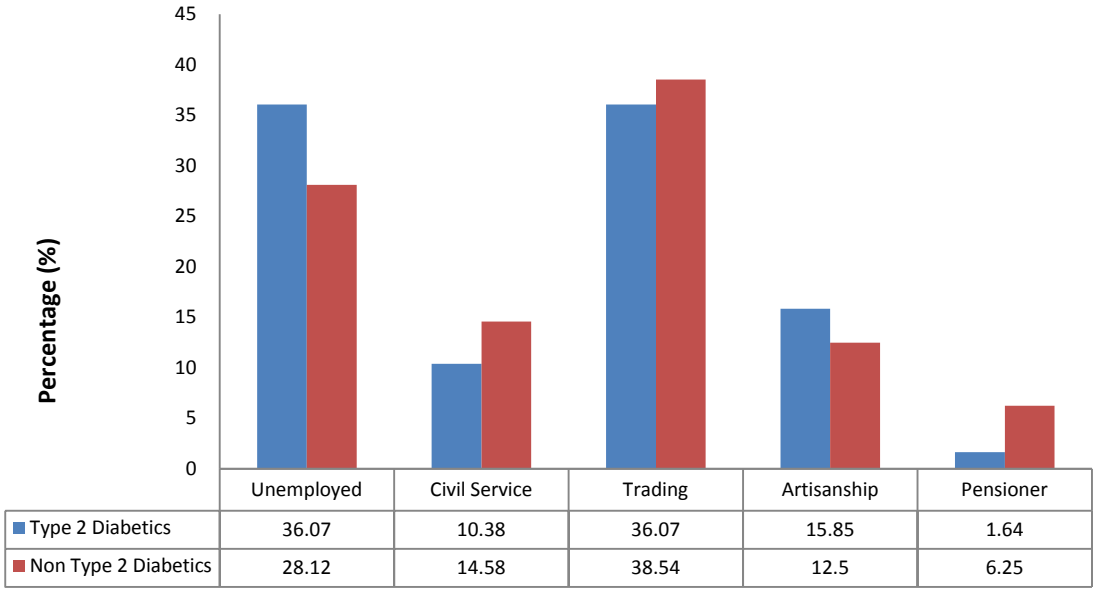
4.1.4 Respondents' Ethnicity

Most of the respondents from both the diabetic (86.3%) and non-diabetic (89.6%) groups were Hausa by tribe. There is no association between type 2 diabetes mellitus and respondents' ethnicity (Yates corrected $X^2 = 0.03$, $p > 0.05$) as shown in Figure 4.3.



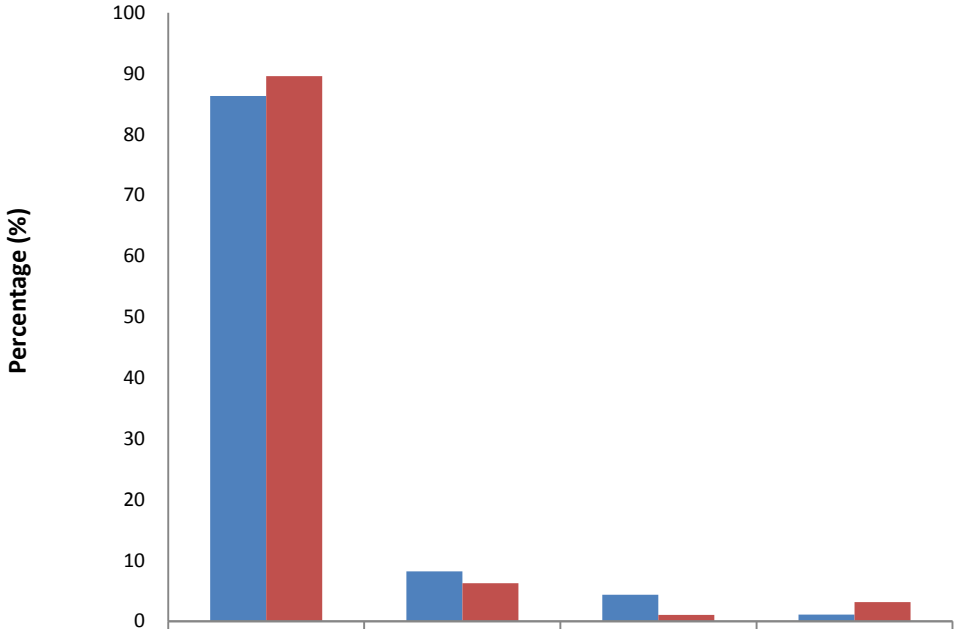
	No Education	Qur'anic only	Primary	Secondary	Post-Secondary
Type 2 Diabetics	4.37	78.14	8.2	3.83	5.46
Non Type 2 Diabetics	3.12	50	11.46	12.5	22.92

Fig 4.1: Educational status of Respondents



	Unemployed	Civil Service	Trading	Artisanship	Pensioner
Type 2 Diabetics	36.07	10.38	36.07	15.85	1.64
Non Type 2 Diabetics	28.12	14.58	38.54	12.5	6.25

Fig 4.2: Respondents' Occupations



	Hausa	Fulani	Kanuri	Others
Type 2 Diabetics	86.34	8.20	4.37	1.09
Non Type 2 Diabetics	89.58	6.25	1.04	3.13

Others: Yoruba, Igbo, Igbira

Fig 4.3: Respondents' Ethnicity

4.1.5 Smoking and alcohol consumption status of the Respondents

Most of the diabetic (86.3%) and the non-diabetic (96.9%) subjects were non smokers and an association was observed between type 2 diabetes and smoking status of the respondents ($X^2 = 7.74$, DF = 1, $P < 0.05$) (Figure 4.4). Similarly, most of the diabetic subjects (96.2%) and all of the non-diabetic subjects did not consume alcohol (Fisher exact $p = 0.05$) as shown in Figure 4.5.

4.1.6 Physical Exercise status of Respondents

More than half of the diabetic subjects (55.2%) and more than half of the non-diabetic subjects (65.6%) did not engage in adequate physical exercise. On the other hand, 19.1% of the diabetic and only 5.2% of the non-diabetic subjects got engaged in adequate physical exercise (Figure 4.6).

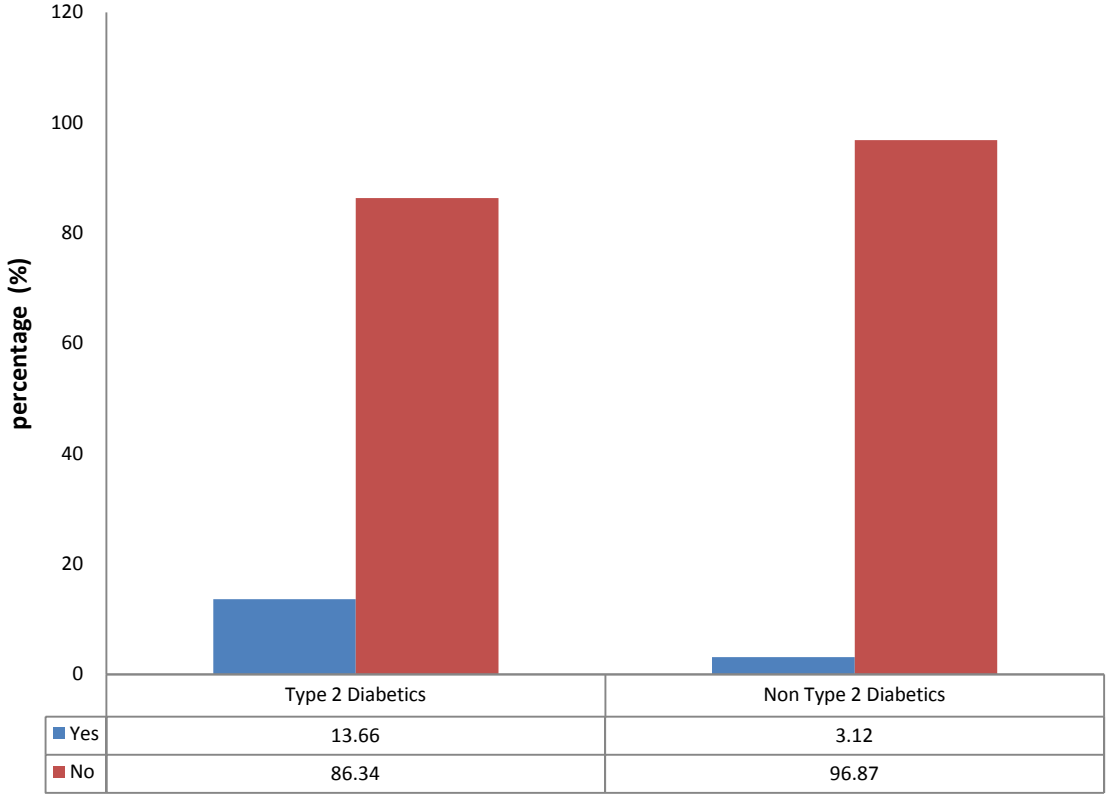


Fig 4.4: Smoking status of Respondents

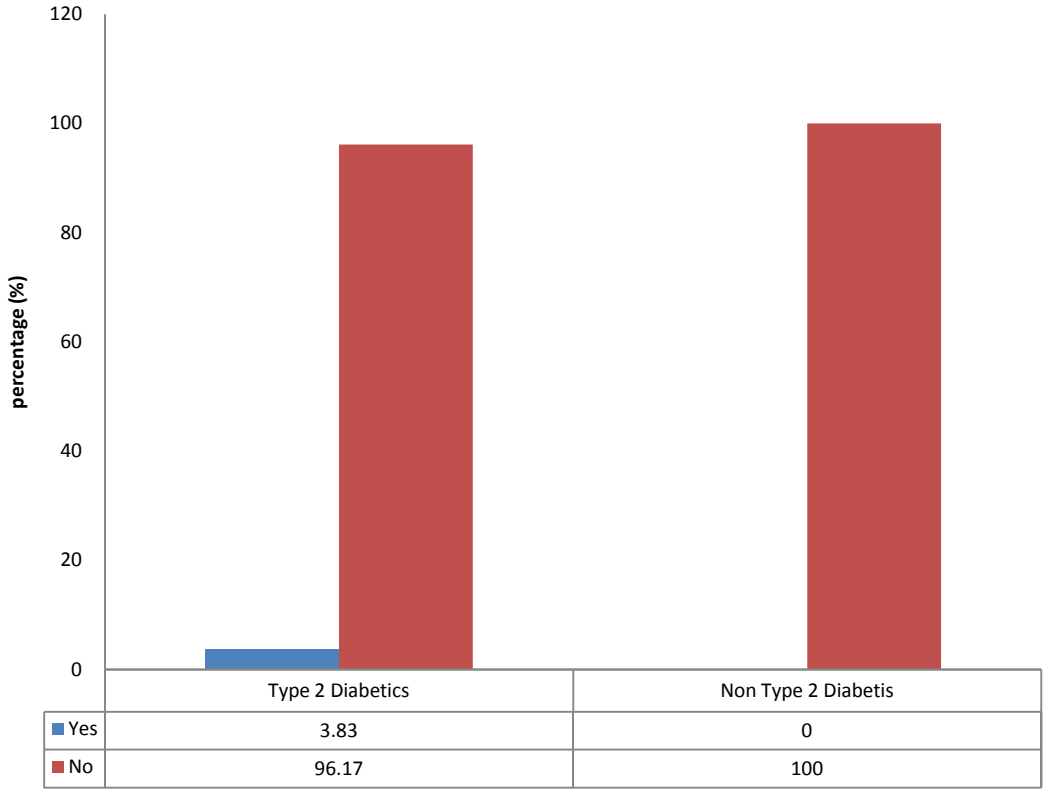


Fig 4.5: Alcohol consumption status of Respondents

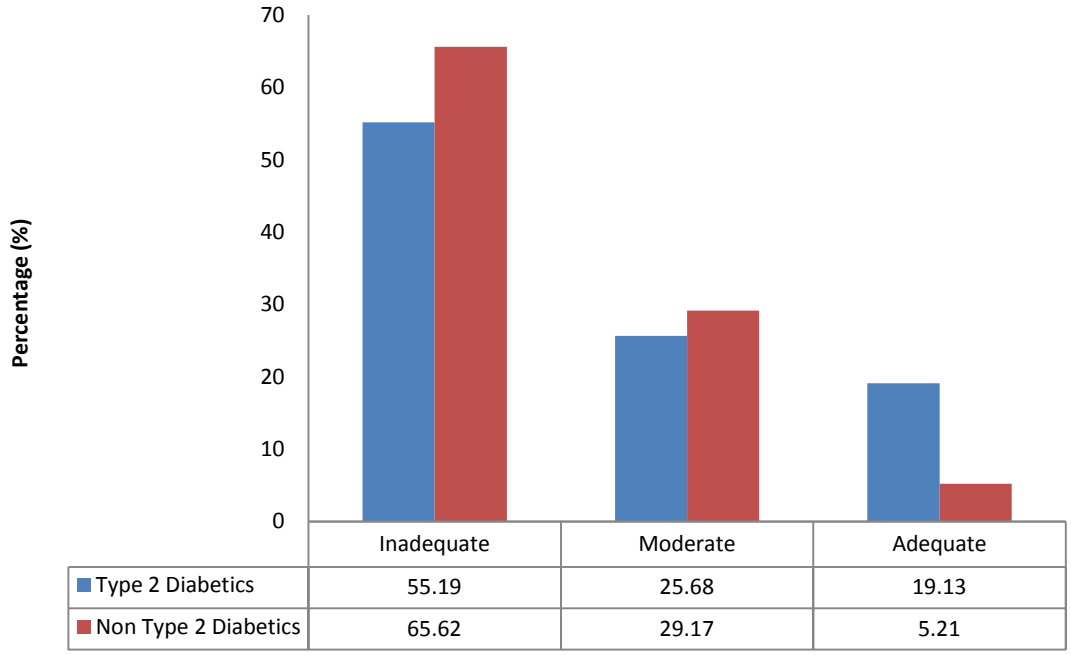


Fig 4.6: Exercise status of the Respondents

4.2 PHYSIOLOGIC AND BIOCHEMICAL PARAMETERS OF RESPONDENTS

4.2.1 The Weight, Height, Body Mass Index, Waist Circumference, Hip Circumference and Waist Hip Ratio of Diabetic and Non-Diabetic Subjects

The comparison in the anthropometric parameters (weight, height, body mass index, waist circumference, hip circumference and waist hip ratio) between the male diabetic subjects and their matched non-diabetic subjects (Table 4.2) were similar in both groups except for the waist hip ratio that was higher in the diabetics (% difference -3.23, $p < 0.05$). In the same vein, when the parameters were compared between the female diabetic subjects and their matched non-diabetics, they were also similar in the two groups except for the body mass index and the waist circumference. Both the body mass index (% diff. 7.62, $p < 0.05$) and the waist circumference (% diff. 10.6, $p < 0.05$) were higher in the female diabetic subjects (Table 4.2).

The variation in weight, height, body mass index, waist circumference, hip circumference and waist hip ratio between the male and female non-diabetics subjects are as highlighted in Table 4.3. The means of weight (% diff. 11.6, $p > 0.05$), height (% diff. 6.0, $p < 0.05$) and waist hip ratio (% diff. 6.4, $p < 0.05$) were significantly higher in male subjects. On the other hand, the means of weight (% diff. 7.0, $p < 0.05$), height (7.8, $p < 0.05$) and waist hip ratio (% diff. 89.6, $p < 0.05$) werey higher in male diabetics compared to their female counterparts, while the means of body mass index (% difference 10.9, $p < 0.05$), waist circumference (% diff. 6.3, $p < 0.05$) and hip circumference (% diff. 11.2, $p < 0.05$) were significantly higher in female diabetics compared to their male counter parts) as shown in Table 4.4.

Table 4.2: Weight (kg), Height (m) Body Mass Index, Hip Circumference (cm) Waist Circumference (cm) and Waist Hip Ratio of the Respondents.

Parameter	Male Non-Diabetic (n=54) Mean ± S.E	Male Type-2 Diabetics (n=78) Mean ± S.E	% difference	Female Non-Diabetic (n=42) Mean ± S.E	Female Type-2 Diabetic (n=105) Mean ± S.E	% difference
Weight (Kg)	70.61± 2.02	69.08 ± 1.39	2.17^{ns}	62.45 ± 1.99	64.21 ± 1.26	-2.82^{ns}
Height (m)	1.67± 0.01	1.68 ± 0.01	-0.60^{ns}	1.57± 0.01	1.54 ± 0.01	1.91^{ns}
Body mass index	25.26± 0.67	24.58 ± 0.49	2.69^{ns}	25.33 ± 0.66	27.26 ± 6.2	-7.62*
Waist circumference(cm)	83.24 ± 1.41	84.22 ± 1.38	-1.18^{ns}	80.98 ± 1.62	89.45 ± 1.27	-10.57*
Hip circumference(Cm)	90.22 ± 1.53	87.83 ± 1.13	2.65^{ns}	93.38 ± 1.92	97.29 ± 1.40	-4.19^{ns}
Waist hip ratio	0.93 ± 0.01	0.96 ±0.01	-3.23*	0.87 ± 0.01	0.99 ± 0.08	-13.79^{ns}

*($p \leq 0.05$) =Significant, ($p > 0.05$)^{ns} = not Significant

Table 4.3: Weight (kg), Height (m) Body Mass Index, Hip Circumference (cm) Waist Circumference (cm) and Waist Hip Ratio in Male and Female non-diabetics

Parameter	Male (n=54) Mean ± S.E	Female (n=42) Mean ± S.E	% difference
Weight (Kg)	70.61± 2.02	62.45 ± 1.99	11.56*
Height (m)	1.67± 0.01	1.57± 0.01	5.99*
Body mass index	25.26± 0.67	25.33 ± 0.66	-0.28 ^{ns}
Waist circumference (cm)	83.24 ± 1.41	80.98 ± 1.62	3.19 ^{ns}
Hip circumference (cm)	90.22 ± 1.53	93.38 ± 1.92	-3.50 ^{ns}
Waist to hip ratio	0.96 ± 0.01	0.87± 0.01	6.45*

*($p \leq 0.05$) =Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.4: Weight (kg), Height (m) Body Mass Index, Hip Circumference (cm) Waist Circumference (cm) and Waist Hip Ratio in Male and Female Type-2 Diabetic Patients

Parameter	Male (n=78) Mean ± S.E	Female (n=105) Mean ± S.E	% difference
Weight (Kg)	69.08 ± 1.39	64.21 ± 1.26	7.05*
Height (m)	1.68 ± 0.01	1.54 ± 0.01	7.78*
Body mass index	24.58 ± 0.49	27.26 ± 6.2	-10.90*
Waist circumference (cm)	84.22 ± 1.38	89.45 ± 1.27	-6.32*
Hip circumference (cm)	87.83 ± 1.13	97.29 ± 1.40	-11.2*
Waist to hip ratio	0.96 ±0.01	0.99 ± 0.08	89.58*

*($p \leq 0.05$) = Significant

4.2.2 Mean values for Pulse rate (min), Systolic, Diastolic and Mean arterial blood pressure (mmHg) in Diabetic and Non-Diabetic Subjects.

There is significant difference in the mean pulse rate ($p < 0.05$) and systolic blood pressure ($p < 0.05$) between the two groups. Both means of pulse rate and systolic blood pressure are higher in the diabetic patients (mean PR ±SE = 86.45 ± 0.90, mean SBP ± SE = 129.7 ± 1.16) compared to the non-diabetics (mean PR ±SE = 80.8 ± 0.90, mean SBP ± SE = 126.15 ± 1.14). However, the mean DBP and MAP are similar between the two groups (Table 4.5).

In evaluating variations of the parameters due to sex, the male and female genders from the diabetic and non-diabetic groups were studied separately, and the male and female diabetic subjects were also examined. It was observed that in both the male and female diabetic and non-diabetic groups there was significant difference only in the mean pulse rate as shown in Table 4.6.

The comparison between the male and female diabetic subjects shows significant differences in the means of pulse rate ($p < 0.05$) and systolic blood pressure ($p < 0.05$) between the groups and both parameters were higher in the male diabetics (mean PR ±SE = 86.7 ± 1.4, mean SBP ± SE = 131.03 ± 1.84), as shown in Table 4.7

Table 4.5: Mean values for Pulse rate (min), Systolic, Diastolic and Mean arterial blood pressure (mmHg) of the Non-Diabetics and Type 2 Diabetic Subjects

Parameter	Non-Diabetics Mean ± S.E	Type 2 Diabetic Mean ± S.E	% difference
Pulse rate (min)	80.77 ± 0.90	86.45 ± 0.90	-7.03*
Systolic blood pressure (mmHg)	126.15 ± 1.14	129.70 ± 1.16	-2.08*
Diastolic blood pressure (mmHg)	78.59 ± 0.61	78.88 ± 0.61	-0.37 ^{ns}
Mean arterial pressure (mmHg)	94.44 ± 0.67	95.82 ± 0.66	-1.46 ^{ns}

*($p \leq 0.05$) =Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.6: Mean values for Pulse rate (min), Systolic, Diastolic and Mean arterial blood (mmHg) in Non-diabetics and Type-2 Diabetic Patients

Parameters	Male Non-Diabetics (n=54) Mean ± S.E	Male Type-2 Diabetics (n=78) Mean ± S.E	% difference	Female Non-Diabetics (n=42) Mean ± S.E	Female Type-2 Diabetics (n=105) Mean ± S.E	% difference
Pulse rate (min)	79.61 ± 1.10	86.7 ± 1.40	-8.91*	82.26 ± 1.50	86.20 ± 1.20	-4.97*
Systolic blood pressure (mmHg)	126.48 ±1.52	131.03 ± 1.84	-3.60 ^{ns}	125.25 ± 1.74	128.71 ± 1.50	-2.39^{ns}
Diastolic blood pressure (mmHg)	79.63 ± 0.84	79.55 ± 1.00	0.10 ^{ns}	77.26 ± 0.86	78.38 ± 0.76	-1.45^{ns}
Mean arterial pressure (mmHg)	95.25 ± 0.91	96.71 ± 1.07	-1.53 ^{ns}	93.42 ± 0.97	95.16 ± 0.85	-1.86^{ns}

*($p \leq 0.05$) =Significant, ($p > 0.05$) ^{ns} not Significant

Table 4.7: Mean values for Pulse rate (min), Systolic, Diastolic and Mean arterial blood pressure (mmHg) in Male and Female Type-2 Diabetic Subjects.

Parameter	Male Type-2 Diabetics (n=78) Mean ± S.E	Female Type-2 Diabetics (n=105) Mean ± S.E	% difference
Pulse rate (min)	86.7 ± 1.4	86.20 ± 1.2	0.58*
Systolic blood pressure(mmHg)	131.03 ± 1.84	128.71 ± 1.50	1.77*
Diastolic blood pressure(mmHg)	79.55 ± 1.00	78.38 ± 0.76	0.91 ^{ns}
Mean arterial pressure(mmHg)	96.71 ± 1.07	95.16 ± 0.85	1.60 ^{ns}

*($p \leq 0.05$) = Significant, ($p > 0.05$) ^{ns} = not Significant

4.3. SERUM LEPTIN, INSULIN AND GLUCOSE LEVELS OF TYPE 2 DIABETIC AND NON-DIABETIC SUBJECTS

4.3.1. Serum Leptin Levels of Type 2 Diabetic and Non-diabetics Subjects

The mean serum leptin levels (± S.E) were 4.7 ± 0.25 and 4.6 ± 0.17 for the diabetic group and the non-diabetics subjects respectively. The difference observed in the means was not statistically significant (% diff. 2.77, $p > 0.05$) as shown in Table 4.8.

Findings from this study also suggest that serum leptin levels vary with lifestyle factors. The mean leptin level is higher among respondents that do not consume alcohol (% diff. 89.8, $p > 0.05$), non-smokers (% diff. 5.2, $p > 0.05$) and in those respondents that do not engage in adequate physical exercise (% diff. 8.8, $p > 0.05$) in-spite of the fact that the difference were not statistically significant (Table 4.9).

This study also investigated for the variations in leptin levels of the diabetic patients and the normal non-diabetics. The mean and standard error of the leptin level was lower in the diabetic males (4.0 ± 0.25) when compared with their counterparts in the non-diabetics (4.2 ± 0.30). However, the difference was not statistically significant (% diff. 4.73, $p > 0.05$) as depicted in Table 4.10. Similarly, the mean and standard error for the leptin levels of the female diabetic patients (5.0 ± 0.40) was lower than that of the female non-diabetics group (5.3 ± 0.40), and the difference was also not statistically significant (% diff.6.23, $p > 0.05$) (Table 4.11).

The comparison of the means of serum leptin in male and female diabetic patients revealed a marked percentage difference in favor of the female diabetic patients albeit that the difference was not statistically significant (% diff. 23.0, $p > 0.05$) as shown in Table 4.12. The distribution according to age and the difference in means of leptin levels for the type 2 diabetic and the non-diabetics subjects is as summarized in tables 15-19. Between 30 and 69 years of age, it was observed that the percentage difference in the means of leptin was more marked and in favour of the type 2 diabetic patients. Thereafter, the percentage difference in mean leptin levels between the two groups became more marked and consistent in the direction of the normal subjects. However, there was no statistically significant difference across the age groups examined except for the group 54-61 that showed percentage difference in the mean leptin levels between the groups (% diff. 3.2, $p < 0.05$), being higher in the non-diabetic than the diabetic subjects (Table 4.13).

The distribution of leptin levels by age group among the diabetic patients is presented in Table 4.14. Internal comparison of the mean levels between the males and females within the group suggest higher levels of the hormone within all age groups of the female patients as evidenced by percentage difference in favour of the females in most of the age groups. However, the difference was statistically significant only in the 46-53 years age group (% diff.53.13, $p < 0.05$).

The mean leptin levels distribution by age in the non-diabetic subjects shows similar trend as in the diabetic group. The levels are higher among the female subjects in most of the age

groups, but the difference was not statistically significant in any of the age groups as shown in Table 4.15.

When the leptin levels were examined among the diabetic and non- diabetic females by their reproductive age groups, serum leptin levels were observed to be higher in the normal subjects across all the age groups as evidenced by the percentage difference in leptin levels between the groups, but the difference were not statistically significant (Table 4.16).

Table 4.8: Mean Serum Leptin, Insulin and Glucose levels of the subjects

Parameter	Non-Diabetics Mean ± S.E	Type 2 Diabetic Mean ± S.E	% difference
Serum Leptin (ng/ml)	4.70 ± 0.25	4.57 ± 0.17	2.77^{ns}
Serum Insulin (μIU/mL)	4.49 ± 0.01	4.46 ± 0.01	0.67*
Serum FBS (mmol/L)	4.67 ± 0.11	9.35 ± 0.21	-100.21*
HOMA- IR score	0.93 ± 0.02	1.85 ± 0.04	-98.92*

*($p \leq 0.05$) =Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.9: Relationship between Lifestyle Factors and Serum Leptin (ng/ml)

Lifestyle factor	n	Mean ± SE	% difference
Alcohol consumption			
Those that consume alcohol	7	3.11 ± 0.18	-89.85 ^{ns}
Those that do not consume alcohol	252	4.46 ± 0.27	
Smoking status			
Smokers	28	4.25 ± 0.43	-5.18 ^{ns}
Non- Smokers	251	4.47 ± 0.15	
Physical exercise			
Inadequate	164	4.79 ± 0.18	8.77 ^{ns}
Moderate/Adequate	115	4.37 ± 0.21	

$(p > 0.05)^{ns}$ = not Significant

Table 4.10: Mean Serum Leptin, Insulin and Glucose levels in Male Non-diabetics and Type-2 Diabetic Patients

Parameter	Male Non-Diabetics (n=54) Mean ± S.E	Male Type-2 Diabetic (n=78) Mean ± S.E	% difference
Serum Leptin (ng/ml)	4.23 ± 0.30	4.03 ± 0.25	4.73 ^{ns}
Serum Insulin (μIU/mL	4.51 ± 0.02	4.46 ± 0.01	1.11*
Serum FBS (mmol/L)	4.83 ± 1.15	9.60 ± 0.36	-98.76*
HOMA- IR	0.97 ± 0.03	1.90 ± 0.07	-95.88*

*($p \leq 0.05$) = Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.11: Mean Serum Leptin, Insulin and Glucose levels in Female Non-diabetics

Parameters	Non-Diabetic females Mean ± S.E (n = 42)	Type-2 Diabetic females Mean ± S.E (n = 105)	% difference.
Serum Leptin (ng/ml)	5.30 ± 0.40	4.97 ± 0.40	6.23 ^{ns}
Serum Insulin(μU/mL)	5.30 ± 0.01	4.46 ± 0.01	15.85 ^{ns}
Serum FBS (mmol/L)	4.46 ± 0.24	9.16 ± 0.01	-105.38*
HOMA- IR	0.89 ± 0.03	1.81 ± 0.05	-103.37*

*($p \leq 0.05$) = Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.12: Mean Serum Leptin, Insulin and Glucose levels in Male and Female Type-2 Diabetic Patients

Parameter	Male Type-2 Diabetics (n=78) Mean ± S.E	Female Type-2 Diabetics (n=105) Mean ± S.E	% difference
Serum Leptin (ng/ml)	4.03 ± 0.25	4.97 ± 0.40	-23.02 ^{ns}
Serum Insulin(μU/mL	4.49 ± 0.01	4.46 ± 0.01	0.67*
Serum FBS (mmol/L)	9.60 ± 0.36	9.16 ± 0.01	4.58*
HOMA- IR	1.90 ± 0.07	1.81 ± 0.05	4.47*

*($p \leq 0.05$) =Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.13: Serum Leptin levels by age group in Non-diabetics and Type 2 diabetic Patients

Age group	Non-Diabetics Mean ± S.E	n	Type 2 Diabetics Mean ± S.E	n	% difference
30-37	4.54 ± 0.58	21	5.76 ± 0.46	19	-26.87 ^{ns}
38-45	4.69 ± 0.49	21	4.33 ± 0.38	41	7.68 ^{ns}
46-53	5.00 ± 0.59	20	5.15 ± 0.41	28	-3.00 ^{ns}
54-61	4.69 ± 0.59	15	4.54 ± 0.32	35	3.20*
62-69	3.61 ± 1.12	5	4.43 ± 0.39	32	-22.71 ^{ns}
70-77	4.54 ± 0.72	9	3.56 ± 0.49	22	21.59 ^{ns}
78-85	5.64 ± 1.40	5	4.42 ± 1.28	6	21.35 ^{ns}
Total		96		183	

*($p \leq 0.05$) =Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.14: Serum Leptin levels by age group in Type-2 Diabetic Patients Males and Females

Age group	Male Mean ± S.E	n	Female Mean ± S.E	n	% difference
30-37	5.39 ± 0.85	9	6.09 ± 0.46	10	-12.99 ^{ns}
38-45	4.09 ± 0.63	17	4.43 ± 0.47	24	-8.31 ^{ns}
46-53	3.84 ± 0.57	10	5.88 ± 0.48	18	-53.13*
54-61	4.71 ± 0.51	12	4.46 ± 0.42	23	5.31 ^{ns}
62-69	3.55 ± 0.59	13	5.03 ± 0.49	19	-41.69 ^{ns}
70-77	2.99 ± 0.40	15	4.75 ± 1.03	7	-58.86 ^{ns}
78-85	5.29 ± 3.55	2	3.38 ± 1.35	4	36.11 ^{ns}
Total		78		105	

*($p \leq 0.05$) =Significant, ($p > 0.05$) ^{ns} = not Significant

Table 4.15: Serum Leptin levels by Age group in Non-Diabetics Males and Females

Age group	Male Mean ± S.E	n	Female Mean ± S.E	n	% difference
30-37	3.49 ± 0.73	11	5.21 ± 0.92	10	-49.28 ^{ns}
38-45	3.89 ± 0.58	11	5.58 ± 0.75	10	-43.44 ^{ns}
46-53	4.56 ± 0.61	15	6.34 ± 1.41	5	-39.04 ^{ns}
54-61	4.32 ± 0.91	6	4.94 ± 0.81	9	-14.35 ^{ns}
62-69	3.95 ± 1.38	4	2.28 ±0.00	1	42.28 ^{ns}
70-77	4.49 ± 1.28	3	4.56 ± 0.96	6	-1.56 ^{ns}
78-85	4.77 ± 1.45	4	9.00 ±0.00	1	-88.68 ^{ns}
Total		54		42	

$(p > 0.05)^{ns}$ = not Significant

Table 4.16: Serum Leptin levels by Reproductive Age group in Female Respodents

Age group	Female Non Diabetics Mean ± S.E	n	Female Type 2 Diabetics Mean ± S.E	n	% difference
30 – 45 (pre-menop)	5.39 ± 0.58	20	4.96 ± 0.38	34	7.98 ^{ns}
46 – 60 (peri-menop)	5.44 ± 0.72	14	5.08 ± 0.33	41	6.62 ^{ns}
61 – 85 (post-menop)	4.83 ± 0.97	8	4.83 ± 0.42	30	0.00 ^{ns}
Total		42		105	

($p > 0.05$) ^{ns} = not Significant,, pre-menop = pre-menopausal, peri- menop =peri-menopausal, post-menop = post-menopausal

4.3.2. Serum Insulin Levels of the Type 2 Diabetic Patients and Non-diabetic Subjects

The mean fasting insulin levels (± S.E) were 4.49 ± 0.01 and 4.6 ± 0.01 for the non-diabetic and diabetic subjects respectively. The % difference in the means was 0.67 in the direction of the non diabetic subjects and the difference was statistically significant ($p < 0.05$). The mean ± S.E of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) score was 0.93 ± 0.02 and 1.85 ± 0.04 for the non- diabetic and the type 2 diabetic groups respectively. The mean value HOMA-IR value was higher in the diabetic group than the non- diabetics, and the difference in means was statistically significant as shown in Table 4.8 (% diff. 98.92, $p < 0.05$).

Thirty eight (39.58%) of the non- diabetics subjects were found to be insensitive to insulin considering a HOMA-IR score of 1 as 100% sensitive whereas up to 97.9% of the type 2 diabetic patients were adjudged insensitive to insulin using this score. On the other hand, considering a HOMA-IR score of 2 as cut-off point, none of the non- diabetic subjects and 58 (38.69%) of the type 2 diabetic patients were insensitive to insulin.

This study also explored for the variations in fasting insulin levels of the diabetic and non-diabetic subjects with sex. The mean and standard error of insulin was lower in the diabetic males 4.46 ± 0.01 than their male counterpart in the non- diabetic group (4.52 ± 0.02), and a statistically significant difference in the means was observed (% diff. 1.11, $p < 0.05$). The mean HOMA- IR score ± S.E for the diabetic and non diabetic males were 1.90 ± 0.07 and 0.97 ± 0.03 respectively, and the HOMA-IR value was more marked in the diabetic group. A statistically significant difference in the mean HOMA-IR value was also observed

between the groups (% diff. 95.88, $p < 0.05$) as depicted in Table 4.10. Similarly, the mean and standard error for the fasting insulin levels of the female diabetic patients (4.46 ± 0.01) was lower than that of the female non- diabetics (5.30 ± 0.01), albeit the fact that the difference was not statistically significant (% diff. 15.85, $p = > 0.05$). The respective mean HOMA-IR ± S.E for the female diabetic and non diabetic groups (1.81 ± 0.05 and 0.89 ± 0.03). The mean score was higher in the female diabetic patients, and the difference in means between the groups was statistically significant (% diff. -103.37, $p < 0.05$) (Table 4.11).

A comparison of the respective means and standard error of fasting insulin levels between the male and the female diabetic subjects (4.49 ± 0.01 and 4.46 ±0.01) found higher mean of fasting insulin in the male patients, and the difference in means was statistically significant (% diff. 0.67, $p < 0.05$). Similarly, the respective mean HOMA- IR scores ± for the female and male patients (1.81 ± 0.05 and 1.90 ± 0.07) were statistically significant with a higher mean in the male diabetic patients (% diff. 4.47, $p < 0.05$) as shown in Table 4.12.

Findings from this study also suggest that the lifestyle factors did not influence the levels of fasting serum insulin. There was no statistically significant difference between fasting insulin levels and alcohol consumption ($p > 0.05$), cigarette smoking ($p > 0.05$) and physical exercise status ($p > 0.05$) as summarized in Table 4.17.

The means fasting insulin between the non diabetic and type 2 diabetic is shown in Table 4.18. There was no significant variation in the mean values between the two groups across the age groups. Similarly, the comparison in fasting insulin levels by age between the non – diabetic and type 2 diabetic males and females showed no statistically significant variation (Tables 4.19 and 4.20).

Table 4.17: Relationship between Lifestyle Factors and Serum Insulin (µIU/ml)

Lifestyle factor	n	Mean ± SE	% difference
Alcohol consumption			
Those that consume alcohol	7	4.46 ± 0.02	-0.22 ^{ns}
Those that do not consume alcohol	272	4.47 ± 0.01	
Smoking status			
Smokers	28	4.46 ± 0.07	-0.22 ^{ns}
Non- smokers	251	4.47 ± 0.10	
Physical exercise status			
Inadequate	164	4.47 ± 0.01	0.00 ^{ns}
Moderate/Adequate	115	4.47 ± 0.01	

$(p > 0.05)^{ns}$ = not Significant

Table 4.18: Serum Insulin levels by age group in Non-diabetics and Type 2 Diabetic Patients

Age group	Non-Diabetics Mean ± S.E	n	Type 2 Diabetics Mean ± S.E	n	% difference
30-37	4.49 ± 0.02	21	4.50 ± 0.02	19	-0.22 ^{ns}
38-45	4.45 ± 0.01	21	4.44 ± 0.01	41	0.22 ^{ns}
46-53	4.53 ± 0.05	20	4.47 ± 0.01	28	1.32 ^{ns}
54-61	4.49 ± 0.02	15	4.47 ± 0.01	35	0.45 ^{ns}
62-69	4.48 ± 0.02	5	4.45 ± 0.01	32	0.67 ^{ns}
70-77	4.52 ± 0.08	9	4.44 ± 0.01	22	1.77 ^{ns}
78-85	4.46 ± 0.01	5	4.46 ± 0.01	6	0.00 ^{ns}
Total		96		183	

$(p > 0.05)^{ns}$ = not Significant

Table 4.19: Serum Insulin levels by age group in non-diabetics Males and Females

Age group	Male Mean ± S.E	n	Female Mean ± S.E	n	% difference
30-37	4.50 ± 0.18	11	4.48 ± 0.04	10	0.44 ^{ns}
38-45	4.46 ± 0.04	11	4.45 ± 0.03	10	0.22 ^{ns}
46-53	4.54 ± 0.02	15	4.52 ±0.11	5	0.44 ^{ns}
54-61	4.45 ± 0.08	6	4.49 ± 0.09	9	-0.90 ^{ns}
62-69	4.47 ± 0.05	4	4.52 ± 0.00	1	-1.12 ^{ns}
70-77	4.71 ± 0.46	3	4.42 ±0.03	6	6.16 ^{ns}
78-85	4.47 ±0.03	4	4.44 ±0.00	1	0.67 ^{ns}
Total		54		42	

$(P > 0.05)^{ns}$ = not Significant

Table 4.20: Serum Insulin levels by Age group in Type-2 Diabetic Patients

Age group	Male Mean ± S.E	n	Female Mean ± S.E	n	% difference
30-37	4.50 ± 0.12	9	4.50 ± 0.10	10	0.22 ^{ns}
38-45	4.44 ± 0.30	17	4.45 ± 0.04	24	0.45 ^{ns}
46-53	4.47 ± 0.10	10	4.47 ± 0.06	18	0.00 ^{ns}
54-61	4.49 ± 0.12	12	4.46 ± 0.06	23	0.67 ^{ns}
62-69	4.44 ± 0.02	13	4.45 ± 0.08	19	-0.23 ^{ns}
70-77	4.44 ± 0.02	15	4.44 ± 0.04	7	-0.23 ^{ns}
78-85	4.48 ± 0.04	2	4.45 ± 0.02	4	0.67 ^{ns}
Total		78		105	

$(P > 0.05)^{ns}$ = not Significant

4.3.3. Serum Lipids Levels of Type 2 Diabetic and Non-Diabetic Subjects

All the lipid variables measured demonstrated higher mean values in diabetic subjects than non-diabetics except for mean serum HDL-c which is higher in the non-diabetic group (Table 4.21). The difference in means of the serum HDL-c between the non diabetic and diabetic groups was statistically significant (% diff. 25.30, $p < 0.05$). The table further shows that the respective means and S.E of total cholesterol levels between the non diabetics and diabetic subjects (4.32 ± 0.10 and 5.39 ± 0.10) was higher in the diabetic patients, and the difference in means was statistically significant (% difference 24.77, $p < 0.05$). Similarly, the means ± SE of serum LDL-c (3.59 ± 0.11) and serum triglyceride (1.32 ± 0.04) for the diabetic group were higher than the respective values for their non diabetic counter parts (2.25 ± 0.11 and 1.05 ± 0.04), with percentage difference of 59.6 and 25.7 respectively. The result revealed a statistically significant difference in the mean values for both parameters ($p < 0.05$) (Table 4.21).

Comparing the lipids profile between the male diabetic patients and their controls, it was observed that there were higher mean values of serum total cholesterol, serum LDL-c and serum triglycerides in the diabetic males than in the male non diabetic subjects, except for the serum HDL-c which was higher in the non- diabetic as depicted in Table 4.22. The diabetic males when compared with the non- diabetic male subjects have significantly higher serum total cholesterol mean ± SE value (5.55 ± 0.14 versus 4.37 ± 0.13, $p < 0.05$). Similarly, when the mean values for the serum LDL-c were compared between diabetic male (3.98 ± 0.14) and non- diabetic male (2.29 ± 0.15), the result demonstrates a statistically significant difference between the two groups (% diff. 73.80, $p < 0.05$). In the

same vein, the mean ± SE of the serum triglyceride between the male diabetic subjects (1.36 ± 0.07) and their male non- diabetic counterpart (1.01 ± 0.05) also observed a statistically significant difference between the two groups (% diff. 34.65, $p < 0.05$). However, although there is a significant difference between the means ± SE of serum HDL-c between the diabetic male group (1.21 ± 0.05) and non- diabetic male group (1.64 ± 0.08), the difference is higher in the male non- diabetic than the male diabetic group as summarized in Table 4.22.

This study also compared the serum lipids between the female diabetic and female non-diabetic groups, and significant difference in the means of serum total cholesterol, HDL-c, LDL-c and triglycerides were also observed. The mean ± SE of serum cholesterol was 5.10 ± 0.14 and 4.26 ± 0.17 for the female diabetic and female non- diabetic groups respectively, with a statistically significant difference (% diff. 19.72, $p < 0.05$), being higher in the non-diabetic than in the diabetic group (Table 4.23).

Comparison between the means and standard error of serum LDL-c for the female diabetic (3.31 ± 0.15 and non- diabetic (2.19 ± 0.16) groups, found a higher mean value in the female diabetic group and a mean difference of 24.40 observed was statistically significant ($p < 0.05$). The difference in the mean values observed in this study between serum triglyceride for the female diabetic (1.29 ± 0.05) and female non diabetics (1.10 ± 0.07) was higher in non- diabetic group and is statistically significant (% diff. 17.27, $p < 0.05$). On the other hand, comparing the mean values for serum HDL-c for the diabetic female group (1.27 ± 0.04) and non- diabetic subjects (1.68 ± 0.07) showed a higher mean value in the diabetic

than the non- diabetic group, and the mean difference is statistically significant (% diff. 24.0, $p < 0.05$) as depicted in Table 4.23.

This study also explored for the variations in lipid levels between the male and female diabetic patients. The mean serum levels for serum total cholesterol, serum LDL-c and serum triglyceride were higher in male diabetics than the female diabetics except for serum HDL-c which is higher in the female diabetic group. The mean and standard error of the serum total cholesterol level was higher in the male diabetic group (5.77 ± 0.14) when compared with that of female diabetic group (5.10 ± 0.14) and the difference was statistically significant (% diff. 11.61, $p < 0.05$). Similarly, the mean and standard error for the serum LDL-c levels of the male diabetic patients (3.98 ± 0.14) was higher than that of the female diabetic group (3.31 ± 0.15), and the difference was also statistically significant (% diff. 16.83, $p < 0.05$). Comparing the mean values of serum triglyceride between the male and female diabetic subject, the mean ± SE for the serum triglyceride of the diabetic male (1.36 ± 0.07) is higher than that of the diabetic female (1.29 ± 0.05).There is statistically significant difference (% diff. 5.15, $p < 0.05$) between the two groups. However, the difference in the means for serum HDL-c between the male diabetic and the female diabetic (% diff. 4.16) favours the female diabetic groups and is statistically significant ($p < 0.05$) as illustrated in Table 4.24.

Table 4.21: Mean Serum Lipid levels of the subjects

Parameter (mmol/L)	Non-Diabetics Mean ± S.E	Type 2 Diabetics Mean ± S.E	% difference
Serum Total cholesterol	4.32 ± 0.10	5.39 ± 0.10	-24.77*
Serum HDL-c	1.66 ± 0.05	1.24 ± 0.03	25.30*
Serum LDL-c	2.25 ± 0.11	3.59 ± 0.11	-59.56*
Serum Triglyceride	1.05 ± 0.04	1.32 ± 0.04	-25.71*

*($p \leq 0.05$) = Significant

Table 4.22: Lipid Profile in Male Non-Diabetics and Type-2 Diabetic Patients

Parameter (mmol/L)	Male Non-Diabetics (n=54) Mean ± S.E	Male Type-2 Diabetics (n=78) Mean ± S.E	% difference
Serum Total cholesterol	4.37 ± 0.13	5.77± 0.14	-32.04*
Serum HDL-c	1.64 ± 0.08	1.21±0.05	26.22*
Serum LDL-c	2.29 ± 0.15	3.98± 0.14	-73.80*
Serum Triglyceride	1.01 ± 0.05	1.36±0.07	-34.65*

* ($p \leq 0.05$) = Significant

Table 4.23: Lipid profile of Female Non-Diabetics and Type-2 Diabetic Patients

Parameter (mmol/L)	Non-Diabetic females Mean ± S.E	Type-2 Diabetic females Mean ± S.E	% difference
Serum cholesterol	4.26 ± 0.17	5.10 ± 0.14	-19.72*
Serum HDL-c	1.68 ± 0.07	1.27 ± 0.04	24.40*
Serum LDL-c	2.19 ± 0.16	3.31 ± 0.15	-51.14*
Serum Triglyceride	1.10 ± 0.07	1.29 ± 0.05	-17.27*

*($p \leq 0.05$) = Significant

Table 4.24: Lipid profile of Male and Female Type-2 Diabetic Patients

Parameter (mmol/L)	Male Type-2 Diabetics (n=78) Mean ± S.E	Female Type-2 Diabetics (n=105) Mean ± S.E	% difference
Serum cholesterol	5.77± 0.14	5.10 ± 0.14	11.61*
Serum HDL-c	1.21±0.05	1.27 ± 0.04	-4.16*
Serum LDL-c	3.98± 0.14	3.31 ± 0.15	16.83*
Serum Triglyceride	1.36±0.07	1.29 ± 0.05	5.15*

*($p \leq 0.05$) = Significant

4.4 RELATIONSHIP BETWEEN SERUM LEPTIN, INSULIN, BLOOD GLUGOSE AND ANTHROPOMETRIC PARAMETERS OF THE RESPONDENTS

The relationship between serum leptin and anthropometric parameters in the diabetic and non- diabetic subjects is described in figures 4.7-4.16.

Figures 4.7 and 4.8 show significant positive correlation between serum leptin and body mass index in both diabetic ($r = 0.36$, $p = < 0.05$) and the non- diabetic ($r = 0.48$, $p = < 0.05$) subjects. As serum leptin increases, there is a corresponding increase in body mass index. Similarly, serum leptin levels are positively correlated with waist circumference in diabetic and non- diabetic groups, as indicated by corresponding increase in waist hip ratio as the serum leptin levels increases. The data from this assessment suggest that the relationship is statistically significant in both the diabetic patients ($r = 0.32$, $p = < 0.05$) and the non-diabetic group ($r = 0.39$, $p = < 0.05$) as shown in Figure 9 and 10.

The relationship between serum leptin levels and waist hip ratio in the diabetic and non-diabetic groups is depicted in Figures 11 and 12. In both illustrations, waist hip ratio increases with increasing serum leptin concentration. However, the relationship is not statistically significant (diabetic subjects: $r = - 0.04$, $p = > 0.05$; non-diabetic group: $r = 0.10$, $p = > 0.05$).

Figures 13 and 14 depict the relationship between serum leptin and serum insulin in diabetic and non- diabetic groups. Serum leptin is positively correlated with serum insulin in the diabetic group although the relationship is not statistically significant ($r = 0.11$, $p = > 0.05$). However, a negative correlation was observed between serum leptin and serum insulin in

the non- diabetic subjects, with decreasing serum insulin level as serum leptin increases, and the relationship is also not statistically significant ($r = - 0.06$, $p = > 0.05$).

The plot between serum leptin and serum fasting blood sugar in diabetic group shows significant negative correlation ($r = - 0.18$, $p = < 0.05$), with a decreasing trend in fasting blood sugar level as serum leptin increases (Figure 15). On the other hand, a statistically significant positive correlation was observed in the non- diabetic subjects between serum leptin levels and serum fasting blood sugar ($r = 0.21$, $p = < 0.05$) as shown in Figure 16. In the same vein, the plot between serum leptin and HOMA-IR score in the diabetic group shows a negative correlation ($r = - 0.18$, $p = < 0.05$) (Figure 17). However a positive correlation was observed in the non-diabetic group ($r = 0.20$, $p = < 0.05$) as shown in Figure 18.

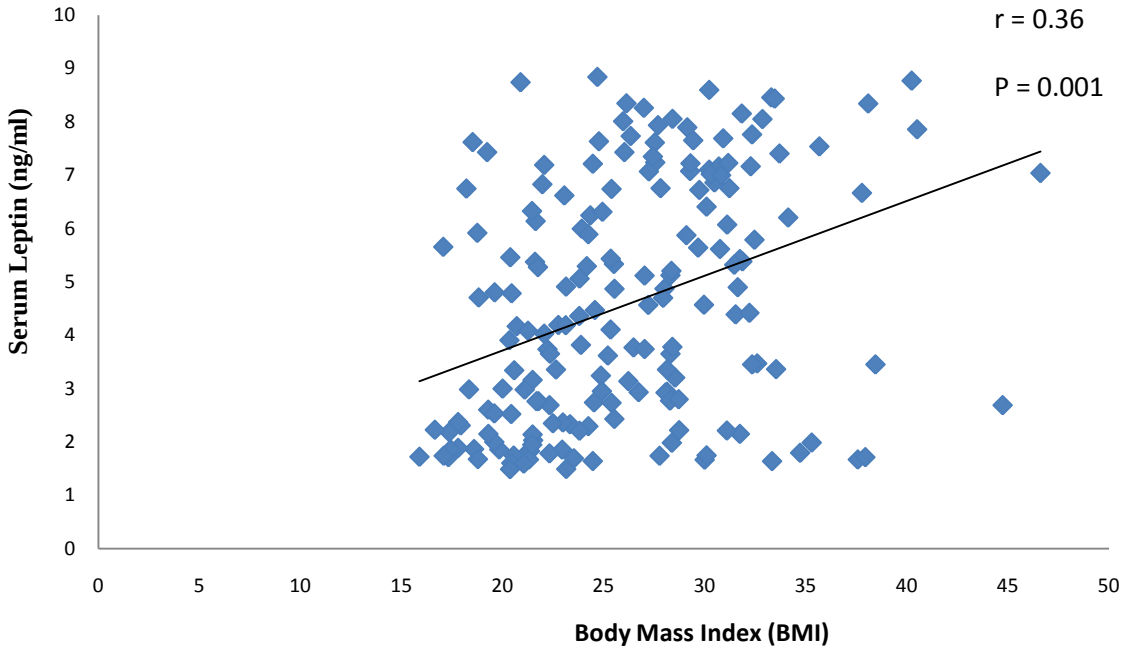


Fig 7: Relationship between Serum Leptin and BMI in Diabetic patients

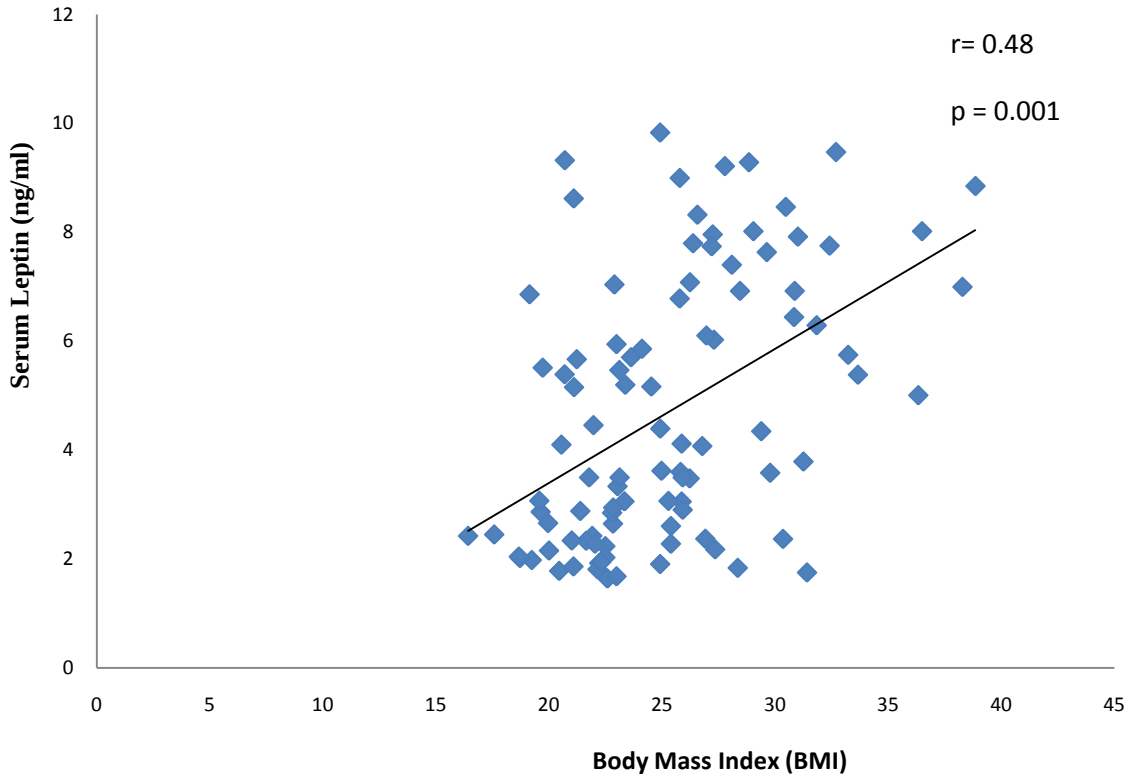


Fig 4.8: Relationship between Serum Leptin and BMI in Non-Diabetic group

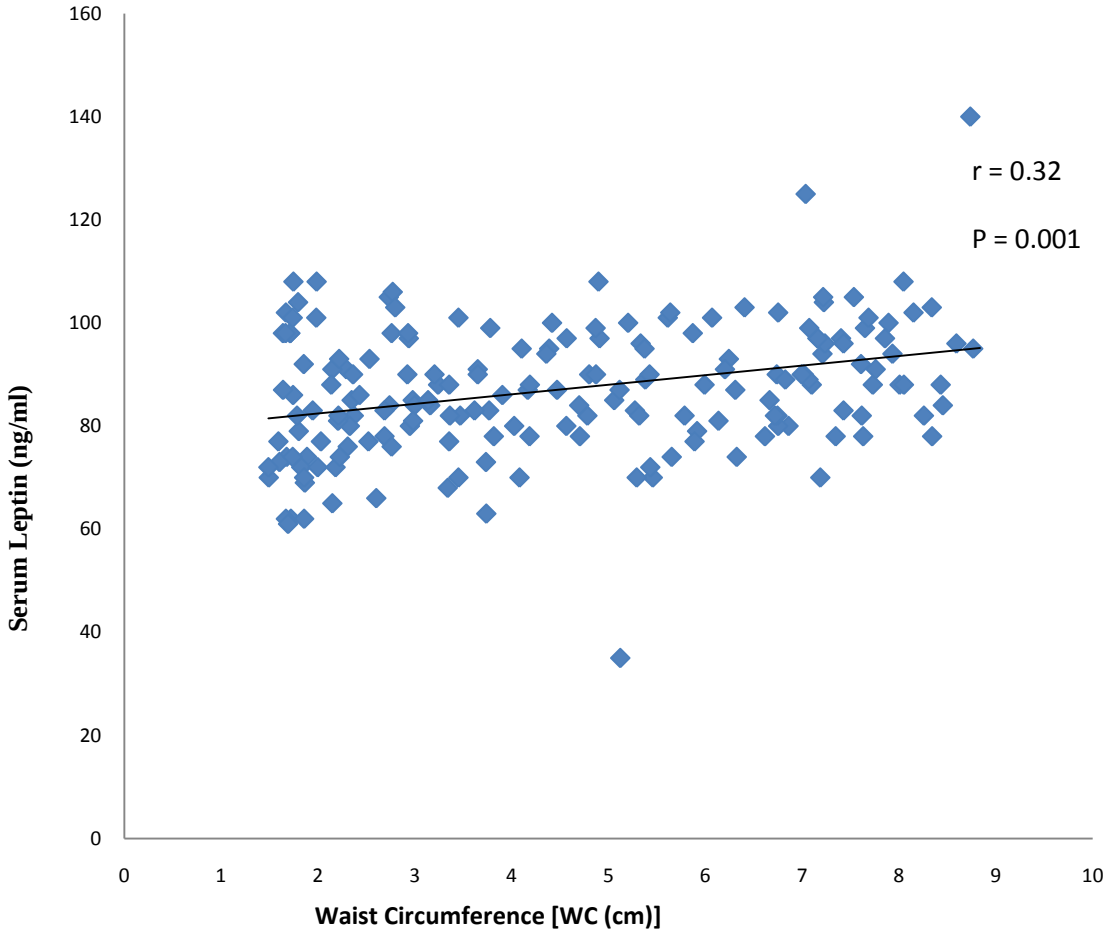


Fig 4.9: Relationship between Serum Leptin and Waist circumference in Diabetic patients

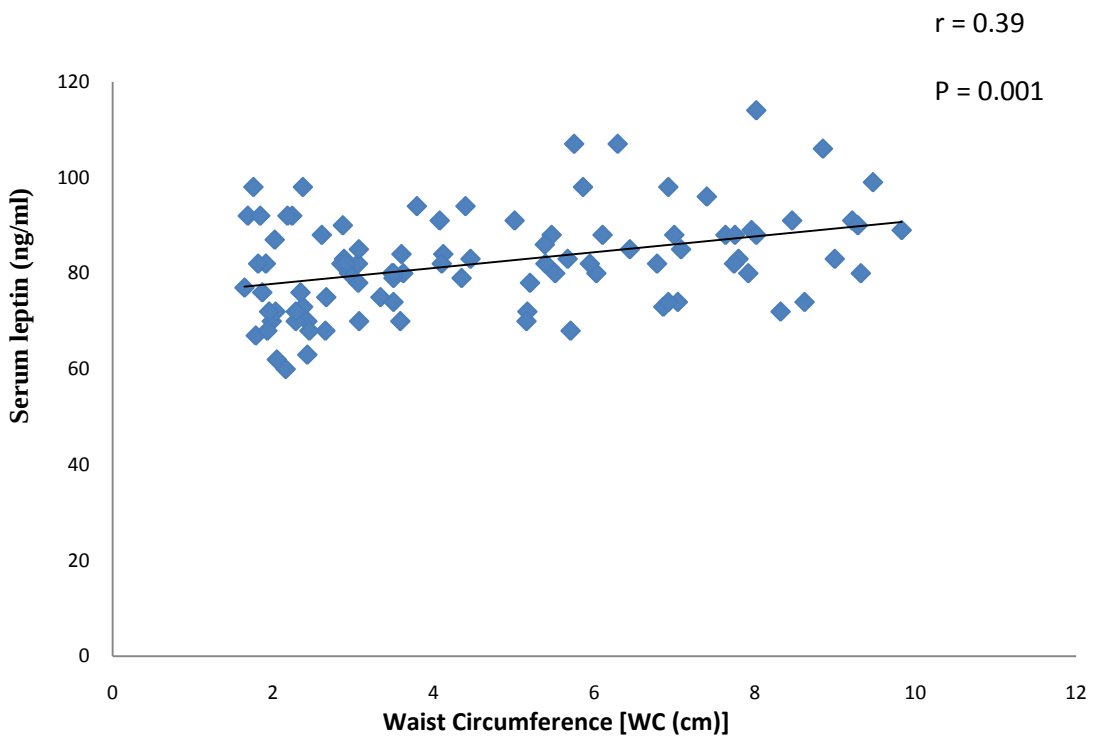


Fig 4.10: Relationship between Serum Leptin and Waist circumference in Non-Diabetic group

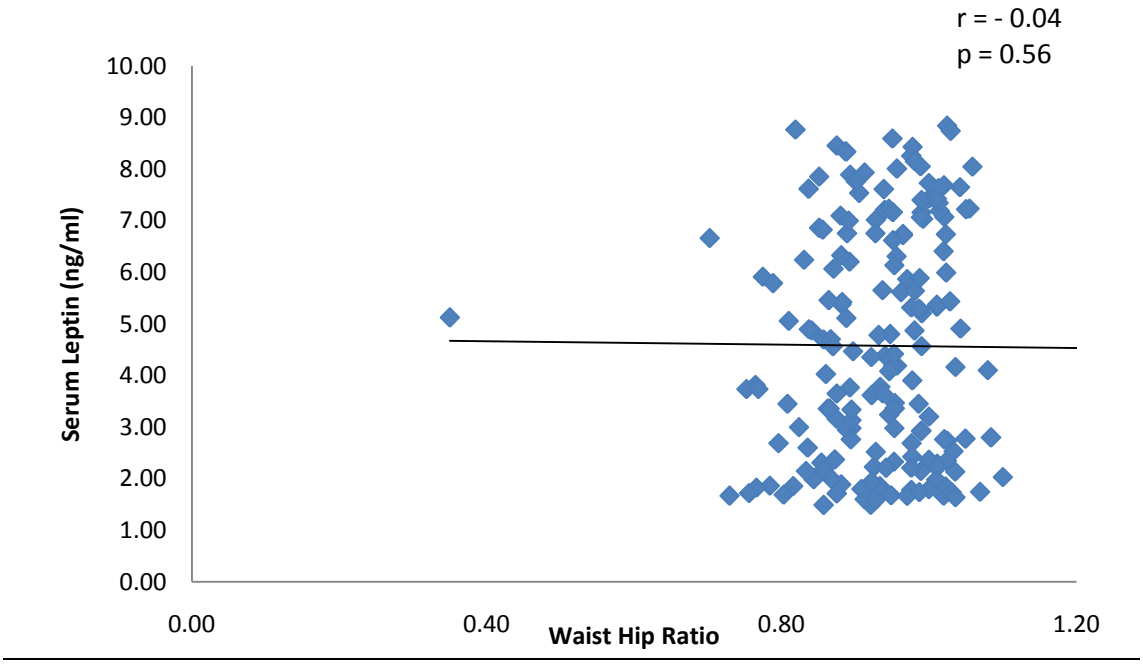


Fig 4.11: Relationship between Serum Leptin and Waist hip ratio in Diabetic group

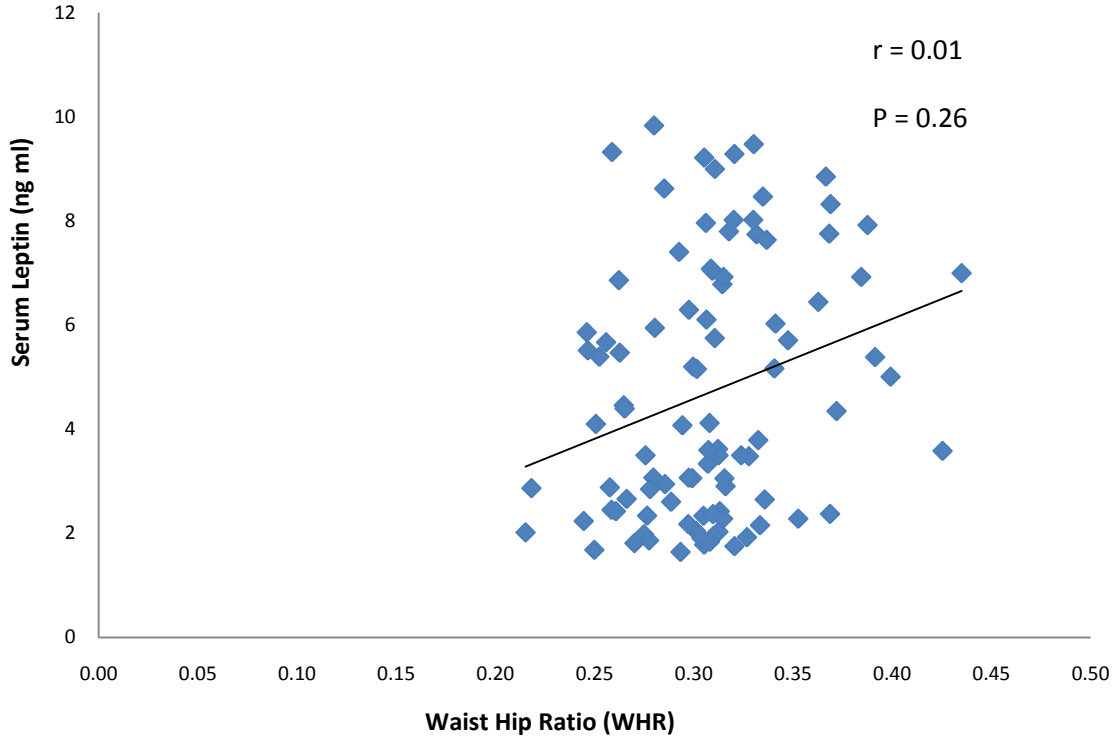


Fig 4.12: Relationship between Serum Leptin and Waist Hip Ratio in Non-Diabetic group

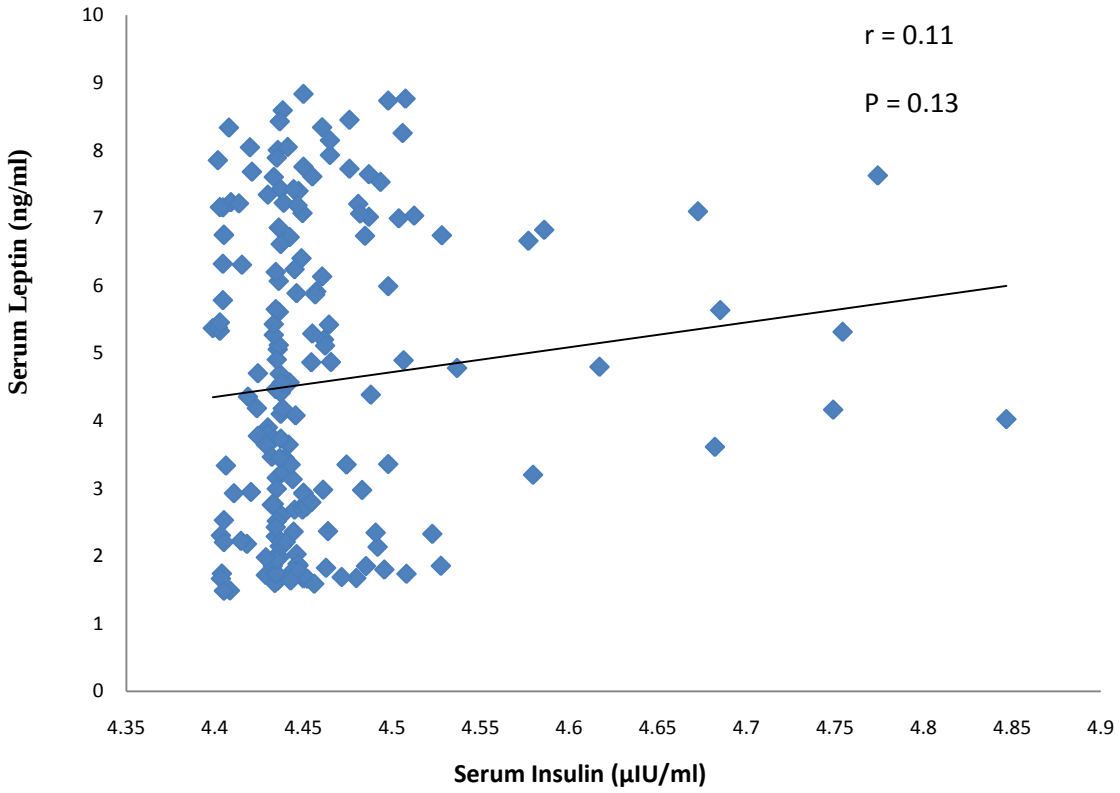


Fig 4.13: Relationship between Serum Leptin and Insulin in Diabetic group

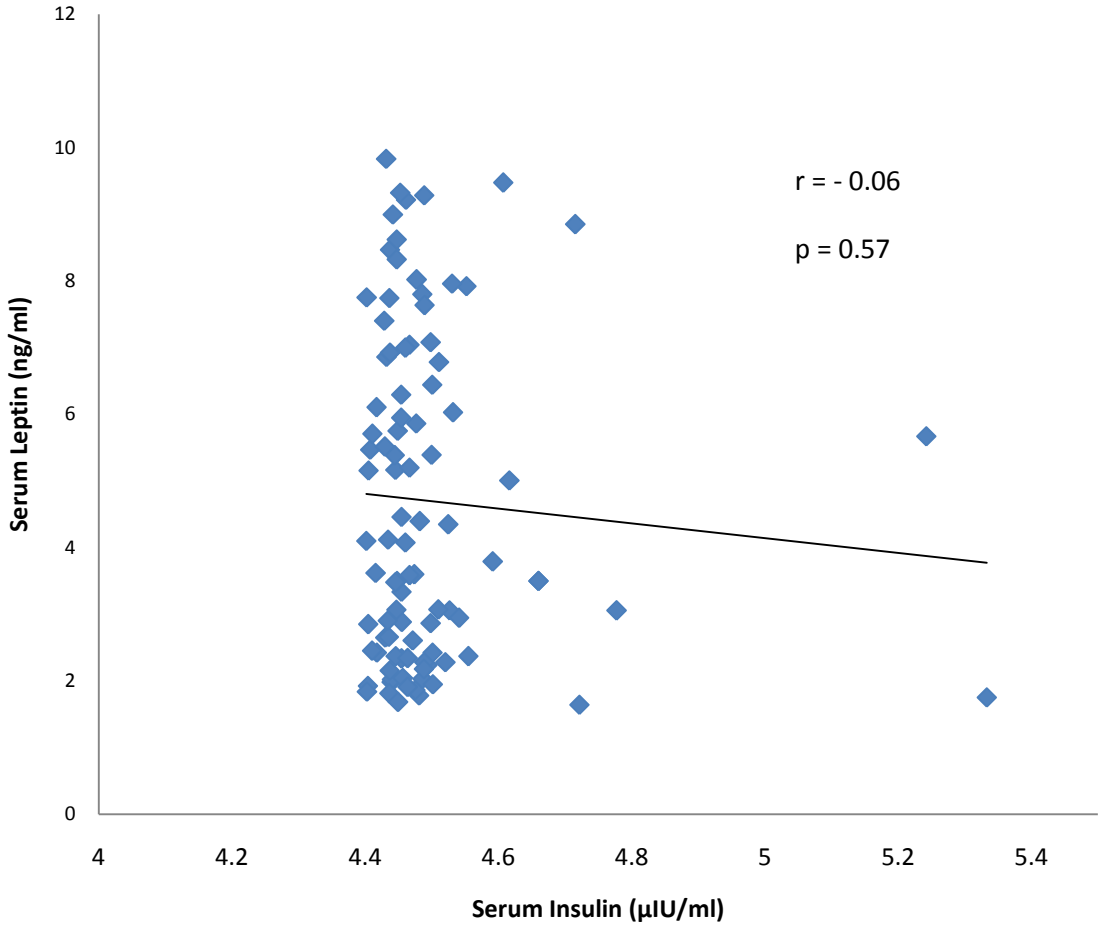


Fig 4.14: Relationship between Serum Leptin and Insulin in Non-Diabetic group

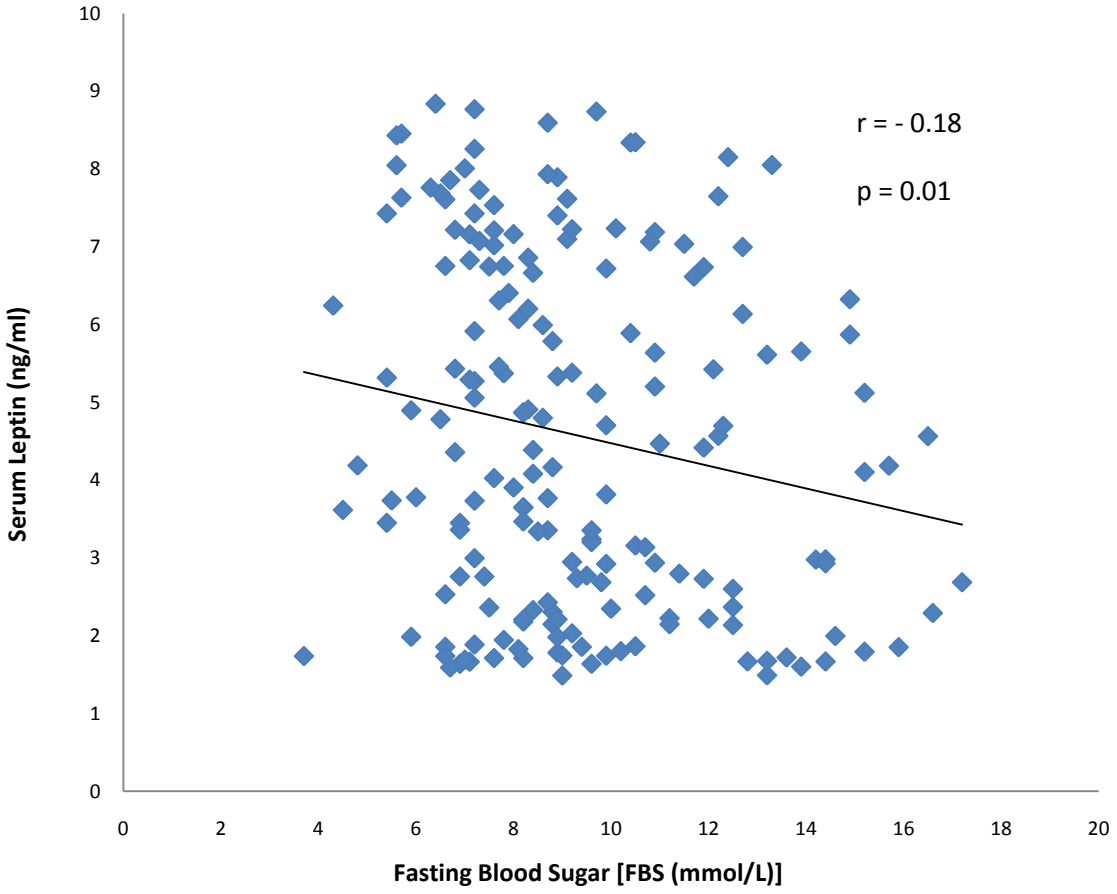


Fig 4.15: Relationship between Serum Leptin and Fasting blood sugar in Diabetic patients

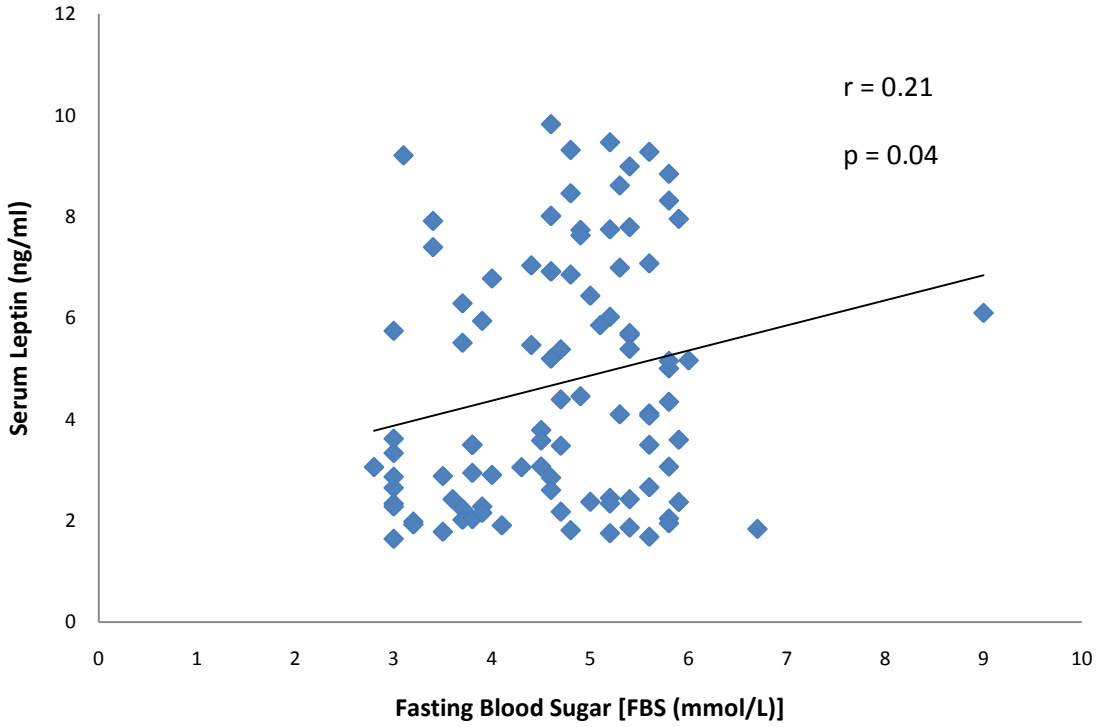


Fig 4.16: Relationship between Serum Leptin and Fasting blood sugar in Non-Diabetic group

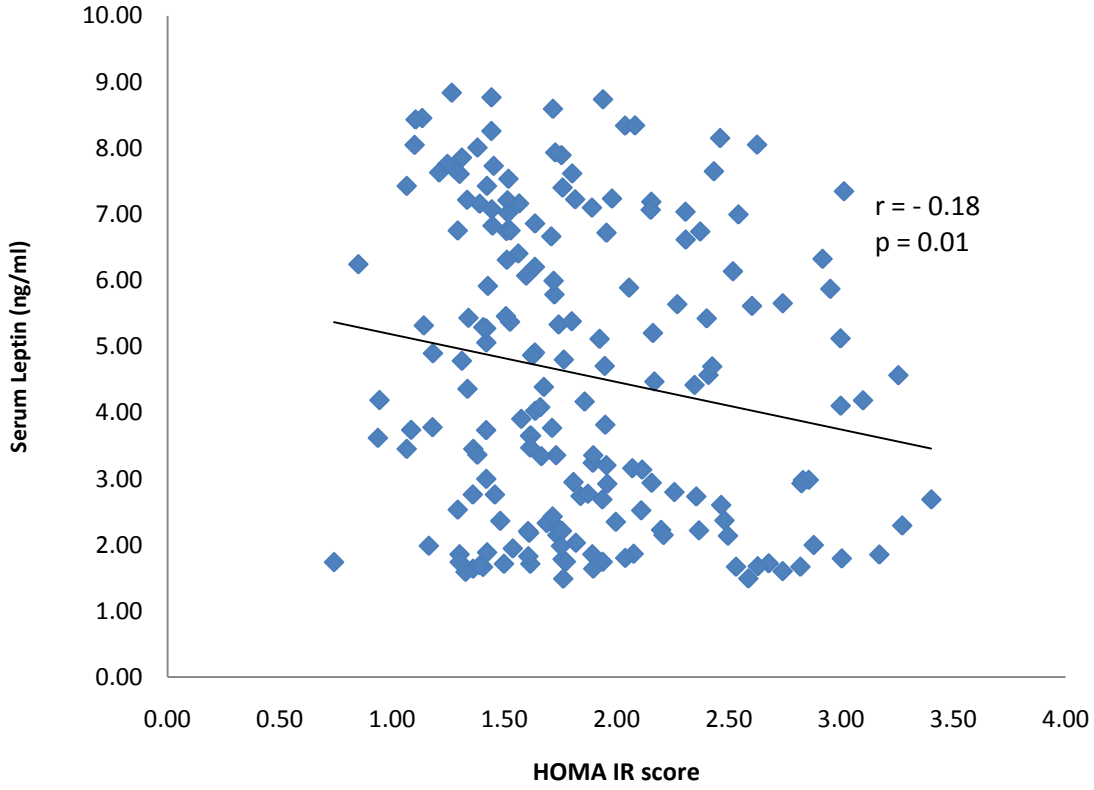


Fig 4.17: Relationship between Serum Leptin and HOMA-IR score in Diabetic patients

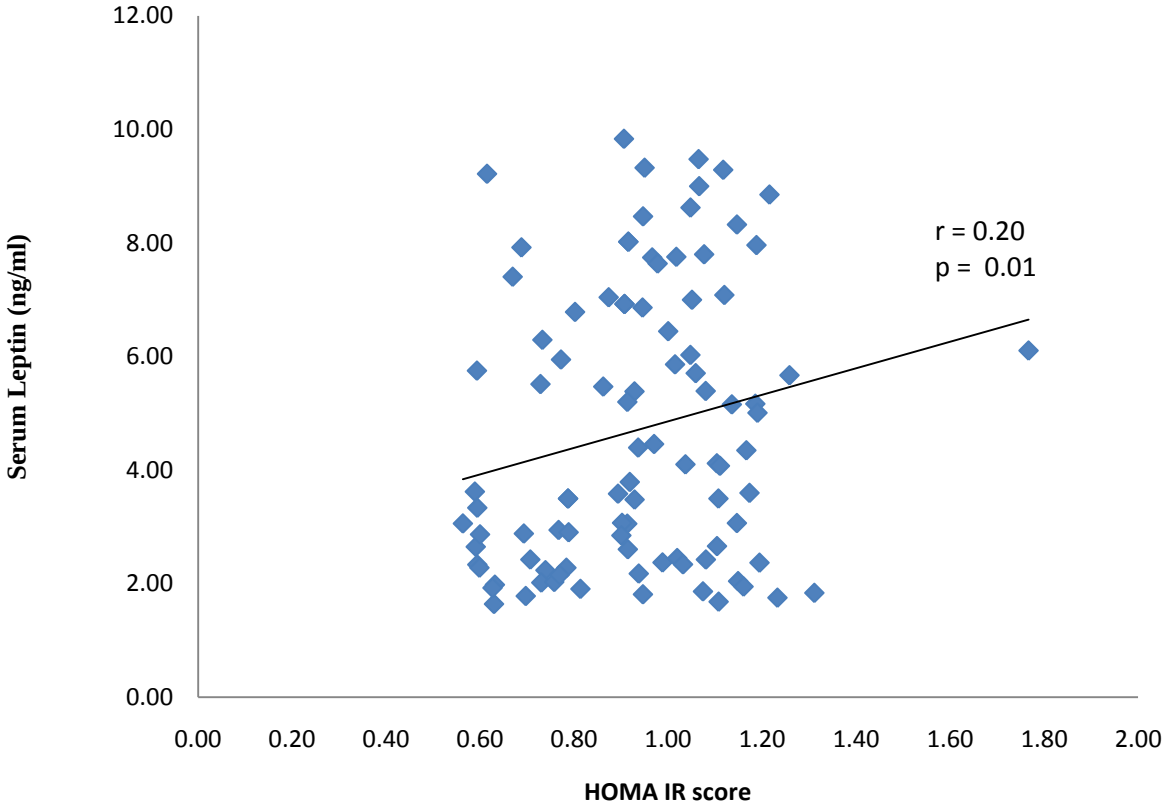


Fig 4.18: Relationship between Serum Leptin and HOMA IR in Non-Diabetic group

When the biological and anthropometric parameters that were significantly correlated with serum leptin were considered in that model for multivariate analysis to identify independent predictors of serum leptin level, it was observed that age, body mass index and waist circumference emerged as the only independent predictors of serum leptin level in the diabetic patients. On the other hand, only body mass index emerged as the independent predictor of serum leptin in the non- diabetic subjects. Fasting blood sugar was the only factor among the biochemical parameters that was significantly correlated with serum leptin level in both the diabetic and non- diabetic patients (Table 4.25).

In exploring the anthropometric and biochemical predictor of serum insulin level, it was observed that none of the anthropometric and biochemical parameters were significantly correlated with serum insulin (Table 4.26).

In determining the independent biochemical predictors of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), statistically significant biochemical parameters were included in a model. The result showed that fasting blood sugar emerged as the only independent biochemical predictor of HOMA-IR score in both the diabetic patients and the control. Interestingly hip circumference and body mass index were the only anthropometric parameters that were significantly correlated with HOMA-IR score in the diabetic and non-diabetic group respectively and as such did not qualify for inclusion into any model (Table 4.27).

Table 4.28 summarizes the anthropometric and biochemical predictors of serum fasting blood sugar. It can be seen from the table that serum cholesterol and triglycerides were the only independent predictors of serum fasting blood sugar in the non- diabetic subjects

Table 4.25: Relationship between Serum Leptin, Anthropometric parameters, Lipid profile, Insulin and FBS among the respondents.

	Non-Diabetics				Type-2 Diabetic Patients			
	Bi-variate analysis		Multiple linear regression		Bi-variate analysis		Multiple linear regression	
Parameter	**r**	**P value**	**'t'-test**	**P value**	**r**	**P value**	**'t'- test**	**P value**
Anthropometry								
Age	0.009	0.93 ^{ns}	-	-	-0.17	0.02*	-2.79	0.006*
BMI	0.48	0.001*	3.29	0.001*	0.36	0.001*	2.62	0.01*
WC	0.39	0.001*	0.81	0.42 ^{ns}	0.32	0.001*	2.14	0.03*
HC	0.29	0.004*	-0.03	0.99 ^{ns}	0.33	0.001*	0.87	0.39 ^{ns}
WHR	0.10	0.26 ^{ns}	-	-	-0.04	0.56 ^{ns}	-	-
Biochemical								
Cholesterol	0.006	0.96 ^{ns}	-	-	0.09	0.20 ^{ns}	-	-
HDL-C	-0.08	0.42 ^{ns}	-	-	-0.13	0.08 ^{ns}	-	-
LDL-C	0.01	0.89 ^{ns}	-	-	0.10	0.16 ^{ns}	-	-
Triglycerides	0.09	0.37 ^{ns}	-	-	0.04	0.58 ^{ns}	-	-
FBS	0.21	0.04*	-	-	-0.18	0.01*	-	-
Insulin	-0.06	0.57 ^{ns}	-	-	0.11	0.13 ^{ns}	-	-
HOMA-I	0.20	0.01*	-0.33	0.74	-0.18	0.01*	1.21	0.23

r = Pearson's correlation coefficient, $p \leq 0.05$*Significant, $p > 0.05$ ^{ns}Not significant.

Table 4.26: Relationship between Serum Insulin, Anthropometric, Lipid Profile, FBS and Leptin.

	Non-Diabetics				Type-2 Diabetic Patients			
	Bi-variate analysis		Multiple linear regression		Bi-variate analysis		Multiple linear regression	
Parameter	**r**	**P value**	**'t'-test**	**P value**	**r**	**P value**	**'t'- test**	**P value**
Anthropometric								
Age	0.07	0.50 ^{ns}	-	-	-0.13	0.07 ^{ns}	-	-
BMI	0.16	0.10 ^{ns}	-	-	0.01	0.90 ^{ns}	-	-
WC	0.19	0.06 ^{ns}			0.004	0.95 ^{ns}	-	-
HC	0.19	0.06 ^{ns}	-	-	-0.004	0.95 ^{ns}	-	-
WHR	-0.002	0.88 ^{ns}	-	-	-0.005	0.95 ^{ns}	-	-
Biochemical								
Cholesterol	-0.07	0.52 ^{ns}	-	-	-0.13	0.07 ^{ns}	-	-
HDL-C	-0.07	0.49 ^{ns}	-	-	-0.03	0.68 ^{ns}	-	-
LDL-C	-0.03	0.79 ^{ns}	-	-	-0.12	0.10 ^{ns}	-	-
Triglycerides	-0.01	0.32 ^{ns}	-	-	-0.005	0.95 ^{ns}	-	-
FBS	0.03	0.73 ^{ns}	-	-	-0.14	0.06 ^{ns}	-	-
Leptin	-0.06	0.57 ^{ns}	-	-	0.11	0.13 ^{ns}	-	-

r = Pearson's correlation coefficient, $p \leq 0.05$*Significant, $p > 0.05$ ^{ns}Not significant.

Table 4.27: Relationship between HOMA- IR, Anthropometric and other Biochemical parameters

	Non-Diabetics				Type-2 Diabetic Patients			
	Bi-variate		Multiple linear regression		Bi-variate		Multiple linear regression	
Parameter	**r**	**P value**	**'t'-test**	**P value**	**r**	**P value**	**'t'- test**	**P value**
Anthropometri								
Age	0.006	0.95	-	-	0.03	0.66	-	-
BMI	0.21	0.03*	-	-	-0.11	0.16	-	-
WC	0.15	0.15	-	-	-0.12	0.11	-	-
HC	0.04	0.72	-	-	-0.15	0.03*	-	-
WHR	0.13	0.21	-	-	0.04	0.58	-	-
Biochemical								
Cholesterol	0.04	0.71	-	-	-0.03	0.66	-	-
HDL-C	0.30	0.003*	-0.78	0.44	-0.03	0.71	-	-
LDL-C	0.08	0.41	-	-	-0.02	0.74	-	-
Triglycerides	0.15	0.14	-	-	0.10	0.19	-	-
FBS	0.99	0.001*	60.98	0.001	1.0	0.001*	307.74	0.001*
Insulin	0.18	0.08	-	-	-0.10	0.19	-	-
Leptin	0.20	0.04*	-0.33	0.74	-0.18	0.01*	1.21	0.23

r = Pearson's correlation coefficient, $p \leq 0.05$*Significant, $p > 0.05$ ^{ns}Not significant.

Table 4.28: Relationship between FBS, Anthropometric and other Biochemical parameters

	Non-Diabetics				Type-2 Diabetic Patients			
	Bi-variate				Bi-variate		Multiple logistic regression	
Parameter	**r**	**P value**	**'t'-test**	**P value**	**r**	**P value**	**'t'- test**	**P value**
Anthropometri								
Age	-0.005	0.96 ^{ns}	-	-	-0.03	0.71 ^{ns}	-	-
BMI	0.19	0.06 ^{ns}	-	-	-0.11	0.15 ^{ns}	-	-
WC	0.12	0.25 ^{ns}	-	-	-0.12	0.10 ^{ns}	-	-
HC	0.01	0.91 ^{ns}	-	-	-0.16	0.04 *	-	-
WHR	0.13	0.21 ^{ns}	-	-	0.04	0.58 ^{ns}	-	-
Biochemical								
Cholesterol	-0.03	0.77 ^{ns}	-	-	-0.03	0.71 ^{ns}	-	-
HDL-C	-0.29	0.004 *	-2.85	0.005 *	-0.03	0.71 ^{ns}	-	-
LDL-C	0.09	0.39 ^{ns}	-	-	-0.02	0.79 ^{ns}	-	-
Triglycerides	0.17	0.10 ^{ns}	-	-	0.09	0.19 ^{ns}	-	-
Leptin	0.21	0.04 *	1.93	0.05 *	-0.18	0.01 *	-	-
Insulin	0.03	0.73 ^{ns}	-	-	-0.14	0.06 ^{ns}	-	-

r = Pearson's correlation coefficient, $p \leq 0.05$*Significant, $p > 0.05$ ^{ns}Not significant.

4.4 RELATIONSHIP BETWEEN LEPTIN, OBESITY AND TYPE 2 DIABETES MELLITUS

Table 4.29 below shows the difference in mean leptin levels between the obese and non-obese diabetic patients studied. Mean serum leptin level was higher in the obese patients, and the difference is statistically significant (% diff. -3.32, $p < 0.05$).

When compared with the obese non- diabetic counterparts, it was observed that mean serum leptin level was higher in the male non- diabetic subjects. Similarly, the mean serum leptin level for the obese female non- diabetic subjects was higher than that of the diabetic patients as depicted in Table 4.30.

Table 4.33 found that out of 62 obese subjects among the respondents, 47 of them are diabetics and only 15 of them are non-diabetics.

Table 4.29: Serum Leptin levels (ng/ml) of Obese and Non- obese Type 2 Diabetic subjects

OBESITY	n	Mean ± SE	% difference
Non- obese	136	4.24 ± 0.18	-3.32*
Obese	47	5.53 ± 0.34	
Total	**183**		

*Significant, $p \leq 0.05$

Table 4.30: Serum Leptin levels of Obese Non-Diabetics and Type-2 Diabetic Patients by Sex

Subjects	**Non-diabetics Mean ± S.E**	**n**	**Type-2 Diabetics Mean ± S.E**	**n**	**% difference**
Male	5.99 ± 0.41	26	4.66 ± 0.70	12	22.20 ^{ns}
Female	7.14 ± 0.61	4	5.68 ± 0.79	10	20.45 ^{ns}
Total		**30**		**22**	

$P > 0.05$ ^{ns}Not significant

Table 4.31: Obesity and Type 2 Diabetes Mellitus

Diabetes	**Obese**	**Non obese**	**Total**
Type 2 Diabetics	47 (75.8)	136 (62.7)	183
Non Diabetics	15 (24.2)	81 (37.3)	96
Total	**62 (100)**	**217 (100)**	**279**

$X^2 = 5.34$, $p = 0.02$, OR (95% C.I) = 0.41 (0.19, 0.89)

CHAPTER FIVE

DISCUSSION

Type 2 diabetes mellitus is one of the fastest-growing health problems in the World, which is reaching epidemic proportion in some regions, as consequence of life-style, lack of exercise, unhealthy diet, obesity and overweight (International Diabetes Federation, 2013). Type 2 diabetes makes up 90-95% of diabetes mellitus worldwide and the current diabetes epidemic is attributable to rising cases of obesity (King *et al*., 1998).

5.1 WEIGHT, HEIGHT, BODY MASS INDEX, WAIST CIRCUMFERENCE, HIP CIRCUMFERENCE AND WAIST HIP RATIO OF THE RESPONDENTS

This study observed similarities in the anthropometric parameters (weight, height, BMI, waist circumference (WC) and hip circumference (HC)) between the male diabetic subjects and their matched non-diabetic subjects except for the waist hip ratio (WHR) which was observed to be significantly higher in the experimental group. Significant higher mean values of BMI and WC was also found in the female experimental group when compared to their controlled counterparts. These findings are consistent with the findings of several investigators that measures of obesity (BMI, WC, WHR) are associated with the risk of developing type 2 diabetes mellitus (Anjana *et al*., 2004).The observed higher values of waist (abdominal circumference) and waist to hip ratio among the male diabetic group compared to their control counterparts supports the findings of Anjana *et al*., (2004) that abdominal fat is more associated with the risk of developing type 2 diabetes than BMI. This is based largely on the rationale that increased visceral adipose tissue is associated with a range of metabolic abnormalities, including decreased glucose tolerance, reduced insulin

sensitivity and adverse lipid profiles, which are risk factors for type 2 diabetes and cardiovascular disease (CVD) (Bello-Sani *et al.,* 2007*).*

Expectedly, the weight, height, body mass index, waist circumference and waist to hip ratio are higher in males then females. Sex differences in body composition are primarily attributable to the action of sex hormones, which drive the dimorphisms during pubertal development. In men, a reduction in free testosterone levels is associated with an increase in fat mass and reduction in muscle mass, and both total and free testosterone levels are inversely associated with obesity (Derby *et al*., 2006).

This study observed that mean pulse rate and systolic blood pressure of the diabetic subjects are higher than in the in the non diabetic group. Large scale studies have shown higher pulse pressure and higher systolic pressure in diabetic than in non- diabetic subjects (Gudmundsdottir *et al*., 2006). Diabetes mellitus is associated with metabolic derangements like elevated plasma lipoproteins more especially in patient with poor glucose control (Onyemelukwe and Stafford, 1983). Higher levels of systolic blood pressure may be associated with augmented oxidation of low-density lipoprotein cholesterol and formation of glycated low-density lipoprotein, which enhance foam cell formation with resultant anatomic and physiologic abnormalities of the vascular endothelium. Also insulin, a glucose regulating hormone augments the reabsorption of sodium and water (obligatory water and sodium reabsorption) from the distal tubules of the kidneys thereby increasing the blood volume and hence systolic blood pressure (Bakari and Onyemelukwe, 2009).

However, the mean diastolic and arterial pressures of both the diabetic and non-diabetic patients in this study were within normal limits.

5.2 SERUM LEPTIN, INSULIN, AND GLUCOSE LEVELS OF TYPE 2 DIABETIC AND NON-DIABETIC SUBJECTS

5.2.1 Serum Leptin levels of Type 2 Diabetic and Non-Diabetic Subjects

Leptin, an adipocyte hormone is a product of human leptin gene. The concentration of the hormone is proportional to body mass adiposity, with higher levels found in individuals with obesity compared with lean weight healthy individuals (Martins *et al*., 2012). Although leptin was originally recognized for its role as a satiety factor, it is now implicated in a wide variety of multisystem biological functions, including the regulation of glucose homeostasis. Emperical evidence suggested that leptin hormone deficiency or leptin receptor resistance manifest with hyperphagia, obesity, type 2 diabetes and neuroendocrine disorders (Wang *et al*., 2014).

In this study, low levels of serum leptin concentration were observed in both the diabetics and the non-diabetic subjects, although the level was slightly higher in the non-diabetic patients. This finding is in tandem with that of Kazmi *et al*., (2010) from Pakistan and Al-shoumer *et al*., (2008) from Saudi Arabia. Similarly, Onyemelukwe *et al*., (2009) from Zaria, Nigeria reported significantly lower leptin levels in wasted HIV infected patients when compared with normal weight HIV infected patients. The low leptin levels observed in the studies above may be attributed to the low adipose tissue mass, as reflected in the BMI of the various subjects irrespective of the cause of the low adiposity. Low leptin levels are

also associated with low insulin levels because insulin is a stimulator of leptin synthesis (Tsai *et al*., 2012). This implies that a decrease in the number of functional producing beta cells occurs in diabetic subjects and as a result, insufficient insulin is produced by beta cells lowering leptin level in type 2 diabetic subjects (Donth *et al*., 2005). Furthermore, subjects with diabetes have increased visceral fat and less subcutaneous fat. In addition, it is known that visceral fat produces less leptin than subcutaneous fat thus subjects with diabetes would be expected to have lower circulating levels than non- diabetic subjects (Montaque *et al*., 2005). This current study also concurs with findings observed by Couillard *et al*., (1997) where sex differences in leptin concentrations were observed with females exhibiting higher leptin levels than males. This may perhaps be due to the effect of estrogen hormone which is known to induce leptin synthesis and androgen, a potent leptin inhibitor. However, Kennedy *et al*., (1997) have associated the higher leptin levels found in females than males of the same adipose tissue mass to be as result of more leptin resistance in the females. Although not statistically significant, findings from this study suggest that serum leptin concentration was influenced by life style factors as reflected by the percentage difference in the mean values between those with positive life style factors (not consuming alcohol, non smokers and engaging in adequate physical exercise) and those without. The findings of this study also reveal that those who do not consume alcohol have higher serum leptin level than those that consume alcohol. It is documented that alcohol consumption predisposes more to central obesity than to subcutaneous tissue deposition, and leptin production is more associated with subcutaneous adipose tissue (Montaque and Rahilly, 2000). In the same vein, non smokers were observed to have higher serum leptin levels than their smoking counterparts. This study is similar to that of Perkin and Fonte (2002) who observed increase

in weight and leptin levels with cessation of smoking after initial weight loss and decrease in leptin when the subjects were made to smoke. This may perhaps be due to the effect of catecolamines on leptin synthesis (Reseland *et al*., 2005). Furthermore, those that engage in moderate/adequate physical exercise had lower serum leptin levels, possibly due to excessive energy expenditure and subsequent burning of the adipose tissue mass from the exercise (Kohrt *et al.,* 1998).

5.2.2 Serum Insulin Levels of Type 2 Diabetic and Non-Diabetic Subjects

Insulin is an anabolic hormone produced by the beta cells of the pancreas in response to glucose and other nutrients (Stephens *et al.,* 2004). Insulin causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, promoting the storage of these nutrients as glycogen in the liver and muscle and to stop using fat as an energy source. Insulin insufficiency or insulin resistance can therefore result in high glucose level in the blood thereby causing diabetes mellitus (Wilcox, 2005).

The serum insulin levels of the diabetic and non diabetic subjects in this study were low. This finding concurs with the levels reported in different studies by Bakari (2005) from Zaria, Northern Nigeria, and with the findings of Ezenwaka *et al*., (1993) and Osei *et al*., (1993) from South Western Nigeria. Similarly, Omar and Asmal (1983) observed in their study on insulin response to oral glucose in young African and Indian non-insulin dependent diabetic patients that African patients showed lower basal and maximal insulin levels and lower insulinogenic indices than Indian patients, despite similar plasma glucose values.

Hyperinsulineamia and hypoinsulinaemia have both been reported in type 2 diabetic patients (Bakari and Onyemelukwe, 2002). Hyperinsulinemia can result from a variety of metabolic diseases and conditions. It is often seen in people with early stage type 2 diabetes mellitus, and where this occurs, it is not the cause of the condition but is only one symptom of the disease. In type 2 diabetes, the cells of the body become resistant to the effects of insulin as the receptors which bind to the hormone become less sensitive to insulin concentrations resulting in hyperinsulinemia and disturbances in insulin release. With a reduced response to insulin, the beta cell of the pancreas secrete increasing amounts of insulin in response to the continued high blood glucose levels resulting in hyperinsulinemia. In insulin resistant tissues, a threshold concentration of insulin is reached causing the cells to uptake glucose and therefore decreases blood glucose levels (Shanik *et al*., 2008).

Hyperinsulinemia can also occur in neonates due to a variety of environmental and genetic factors. If the mother of the infant is a diabetic, and does not properly control her blood glucose levels, the hyperglycemic maternal blood can create a hyperglycemic environment in the fetus. To compensate for the increased blood glucose levels, fetal pancreatic beta cells can undergo hyperplasia. The rapid division of beta cells results in increased levels of insulin being secreted to compensate for the high blood glucose levels. Following birth, the hyperglycemic maternal blood is no longer accessible to the neonate resulting in a rapid drop in the newborn's blood glucose levels. As insulin levels are still elevated this results in hyperinsulineamia. Furthermore, congenital hyperinsulinism and drug induced hyperinsulinemia also occur (Arnoux *et al*., 2010). On the other hand, hypoinsulinemia as

observed in this study is the occurrence of low fasting plasma insulin. Empirical evidence suggests that relative or absolute insulin deficiency, with or without impaired tissue insulin sensitivity, can lead to the development of type 2 diabetes. This scenario is best exemplified by patients with maturity onset diabetes of youth (MODY) (McCarthy *et al*., 2000). This familial subtype of type 2 diabetes is characterized by early age of onset, autosomal dominant inheritance with high penetrance, mild to moderate fasting hyperglycemia, and impaired insulin secretion. The defect in MODY individuals is impaired insulin secretion in response to glucose and other secretagogues leading to insulin deficiency. Glucotoxicity and lipotoxicity are among the acquired defects that can lead to impaired insulin secretion. The glucotoxicity hypothesis is supported by the observation that improved glycemic control, leads to enhanced insulin secretion. Chronic beta cell exposure to elevated fatty acyl-CoA inhibits insulin secretion through operation of the Randle cycle. Other factors that impair pancreatic beta cells and ultimately reducing insulin synthesis are amylin, incretin, increasing age, hexosamines and tumor necrosis factor alpha. Beta cell mass is also a determinant of insulin secretion and 20% to 40% reduction in beta cell mass has been observed in long standing type 2 diabetics. Studies have also shown that low birth weight is associated with the development of type 2 diabetes in a number of populations as a result of impaired insulin secretion or reduced beta cell mass (Erikson, 2003).

Insulin resistance, a condition in which the body produces insulin but the cells fail to respond to insulin can ultimately cause impaired insulin secretion leading to hypoinsulinemia. This study observed lower insulin levels in the diabetic patients when compared with their non-diabetic counterparts and higher degree of insulin resistance in the

diabetic patients. Although a large body of clinical and experimental evidence documents that hyperinsulinemia and insulin resistance precedes the onset of type 2 diabetes, some have suggested that it is virtually impossible to develop diabetes due to the severity of insulin resistance found in most type 2 diabetic patients unless the capacity to secrete additional amounts of insulin to compensate for the insulin resistance is impaired (Reaven, 1995).

The homeostasis model assessment of insulin resistance (HOMA – IR), which was used in this study to assess insulin resistance in the subjects this study has observed a higher mean (HOMA-IR) score of 1.85 ± 0.04 among the diabetic patients than the 0.93 ± 0.02 observed among the non-diabetic group. The study observed that when HOMA-IR score of 1 was considered as 100% sensitive, about 97.9% of the diabetic patients and 39.58% of the non-diabetic subjects had insulin resistance. Nevertheless, none of the non-diabetic subjects and 58 (38.69%) of the type 2 diabetic patients were insensitive to insulin when 2 was considered as the bench mark using the study of Bonora *et al*., (2000) who found normal HOMA IR of 2.06 ± 0.14 in normal non-diabetic population. The observed higher value of HOMA IR score in the diabetic patients represents greater degrees of insulin resistance in the diabetic patients than the non-diabetic subjects. Reaven *et al*., (1989), in their study "Insulin secretion and action in non insulin-dependent diabetes mellitus", also observed that type 2 diabetic patients have lower insulin sensitivity than the non- diabetic group. Similarly, Bakari, *et al*., (2005) also reported lower insulin resistance among diabetic patients in Zaria. However, this study found that all the non- diabetic subjects were sensitive to insulin when HOMA-IR score of two was considered as bench mark, contrary to that of

Bakari *et al*., (2005) who found that 19.4% of the non-diabetic subjects were insulin resistant, having HOMA IR score of more than two. Nevertheless when one was considered as the bench mark, about 38% of the non diabetic subjects were found to be insensitive to insulin thereby showing similarity with the report of Raeven *et al*., (1989) that about 25% of the general European population had insulin resistant.

The HOMA IR score finding from the current study corroborates its usefulness as a diagnostic tool for insulin sensitivity and in low resource setting can be used to mirror the expensive and more sophisticated Euglycaemic glucose clamp technique for assessing insulin resistance. Determination of insulin resistance is useful in clinical practice to differentiate between beta cell dysfunction and insulin resistance in type 2 diabetics that show non response to treatment despite increased dosages of drugs and/ or insulin. In such cases once established insulin resistant, leptin replacement may perhaps be of help to the patients since it has been shown that small doses of leptin significantly improves insulin synthesis and secretion in the adipose tissue and also improves insulin sensitivity.

When the mean HOMA-IR scores obtained in this study were compared between the males and female diabetics, it was observed that the males showed more insulin resistant than the females. Fat accumulation in different parts of the body is sexually dimorphic. In humans, men accumulate more visceral fat, whereas women accumulate more subcutaneous fat and have a higher percentage of body fat compared to men. It has been shown that visceral fat is more associated with insulin insensitivity than the subcutaneous fat. Visceral adiposity represents approximately 20 and 6% of total body fat in males and females respectively

(Ross *et al*., 1992; Ross *et al*., 1993). The increased sensitivity to insulin and lipogenesis observed in adipocytes from females in a study by Macotela *et al*., (2009) may account for their lower level of insulin resistance and diabetes risk despite similar or higher fat content than males.

The hypoinsulinemia observed in this study could be associated with significant low leptin levels and significant insulin resistance observed in the diabetic group, as insulin resistance is one of the causes of beta cell dysfunction. Although low insulin levels were observed among both diabetic and control groups in this study, the lower levels among the control compared with the white population could be associated with differences in the genetic, environmental and life style factors associated with that condition. Intake of high total and saturated fat intake is known to be associated with higher plasma insulin concentration and high dietary fiber diet is associated with hypoinsulinemia (Marshall *et al*., 1997).

Higher levels of insulin among the non-diabetic subjects observed in this study is in consonance with the finding of Bakari (2004) in his study of "fasting plasma insulin levels and blood pressure among type 2 diabetic Nigerians". The similarity in the findings between the current study and that of Bakari (2005), corroborates the possibility that the two study populations may share causal factor for diabetes mellitus owing to their similarity in ethnicity and environmental factors.

However, this study observed that life style factors like alcohol ingestion, cigarette smoking and physical exercise do not seem to influence serum insulin concentration. This study

contradicts that of Piatti *et al*., (2014) who in their study observed lower insulin levels among smokers compared to non-smokers. Nicotine, a constituent of cigarette is known to be associated with increase in abdominal fat accumulation and hence insulin resistance, thereby increasing the risk of type 2 diabetes mellitus.

5.2.3 Serum Lipid Levels of Diabetic and Non- Diabetic Subjects

Plasma lipoproteins include chylomicrons, very low density lipoproteins, low density lipoproteins and high density lipoproteins. These lipoproteins transport triglycerides, phospholipids, cholesterol and cholesterol esters. Insulin resistance or insulin deficiency affects enzymes and pathways of lipid metabolism resulting in increased triglycerides, low density lipoprotein - cholesterol and decreased high density lipoprotein - cholesterol which in turn results into significant vascular complications of diabetes mellitus. This study observed higher levels of serum total cholesterol, low density lipoprotein and triglycerides in the diabetic subjects than in the non diabetic control, but higher high density lipoprotein in the control group. This cluster of lipid abnormalities found in type 2 diabetes mellitus carries a high risk of atherosclerosis, and cardiovascular disease, especially coronary heart disease (CHD) and stroke, and it is by far the leading cause of death among patients these patients. The observed higher levels of triglycerides in the diabetic group when compared to that of the non- diabetic group in the study is similar to that of Bello- Sani *et al*., (2007) from Zaria. Similarly, Onyemelukwe *et al*., (1981) also from Zaria, reported higher levels of triglycerides in the diabetic group than the control. The similarity of this finding with that of Bello-Sani *et al*., (2007) and that of Onyemelukwe *et al*., (1981) in Zaria Northern Nigeria,

may be due to the similarities in diet and life style which are among the factors that influence plasma lipids.

Type 2 diabetic patients show high levels of triglycerides because to a large extent hepatic metabolism of triglycerides is controlled by insulin. This implies that there is more pool of triglycerides in the blood with inadequate insulin action. Through the action of cholesterol ester transfer protein, TGs are transferred from VLDL-c to HDL-c, creating TG-rich HDL-c particles, which are hydrolyzed by hepatic lipase and rapidly cleared from plasma (Hopkins *et al*., 1986). A similar cholesterol ester protein-mediated transfer of TGs from VLDL-c to LDL-c contributes to the formation of small dense LDL-c particles (Berneis and Krauss, 2002). These small dense lipoprotein particles are taken up by arterial wall macrophages resulting in atherogenesis (Gowri *et al*., 1999).

Higher levels of low density lipoprotein and lower high density lipoprotein observed in white population than in the Africans and African Americans may be owing to lower hepatic lipase activity in African-Americans. In addition, triglyceride (TG) levels are lower in both African-American men and women than Whites, and may be related to increased activity of lipoprotein lipase in African-Americans (Morris *et al*., 2009). This is perhaps the reason for the higher prevalence of coronary heart disease in the white than in the black population.

Comparing the parameters between males and females diabetics, total cholesterol levels, LDL-c levels and triglyceride levels are higher in the males than the females while the HDL-c is higher in the females than the males suggesting that the males are more at risk of developing vascular complications diabetics than the female diabetics.

5.3 RELATIONSHIP BETWEEN LEPTIN, INSULIN, FASTING BLOOD GLUCOSE AND ANTHROPOMETRIC PARAMETERS OF THE TYPE 2 DIABETIC AND NON-DIABETIC

This study observed positive correlation between leptin and body mass index in both diabetic and non diabetic subjects. Leptin, an adipocyte hormone is mostly synthesized in the white adipose tissue and the concentration of the hormone in blood correlates with body fat mass. It has been found that the higher the body mass, the higher the circulating leptin concentration (Considine *et al*., 1996; Schwartz *et al*., 1996). This observation is consistent with findings of studies conducted among both white and black populations (Monti *et al*., 2006; Ruhl *et al*., 2007; Martins *et al*., 2012). Since leptin levels increases with increase in BMI, leptin levels can be projected from BMI using the tredline formula for the relationship.

The observed correlation between leptin and waist circumference (abdominal circumference) both among the study and control groups in this study suggest that the higher the waist (abdominal) circumference, the greater the leptin concentration. This finding is expected because of the large deposits of white adipose tissue in the abdomen, and leptin is a known adipocyte hormone. Positive correlation between waist circumference and leptin has also been reported from Saudi Arabia (Bahathiq, 2010). Similarly, another study on male and female teenagers in Gaza, Palestine showed significant positive relationship between leptin and waist (abdominal) circumference (Taleb *et al*., 2014). Furthermore, the Gaza study also observed increasing leptin concentration as waist hip ratio increases, although the relationship was not statistically significant. In addition, the finding of this

study that BMI and waist circumference emerged as independent predictors of leptin concentration following multivariate analysis further corroborates the interactions between leptin hormone and anthropometric parameters, specifically BMI and WC.

Both generalized and abdominal obesity are associated with increased risk of morbidity and mortality and constitute the main cause of obesity-related deaths is CVD. Although there is grounded controversy on which single anthropometric measure predicts the risk of developing non communicable diseases like type 2 diabetes mellitus and cardiovascular diseases Anjana *et al*., (2004) in their study titled "Visceral and Central Abdominal Fat and Anthropometry in Relation to Diabetes in Asian Indians" reported that abdominal obesity is a better predictor of these diseases.

This study observed increase in leptin levels with increasing insulin levels in diabetic patients although the association was not statistically significant. Leptin increases with increasing insulin levels because the former is known to inhibit Insulin synthesis, and insulin is an important stimulator of leptin production as highlighted earlier. Leptin and insulin, although they are two hormones with different receptors, they exert an overlapping effect in the arcuate nucleus in the hypothalamus which is an area involved in energy homeostasis (Benomar *et al*., 2005). In the non diabetic control group however, an inverse relationship was observed between leptin and insulin, a finding very difficult to explain. Interestingly, Khokhar *et al*., (2013) in India similarly reported negative relationship between leptin and insulin among their non-diabetic control subjects.

The negative correlation between leptin and FBS among the diabetic subjects in this study may perhaps be due to the role of leptin in glucose homeostasis, reducing blood levels towards the normal values. Low leptin levels found in this study may perhaps be the reason of hyperglyceamia as a result of its effect on insulin synthesis and or insulin sensitivity. This finding is similar to the findings of Koch *et al*., (2010) in their study, Leptin rapidly improves glucose homeostasis in obese mice by increasing hypothalamic insulin sensitivity. Although, it was reported by previous studies that the main function of leptin was to control food intake and energy expenditure (Pelleymounter *et al*., 1995; Muzzin *et al*., 1996; Schwartz *et al*., 1996; Farooqi *et al*.,., 1999; Yu *et al*., 1997), Hedbacker *et al*., in their findings suggested that leptin is more potent at regulating glucose levels in blood than it is at suppressing appetite.

In determining predictors of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), FBS was the only independent predictor of HOMA-IR found. HC and leptin were found to be correlated with (HOMA-IR) score in the diabetic group. In the non diabetic group, BMI, HDL-c, FBS and Leptin correlated with (HOMA-IR) score. HDL-c and leptin were the only independent predictors of FBS in the non--diabetic group while only HC and leptin correlated with FBS in the diabetic group.

5.4 RELATIONSHIP BETWEEN LEPTIN, OBESITY AND TYPE 2 DIABETES MELLITUS

Leptin, an adipocyte hormone is synthesized mainly in the white adipose tissue as earlier stated. The concentration of the hormone in the body is proportional to the adipose tissue mass as reported by previous studies (Considine *et al*., 1996). High concentration of the hormone in the body of individuals predisposes to leptin resistance leading to reduce function of leptin. Hyperleptinemia and leptin resistance has been reported in obese individuals. And these individuals are reported to manifest with hyperphagia, insulin resistance, decreased energy expenditure, severe obesity and insulin resistance (Zhang *et al*., 1994). This study observed significant higher leptin levels in obese subjects than non-obese subjects as observed in previous studies among diabetes and normal subjects (Ruhl *et al*., 2007).

Obesity is a modifiable risk factor for type 2 diabetes mellitus because of the co-existence of insulin resistance in obese individual. Cross sectional studies have shown that insulin resistance plays a major role in the pathogenesis of type 2 diabetes mellitus. This study has found that about 97% of the diabetic patients are resistant to insulin and 75% of the patients are obese. A significant association was observed between type 2 diabetes mellitus and obesity in the study. Although the study found higher levels of leptin in obese than in non obese subjects, the level of the hormone is lower among the obese diabetic than non obese diabetic. This may perhaps explain why there is significant percentage of insulin resistance among the diabetic subjects.

Leptin hormone has pleotrophic function because of the multiple receptor sites in the body. Apart from its established role in food intake and energy expenditure, leptin has been found to play a role in glucose homeostasis (Morton and Schwartz 2011), through its action centrally in the brain and peripherally on the pancreas, adipose tissue and skeletal muscle. Leptin deficiency or leptin resistance has been shown to be associated with type 2 diabetes mellitus and leptin replacement reversed the hyperglycemia independent of its effect on weight loss.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMENDATIONS

6.1 SUMMARY

The mean serum leptin levels for the diabetics and non diabetics was observed to be 4.57 ± 0.17 ng/ml and 4.70 ± 0.25ng/ml respectively ($p > 0.05$) and the mean insulin level for the diabetic and non diabetic groups are 4.70 ± 0.25 μU/mL and 4.49 ± 0.01 μU/mL respectively ($p < 0.05$). A positive correlation was observed between leptin and body mass index (BMI) in both type 2 diabetic patients ($r = 0.36$, $p < 0.05$) and non-diabetics ($r = 0.48$, $p < 0.05$). A positive correlation was also found between leptin and waist circumference (WC) in both type 2 diabetic patients ($r = 0.32$, $p < 0.05$) and non-diabetics ($r = 0.39$, $p < 0.05$). Hip circumference (HC) also correlated positively with leptin in both the diabetics ($r = 0.33$, $p < 0.05$) and non-diabetics. The study also found a positive correlation between leptin and FBS in the non-diabetic group ($r = 0.21$, $p < 0.05$) but a negative correlation in the diabetic group($r = - 0.18$, $p < 0.05$). A correlation was also found between leptin and HOMA-IR in both the diabetic ($r = - 0.018$, $p < 0.05$) and non-diabetics ($r = 0.20$, $p < 0.05$). However there was no significant relationship between leptin and insulin in both type 2 diabetic patients ($r = 0.11$, $p > 0.05$) and non-diabetics ($r = - 0.06$, $p < 0.05$). Age, BMI and WC emerged as the independent predictors of leptin in the diabetic group while BMI, WC and HC emerged as independent predictors of leptin in the non-diabetics. The study also found higher serum total cholesterol, total triglycerides and LDL-c in the diabetic and higher HDL-c in the non diabetic group. Insulin resistance was also found to be higher in the diabetic group.

6.2 CONCLUSION

This study observed low leptin and insulin levels in the diabetic subjects. Serum leptin levels ware significantly higher among obese diabetic patients than their lean counterparts.

Using the HOMA-IR score of one as standard for assessment of Insulin resistance, about 97% of the diabetic subjects were found to be insulin resistant and none of the non-diabetic group showed resistance to insulin. Insulin resistance was more among the males diabetic patients compared to their female counterparts.

The study also found higher WHR in the male diabetic group and higher BMI and WC in the female diabetic patients. Comparing males and females, the females had higher BMI, WC and HC than the male diabetic patients. A positive correlation was observed between leptin and BMI, FBS, WC and HC in the non-diabetic group and only BMI was found to be an independent predictor of leptin in that group. On the other hand, age, BMI, WC, HC and FBS correlated positively with leptin in the diabetic patients; and age, BMI and WC emerged as independent predictors of leptin in that group. Furthermore, FBS and leptin were positively correlated with HOMA-IR score in both diabetic and non-diabetic group, and FBS was found to be an independent predictor of insulin resistance among the diabetic patients.

This study also observed that serum total cholesterol, serum triglycerides and serum LDL-c were higher in the diabetic subjects except for the HDL-c which was higher in the non-diabetic group, and when compared between males and female diabetic subject the reverse was the case.

6.3 RECOMMENDATION

In view of the findings of this study, it is recommended as follows:

1. The findings of low leptin and insulin levels and high insulin resistance among the type 2 diabetic patients in this study suggest that leptin replacement may be beneficial to this group. Further studies are therefore recommended in order to establish its utility in the clinical management of type 2 diabetes mellitus.
2. The findings of dyslipideamia among the diabetic subjects studied shows that they are at high risk of developing vascular complications. Thus it is recommended that the triad of health hazard awareness campaigns, dietary advice and gentle physical exercises be packaged as part of routine clinical care for this vulnerable group.
3. Based on the finding that majority of the type 2 diabetic patients were obese, and type 2 diabetes mellitus was strongly associated with obesity, the health ministries and relevant development partners should package an awareness campaign to the general public on health hazards of obesity.

SIGNIFICANT CONTRIBUTION OF THIS WORK TO SCIENTIFIC KNOWLEDGE

1. The study found a positive relationship between BMI and leptin. BMI may therefore be used to project leptin levels using trend line formula for the relationship ($y = 0.2462x - 1.5263$) where y = leptin concentration in ng/ml and x = BMI
2. The study also found low leptin levels in type 2 diabetic patients in Kano.

3. The finding of 97% of the diabetic subjects is insulin insensitive and 38.95 the non diabetic subjects are insulin sensitive based on the HOMA-IR score of one as cut off.

4 Type 2 diabetic patients in Kano exhibits higher levels of triglycerides, total cholesterol, low density lipoproteins and low high density lipoproteins compared to non-diabetic subjects.

5 This study observed hypoinsulinemia in type 2 diabetic patients in Kano.

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APPENDICES

Appendix 1: Questionnaire

THE RELATIONSHIP BETWEEN DIABETIC PROFILE, LEPTIN AND ANTHROPOMETRIC MEASURES IN DIAGONOSED TYPE 2 DIABETIC PATIENTS IN KANO

A. SOCIODEMOGRAPHIC DATA

Age ( )

Sex ( )

Ethnicity ( )

Educational level

None ( ) Qur'anic only ( ) Primary ( )

Secondary ( ) Post secondary ( )

Occupation

Unemployed ( ) civil servant ( ) business man ( ) others ---------- (specify)

Marital status

Married ( ) single ( ) widow ( ) divorcee ( ) widower ( )

Religion

Islam ( ) christianity ( ) traditional ( ) paganism ( )

B. LIFE STYLE AND MEDICAL HISTORY

Have you ever smoked cigarette?

Yes ( ) No ( )

Do you take alcohol?

Yes ( ) No ( )

If yes, how many bottles do you take in a day? ----------

Concerning physical exercise, which of the following best describes you statue?

1. In adequate (brisk walk/stroll for less than 30 minutes and less than five days in a week)

2. Moderate (if a farmer or herdsman)

3. Adequate (brisk walk/stroll for up to 30 minutes and at least five days in a week)

Which of the following describes your regular diet?

Mostly high energy diet (like rice cassava, yam e.t.c) ( )

Mostly low energy diet (like fish, chicken, vegetables e.t.c) ( )

Do you often eat in between meals?

Yes ( ) No ( )

At what age were you diagnosed diabetic? ----------

Is any member of your family other than you diagnosed diabetic?

None ( ) parents ( ) grand parent(s) ( ) siblings ( ) other relatives ( ) don't know ( )

Do you suffer any of the following illnesses?

Hypertension yes ( ) no ( )

Peptic ulcer disease yes ( ) no ( )

Asthma yes ( ) no ( )

Heart disease yes ( ) no ( )

Lung disease yes ( ) no ( )

Other chronic disease yes ( ) no ( )

Do you use any of the following drugs routinely?

Steroids eg prednisolone yes ( ) no ( )

Peptic ulcer medications eg omeprazole yes ( ) no ( )

What do you use for diabetic control?

Exercise only ( )

Insulin and exercise ( )

Oral hypoglycemic and exercise ( )

Insulin, oral hypoglycemic and exercise ( )

C. VITAL SIGNS AND ANTHROPOMETRIC MEASUREMENTS

Pulse rate ----------

Blood pressure ----------

Weight ----------

Height ----------

Body Mass Index (BMI) ----------

Waist (abdominal circumference) ----------

Hip circumference ----------

Waist to hip ratio ----------

D. BLOOD ANALYSIS

Fasting blood sugar ----------

Serum insulin ----------

Serum leptin ----------

Serum Triglycerides ----------

Serum cholesterol

Serum HDL

Serum LDL

Appendix 2: Informed Consent Form

TITLE OF PROJECT: Study of the relationship between leptin and anthropometric parameters in type 2 diabetic patients in Kano.

INTRODUCTION

You are being asked to participate in a clinical study on the relationship between leptin and anthropometric parameters in type 2 diabetic patients in Kano. From the information collected we hope to learn more about the hormones insulin and leptin in diabetic patients.

PROCEDURE

If you agree to participate in the study, we will ask for your demographic details and medical history including onset and duration of your diabetes, history of medications, dietary and family history of diabetes, and of associated medical conditions (co-morbidities). We would also measure your weight and height, abdominal and hip circumferences; and also your pulse and blood pressure. We will finally take a 5 ml sample of your blood to examine your blood sugar, lipid (fats), and the insulin and leptin hormones that control your blood sugar.

RISKS AND BENEFITS: There are no anticipated risks associated with this study, apart from the pain encountered while taking blood sample.

PAYMENTS: You will not be paid to participate in the study.

PARTICIPANTS RIGHTS: Your participation is voluntary and you have the right to withdraw your consent at any time without giving any reason. Your identity will not be disclosed in any published and written material resulting from this study.

CONSENT

If you decide to take part in the study, kindly sign or thumb print in the space provided below:

I

……confir

m that the purpose and procedure of this study was thoroughly explained to me. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. I also understand that my medical notes and data from the study may be looked at by responsible individuals. I have given my permission to these individuals to have access to my records. I therefore I agree to take part in the above clinical study.

Signature/ Thumbprint…… Date……

HAUSA VERSION OF CONSENT FORM

TAKARDAR NEMAN YARDAR BINCIKE

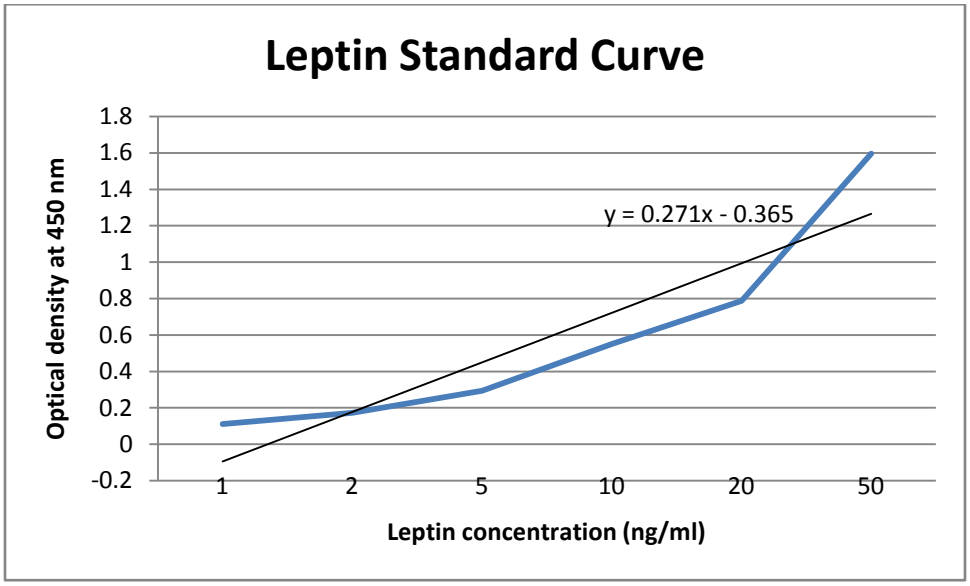
Sunan bincike: Dangantakar sinadarin leptin da yanayin girman jiki a chikin masu chiwon sukari a Kano.

Na tabbatar da karantawa kuma anyi min bayani cikakke kuma na fahimci bayanan da aka bani. Na samu damar tambaya da amsa yadda ya kamata kuma anyi min bayani cikakke. Na

kuma gane amincewata ta kashin kaina ce, amma zan iya janye amin cewar tawa duk sanda naso, ba tare da wani abu yasame ni ba ko ya biyo baya. Na kuma gamsu da cewa dukan bayanai na, za'a iya duba su, kuma na amince a duba din. Kuma na yarda in shiga wannan binciken.

Sa hannu …… Kwanan Wata……..

Appendix 3: Leptin Standard Curve



Appendix 4: Insulin Standard Curve

