COMPARISON OF LEPTIN LEVELS IN THIN TYPE 2 DIABETES MELLITUS, OBESE TYPE 2 DIABETES MELLITUS, THIN NON-DIABETIC AND OBESE NON-DIABETIC.

Dissertation submitted in

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BIOCHEMISTRY – BRANCH XIII

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

COIMBATORE

APRIL – 2016

CERTIFICATE

This is to certify that the dissertation titled **"COMPARISON OF LEPTIN LEVELS IN THIN TYPE-2 DIABETES MELLITUS, OBESE TYPE-2 DIABETES MELLITUS, THIN NON-DIABETIC AND OBESE NON-DIABETIC"** submitted by **Dr.J.Sowndharya** is an original work done by her at PSG Institute of Medical Sciences and Research, Coimbatore. This work was done under the guidance of Dr.G.Jeyachandran, Professor & Head, Department of Biochemistry, PSG Institute of Medical Sciences and Research.

Dr.S.Ramalingam
Dean
PSG IMSR

Dr.G.Jeyachandran
Professor & Head
Department of Biochemistry
PSG IMSR

PLACE:

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DECLARATION

I solemnly declare that this dissertation **" COMPARISON OF LEPTIN LEVELS IN THIN TYPE-2 DIABETES MELLITUS, OBESE TYPE-2 DIABETES MELLITUS, THIN NON-DIABETIC AND OBESE NON-DIABETIC"** was written by me in the Department of Biochemistry, PSG Institute of Medical Sciences and Research, Coimbatore under the guidance of **Dr.G.Jeyachandran**, Professor and Head, Department of Biochemistry, PSG Institute of Medical Sciences and Research.

This dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the university regulations for the degree of M.D. Biochemistry – Branch XIII examinations to be held in April 2016.

Place:

Date: **Dr.J.SOWNDHARYA**

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ABBREVIATIONS:

ADA - American Diabetes Association

AGE - Advanced Glycation End products

BMI - Body mass index

CETP – Cholesteryl ester transfer protein

JNK - c-Jun N-terminal kinase

CT - Computed tomography

DM – diabetes mellitus

FPG - Fasting Plasma Glucose

HbA1c - Glycated hemoglobin

HDL - High Density Lipoprotein

HRP – Horse Radish Peroxidase

IFG - Impaired Fasting Glucose

IGT - Impaired Glucose Tolerance

IRE - 1α - Inositol requiring kinase-1α

IRS -1 &2 - Insulin receptor substrate 1 and 2

IL – Interleukin

JAK-STAT – Janus kinase – signal transducers and activators of transcription

LDL – Low Density Lipoprotein

MRI – Magnetic Resonance Imaging

MC4R – Melanocortin 4 receptor

MODY –Maturity Onset Diabetes of the Young

PC-1- Prohormone convertase 1

PLC – Phospholipase C

PKC – Protein kinase C

POMC – Pro-opiomelanocortin

SDF -1 – Stromal cell derived factor 1

TMB – Tetramethylbenzidine

TNF – Tumor necrosis factor

TrkB – Tropomysin receptor kinase B

UCP1- Uncoupling protein 1

VLDL – Very Low Density Lipoprotein

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To
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The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 30th May, 2014 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your study proposal entitled:

"Comparison of leptin levels in obese type 2 diabetes, thin type 2 diabetes, obese non-diabetic and thin non-diabetic"

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1. Duly filled application form
2. Proposal
3. Informed Consent Forms (Ver 1.1)
4. Data collection tool
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After due consideration, the Committee has decided to approve the study.

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Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
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Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
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Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes

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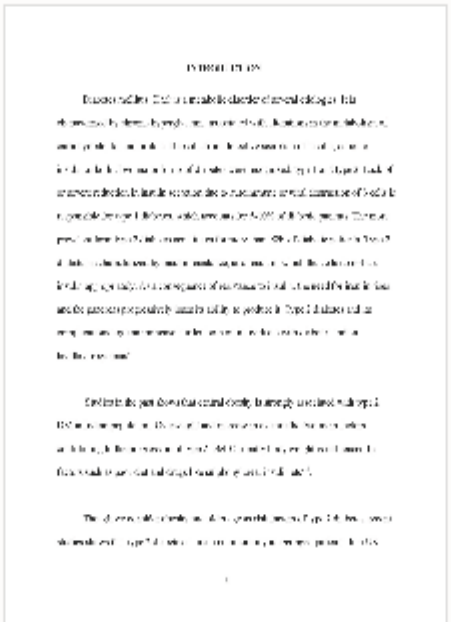


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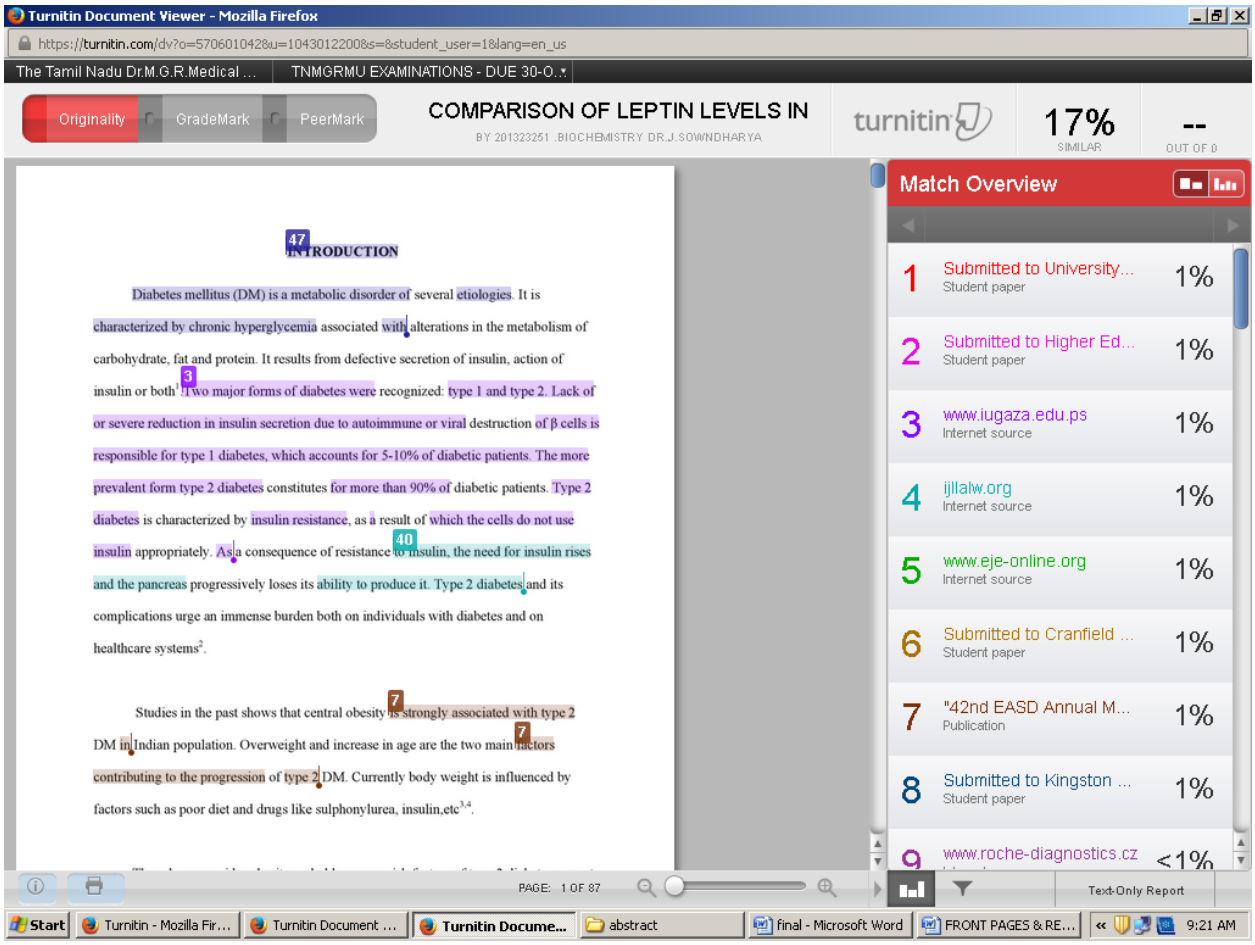
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INTRODUCTION
Diabetes mellitus (DM) is a metabolic disorder of several etiologies. It is characterized by chronic hyperglycemia associated with alterations in the metabolism of carbohydrate, fat and protein. It results from defective secretion of insulin, action of insulin or both[1].Two major forms of diabetes were recognized: type 1 and type 2. Lack of or severe reduction in insulin secretion due to autoimmune or viral destruction of β cells is responsible for type 1 diabetes, which accounts for 5-10% of diabetic patients. The more prevalent form type 2 diabetes constitutes for more than 90% of diabetic patients. Type 2 diabetes is characterized by insulin resistance, as a result of which the cells do not use insulin appropriately. As a consequence of resistance to insulin, the need for insulin rises and the pancreas progressively loses its ability to produce it. Type 2 diabetes and its complications urge an immense burden both on individuals with diabetes and on healthcare systems[2].
Studies in the past shows that central obesity is strongly associated with type 2 DM in Indian population. Overweight and increase in age are the two main factors contributing to the progression of type 2 DM. Currently body weight is influenced by factors such as poor diet and drugs like sulphonylurea, insulin,etc[3,4].
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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of several etiologies. It is characterized by chronic hyperglycemia associated with alterations in the metabolism of carbohydrate, fat and protein. It results from defective secretion of insulin, action of insulin or both[1].Two major forms of diabetes were recognized: type 1 and type 2. Lack of or severe reduction in insulin secretion due to autoimmune or viral destruction of β cells is responsible for type 1 diabetes, which accounts for 5-10% of diabetic patients. The more prevalent form type 2 diabetes constitutes for more than 90% of diabetic patients. Type 2 diabetes is characterized by insulin resistance, as a result of which the cells do not use insulin appropriately. As a consequence of resistance to insulin, the need for insulin rises and the pancreas progressively loses its ability to produce it. Type 2 diabetes and its complications urge an immense burden both on individuals with diabetes and on healthcare systems[2].

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Though we consider obesity and older age as risk factors of type 2 diabetes, recent studies shows that type 2 diabetes is much common in younger aged patients. In a US

based study 33% increase in the incidence and prevalence seen during the past decade is due to type 2 diabetes occurring in children and young adults. In 1992, it was rare for most pediatric centers to have patients with type 2 diabetes. By 1994, type 2 diabetes accounted for up to 16% of new cases of pediatric diabetes in urban areas, and by 1999, it accounted for 8–45% of new cases depending on geographic location[5].

The association between obesity and type 2 diabetes also has to be reexamined. Not all type 2 diabetics are obese and not all obese are type 2 diabetic. In an Indian study on the prevalence of obesity in type 2 diabetes, it was found that only 20% are obese (BMI above 30), 26.7% are overweight (BMI 26-30), 43.3% normal weight (BMI 20-25) and interestingly 10% are underweight (BMI below 20). Many studies focus rather on central obesity based on waist hip ratio instead of obesity based on BMI, mainly due to lack of convincing association. In the same, Shilpi Sharma and Shashi Jain study, 85% of Female type 2 diabetic had abdominal obesity. Still, only 40% of males with type 2 diabetes had abdominal obesity. So, type 2 diabetes may represent a heterogeneous group and may not be equated to obesity[6].

Leptin, the product of ob gene, is a peptide that is strongly correlated with adiposity. Leptin, a protein hormone made of 167 aminoacids is produced by adipocytes. It is considered to have a role in regulation of body weight and energy metabolism[7]. Leptin along with other adipokines influences insulin sensitivity and is established to be responsible for the pathogenesis of obesity associated disorders[8]. Increased level of serum Leptin is considered as a component of metabolic syndrome[9]. It was suggested that

resistance to Leptin in β-cells results in hyperinsulinemia due to the inhibitory effect of Leptin on insulin secretion. This may lead to the exhaustion of β-cells in the pancreas leading to the progression of Type 2 DM[10]. Previous studies showed a strong positive correlation of Leptin with body mass index (BMI) and body fat in non-obese and obese subjects with Type 2 diabetes mellitus[11].

This study is an attempt to understand the thin built patients with type 2 diabetes mellitus. As obesity and type 2 diabetes are tightly linked, whether any of the lipokines got dysregulated in some thin individual leading to type 2 diabetes is the suspicion. Out of all lipokines, Leptin is very well associated with obesity. So, in this study we wanted to examine whether Leptin level is elevated in thin diabetic. That is, whether Leptin level has association with type 2 diabetes independent of obesity status, will be examined in this study. This question will be answered by studying the association between obesity, Leptin levels and type 2 diabetes.

AIM & OBJECTIVES

Aim:

To compare the levels of serum Leptin in thin type-2 diabetes mellitus, thin non-diabetic, obese type-2 diabetes mellitus & obese non-diabetic.

Objectives:

1. To form four groups namely obese type 2 diabetic (n=40), thin type-2 diabetes mellitus (n=40), obese non-diabetic (n=40) and thin non-diabetic (n=40) according to inclusion and exclusion criteria.
2. To collect relevant data and sample for Leptin estimation.
3. To estimate Leptin using ELISA method.
4. To compare the data between 4 groups.
5. To analyze the correlation between the quantitative parameters.

REVIEW OF LITERATURE

Diabetes mellitus is the major non-communicable disease which is prevalent worldwide. It is a multifactorial metabolic disorder characterized by chronic hyperglycemia. It is associated with alterations in carbohydrate, protein and fat metabolism due to defective insulin secretion or action or both[12.]

Epidemiology of diabetes mellitus:

Worldwide occurence of diabetes mellitus in adult population (20-79 years old) is estimated to be around 8.3% with 382 million people suffering from diabetes .North America and the Caribbean region has got the highest prevalence of disease (11%) followed by the Middle East and North Africa (9.2%)[13] . Type 2 DM accounts for approximately half of adolescent diabetes in the United States, and one-third of these cases were undiagnosed[14]. It was estimated that nearly 1 million Indians die due to diabetes every year with the average age of onset being 42.5 years and it is expected that by 2030 incidence will increase possibly due to increased prevalence of obesity and lack of physical activities[15,16]. Prevalence of diabetes mellitus is higher in men less than 60 years of age when compared to women at older ages[17]. Majority of people with diabetes are in 45 to 64 years of age in developing countries, whereas in developed countries most of them are greater than 64 years of age[18]. The prevalence of Type 2 diabetes is high and it is 4-6 times elevated in urban parts of India than in rural sector[19]. At present, India stands next to China with 63 million population diagnosed with diabetes due to obesity[20].

Criteria for diagnosing diabetes mellitus[21]:

- Glycated hemoglobin ≥ 6.5% or
- Fasting Plasma Glucose (FPG) ≥ 126 mg/dL or
- 2-hour plasma Post prandial glucose ≥ 200mg/dL during an Oral Glucose Tolerance Test or
- Random plasma Glucose ≥ 200 mg/dL with signs and symptoms of hyperglycemia.

Type 2 diabetes mellitus:

The major prevalent type of diabetes is type 2. It affects greater than 90% of the population suffering from diabetes globally. There is a rapid increase in the number of diabetic patients and this fiery growth is noted in both rural and urban areas. It is characterized by excessive hepatic glucose production, variable degree of resistance to insulin action, decreased insulin secretion, and abnormalities in fat metabolism.

Risk factors for type 2 diabetes mellitus[21]:

Sedentary life, lack of physical activity, diet, lifestyle changes and related epidemiological conversion has been established as risk factors for type 2 DM. Other major risk factors are listed below.

- Family history of diabetes mellitus

- Overweight with Body Mass Index, BMI ≥ 25 kg/m^2
- Decreased physical activity
- Ethnicity
- Previously identified to have IFG/IGT
- Blood pressure $\geq$ 140/90 mm of Hg
- Triglycerides $\geq$ 250 mg/dL
- High Density Lipoprotein (HDL) $\leq$ 35 mg/dL
- Previous History of Gestational diabetes mellitus
- Polycystic ovary syndrome.

Pathogenesis of Type-2 DM:

Type-2 DM is found to result from a synergy between genetic and acquired etiologies. This adversely influences the function of beta cell and also on the insulin sensitivity of the tissues[22]. For several years it was notorious whether the derangements in beta cell function or resistance to the insulin action was the primary underlying causal element. The focus on insulin resistance being the key defect has been traced back to the classic studies of Himsworth and Kerr[23]. Insulin resistance was found to result from a blend of genetic as well as environmental factors.

Insulin resistance:

It is defined as the deprivation of insulin to exert its biological functions at effective circulating levels in normal subjects[24]. It is found in non-diabetic individuals who are obese and in patients with Type-2 DM. It is a distinctive sign of defective action of insulin. There is an extensive scientific continuum of resistive action of insulin varying from euglycemic status with a remarkable augmentation in insulin secretion by the pancreatic islet cells to hyperglycemia in spite of increased doses of exogenous insulin.

Loss of beta-cell function:

The impairment of beta cell function is allied with the insulin resistance mediated increased beta-cell demand which is required for developing increased glucose values in fasting status. The foremost flaw is loss of secretion of insulin that is induced by glucose labelled as selective glucose insensitivity. Excess increase in blood glucose levels renders the beta cells insensitive to glucose. The extent of beta cell dysfunction is found to be associated with both glucose concentration and duration of hyperglycemia. The normal pulsatile secretion of insulin is also found to be disturbed.

Diabetogenes:

Genetic factors also pave way to the progression of diabetes. Genes that affect beta cell apoptosis, beta cell regeneration, sensing glucose levels, ion channels, energy transduction, microtubules or microfilaments, metabolism of glucose and other islet proteins mandatory for the synthesis, binding, progress and discharge of secretory granules[25]. Until recently, only a small number of polymorphisms have been identified as

risk factors: One involves amino acid genetic variations in the peroxisome proliferator activated receptor gamma which is expressed in target tissues of insulin action and beta cells. Second involves the gene encoding a cysteine protease namely calpain-10, which modulates insulin release as well as insulin actions on skeletal muscle and adipose tissue[26]. Numerous genetic factors along with exogenous factors like environmental agents together contribute to the increased glycemic state of the individual. However, despite extensive work, the genes leading to Type-2 diabetes remains indefinite.

Environment:

Environmental factors ranging from dietary habits to level of physical activity are chief determinants in the development of Type-2 DM. Obesity is associated with insulin insensitivity and is the most significant predictive risk factor for development of Type-2 DM. It is found to be mediated by a variety of factors released from adipose tissue such as leptin, adiponectin and many others which adversely affect functions of beta cell. An inverse relationship exists between the level of physical activity and the prevalence of Type-2 DM. Exercise is thought to produce an increased sensitivity to insulin in skeletal muscle and adipose tissue.

Dyslipidemia in diabetes mellitus:

Insulin resistance in the perception of glucose metabolism leads to impairment in the suppression of endogenous glucose production under basal and fasting conditions. It also leads to reduced peripheral uptake of glucose. Insulin suppresses the production of very low density lipoprotein (VLDL). Resistance to this action of insulin increases circulating serum triglycerides levels. Resistance to insulin action in the adipose tissue

increases the discharge of non-esterified fatty acids (NEFA) both to the liver and skeletal muscle. This impairs the actions of insulin on glucose metabolism in these tissues. Evidence from previous studies suggested that insulin normally suppresses the production of VLDL, especially VLDL -1 apo-B particles from the liver[27]. This effect is brought about by decrease in availability of non-esterified fatty acids and also a direct effect of insulin on the liver cells where it inhibits the assembly and synthesis of VLDL particles[28]. In discrepancy to normal subjects, insulin fails to restrain VLDL apo- B production in those with Type-2 DM, though insulin profoundly lowers NEFA concentrations. Overproduction of VLDL and the defective insulin mediated inhibition of VLDL production are found to be one main causative reason for the increase triglyceride concentrations in subjects with insulin resistant Type-2 DM[29].

Reduction in HDL levels in patients with insulin resistance accompanied by elevated triglycerides. Increased switching of triglycerides and cholesteryl esters, between high density lipoproteins and others that are rich in triglyceride, by cholesterol ester transfer protein (CETP) in conditions producing a hypertriglyceridemic state. Finally the HDL particles are enriched with triglycerides which make them susceptible to the action of hepatic lipase thereby clearing HDL from the systemic circulation at an increased pace. Further suboptimal activity of lipoprotein lipase (LPL) leads to fall in HDL cholesterol levels by diminishing the inter-exchange of HDL-3 to HDL-2 particles[30].

Prominent rise in the level of VLDL particles in diabetic patients elevates the transfer of cholesterol esters and triglycerides between VLDL and LDL, mediated by

CETP. The increase in triglyceride level in LDL particles creates an environment susceptible for the action of hepatic lipase[31]. This enzyme hydrolyses triglycerides present in LDL thereby increasing their mass. The above said events are the reason for Type-2 DM individuals having small and dense LDL compared with individuals without diabetes[32]. The association between insulin resistance and cardiac disease are due to the atherogenecity of smaller and denser LDL[33].

Glycated haemoglobin (HbA1c**):**

It is a well-known indicator of glucose levels in blood over a prolonged phase of time. Biochemically, glycation is the attachment of a sugar moiety to amino groups of proteins which occurs spontaneously without the need for an enzyme. Adult hemoglobin is composed of four polypeptide chains, two alpha and two beta chains. Glycated hemoglobin is formed by reaction of glucose with the amino terminal valine residue of any one of the β-chains of HbA to produce an unsteady Schiff base. This unstable Schiff base is reversible. This undergoes Amadori rearrangement to form a stable conjugate referred to as glycated haemoglobin. This reaction when it occurs between glucose and other proteins leads to the synthesis of advanced glycation end products (AGE) which contributes to the micro and macro vascular complications of DM. Adhesion of plasma proteins in the altered blood vessels contributes to accumulation of LDL and consequent atherosclerosis. The cut-off limit was set at a level of 6.5%.The American Diabetes Association acknowledged this decision. The traceability of the method should be to the Diabetes Control and Complications Trial reference assay[34].

Obesity:

Obesity is defined as the condition of surplus mass of adipose tissue. Obesity is efficiently associated with morbidity and mortality. The body mass index (BMI) is commonly employed to measure obesity. This is calculated by the formula which uses height and weight. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2).Other methods to quantify obesity are anthropometry (skin-fold thickness), densitometry (underwater weighing),Computed tomography (CT) or Magnetic Resonance Imaging (MRI), and electrical impedance[35].

World Health Organisation (WHO) has given the cut –off values for classifying persons based on body mass index. Under this they are classified as underweight, normal weight, over weight and obese persons. These values given for Body Mass Index are not dependent on age. The given values are same for both sexes. Due to variations in body proportions, body mass index will not correspond to the same degree of fat. The adverse effects of health are continuous with increasing BMI. The associations between body mass index and health risks are different in different populations.

Table 1: BMI BASED CLASSIFICATION OF WEIGHT IN ADULTS[36,37]

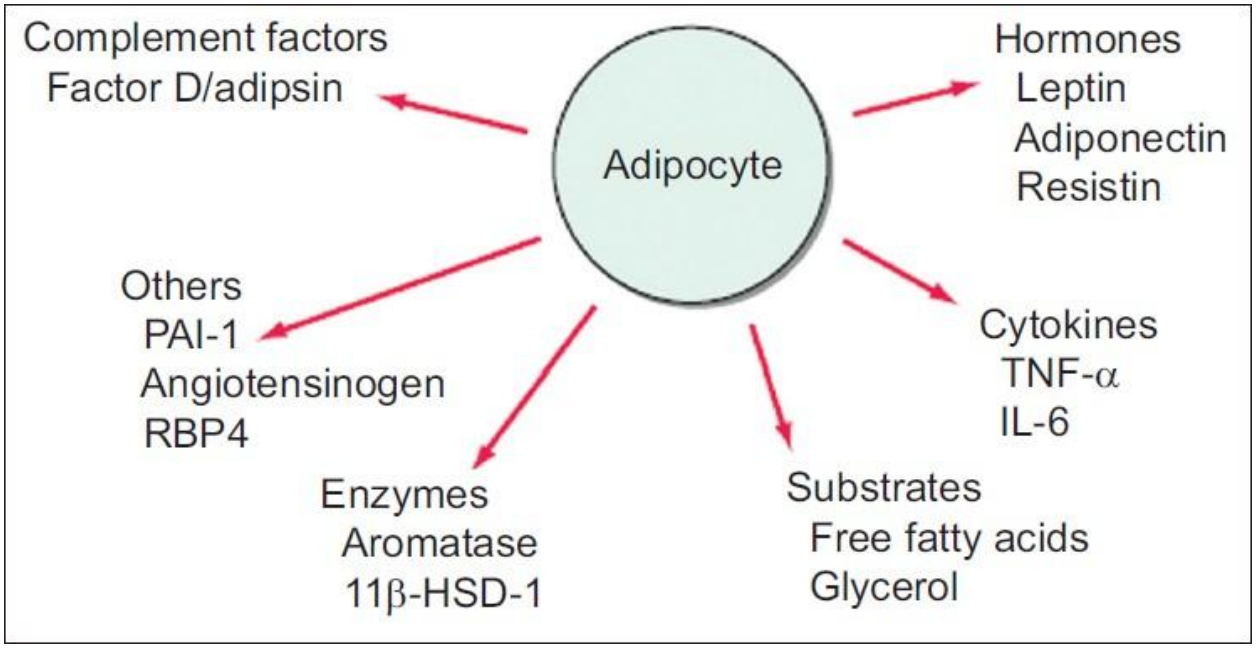
Classification	**Body mass index(kg/m^2)**	
	Principal cut-off points	**Additional cut-off points**
Underweight-Thin	**<18.50**	**<18.50**
Severe	<16.00	<16.00
Moderate	16.00 - 16.99	16.00 - 16.99
Mild	17.00 - 18.49	17.00 - 18.49
Normal range	**18.50 - 24.99**	**18.50 - 22.99**
		23.00 - 24.99
Overweight	**≥25.00**	**≥25.00**
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	**≥30.00**	**≥30.00**
Class I Obese	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Class II Obese	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Class III Obese	≥40.00	≥40.00

The deposition of fat in varying anatomic locations also has its own effect on morbidity. Among the distribution, intra-abdominal and abdominal subcutaneous fat is given a distinct importance. The determination of abdominal fat is done by measuring waist-to-hip ratio. If the ratio is more than 0.9 in women and more than 1 in men is abnormal. If an abnormal waist to hip ratio exists, then a person is more prone to develop many complications. The major complications are resistance to insulin, diabetes, increase in blood pressure, lipid profile and androgen secretion in women. Intra-abdominal and/or upper body fat is more strongly correlated to these complications than to overall adiposity. Due to excess fat storage, more free fatty acids are discharged into the portal circulation. This will have an adverse metabolic action on the liver.

The Adipocyte and Adipose Tissue:

The function of adipocytes as endocrine cell is to release various molecules. This includes Leptin which helps in maintaining equilibrium of energy, cytokines like tumor necrosis factor and interleukin-6, complement factors likefactor D (adipsin), plasminogen activator inhibitor I which is a prothrombotic activator, and angiotensinogen involved in maintaining blood pressure. This is carried out in a synchronized manner.

FACTORS SECRETED BY THE ADIPOCYTES:



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Jameson JL, Loscalzo J.Harrison's Principles of Internal Medicine, 17th edition: Chapter 74 Biology of Obesity. Page: 464.

Etiology of Obesity[35]:

Environmental factors:

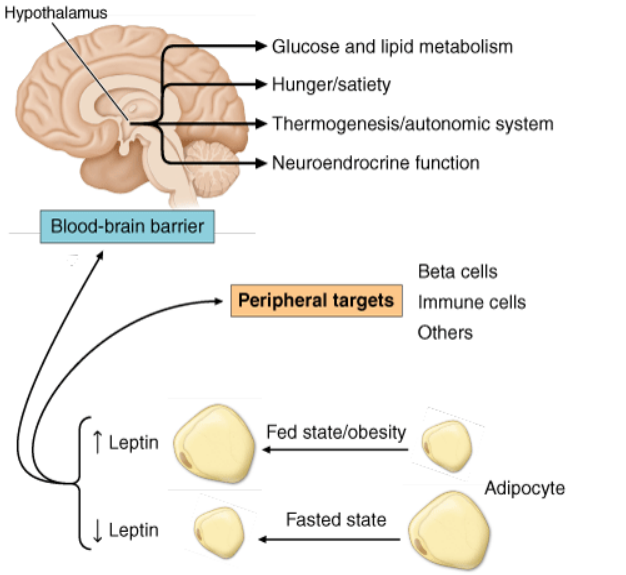
The body weight is inherited in the same manner as that of height. Both the genes and the environment plays an important role in becoming obese. Among the genes and environment, environment plays an important role in obesity. Another important factor in gaining obesity is that the genes will increase the propensity of obesity with respect to

specific diets and nutrition. One more factor playing a key role in obesity is the cultural factors. This is related to the accessibility to the diets and also the composition of the diet. It is greatly influenced by the level of physical work. Obesity is greatly promoted by diets which are rich in high fats particularly which is combined with simple carbohydrates contrasting complex carbohydrates.

Genetic factors:

Obesity is also related to some genetic syndromes caused by mutations. One such is the mutation of ob gene. Mutations affecting Leptin and its receptor have been discovered in many families presenting with morbid, early onset obesity. This paves way for illustrating the biological association of Leptin in human beings.

PHYSIOLOGICAL ROLE OF LEPTIN:



Other gene mutations linked to obesity are:

POMC	Pro-opiomelanocortin, a precursor of several hormones and neuropeptides
MC4R	Type 4 receptor for MSH
PC-1	Prohormone convertase 1
TrkB	TrkB, a neurotrophin receptor

Syndromes linked with obesity are Cushing's Syndrome, Hypothyroidism, Insulinoma, Craniopharyngioma and Other Hypothalamic Disorders.

Pathogenesis of Obesity[35]:

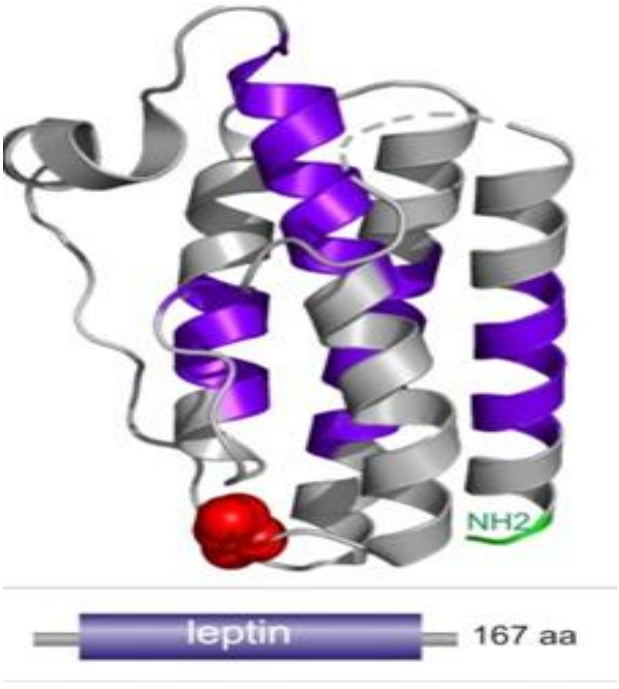
Excessive intake of energy, diminished expenditure of energy, or both leads to the development of obesity. Measuring both the parameters is necessary for finding the cause of obesity. The body weight is maintained by the mechanisms involving adipose tissue and hypothalamus. This physiological action is maintained by a system which acts as a sensor in adipocytes that reveals the lipid stores and their receptor/ "adipostat" being present in the centres of hypothalamus. During the fat in the body being used up, the adipostat signalling will be low, and the response shown by hypothalamus is by triggering hunger and declining the energy expenditure to save energy. Opposite effect occurs when the fat stores are in excess. The molecular basis for this physiological notion

has been arrived at following the breakthrough finding of the *ob* and *db* genes and their respective products namely Leptin and its receptor.

Leptin:

Leptin is a 16 kilo Dalton protein hormone made up of 167 amino acids. It is derived from the Greek word 'leptos' which means lean. It is coded by ob gene present on chromosome 7 in humans. It plays a vital part in regulation of food ingestion, balancing energy and functions as a metabolic and neuroendocrine hormone. The foremost function of leptin is in regulating body weight.

STRUCTURE OF LEPTIN



Source: Essan et al. A Review: Leptin Structure and Mechanism actions. BEPLS April 2014; Vol 3 (5):185 -192.

Leptin is one of the most important hormone secreted by adipocytes into the bloodstream[38]. Two circulatory forms of Leptin have been found. One as the free form in plasma and other is associated with Leptin binding protein. The latter includes a type of Leptin receptor which is soluble. It is opted that, the major part of Leptin is found as a bound form in thin built individuals and in obese individuals as free form[39] .

Central actions:

Leptin receptors were located in the choroid plexus and hypothalamus, a region regulating intake of food, appetite and weight. Lepti binds to its receptor and acts mainly to inhibit hunger by the following mechanisms[40,41]:

1. It opposes the effects of Neuropeptide Y, which is secreted by cells of gut and hypothalamus and serves to stimulate hunger.
2. It has antagonistic action of Anandamide which also promotes hunger and it exhibits its action by binding to the same receptors as Tetrahydrocannabinol.
3. It enhances the synthesis of alpha -melanocyte stimulating hormone which functions as a hunger suppressant.
4. It acts on receptors in the Mediobasal hypothalamus and controls food ingestion and energy expenditure[42].

These mechanisms produce neuronal signals which make a person to consume less food and to metabolize more amounts of fuels[43].

Peripheral Actions:

Leptin also exerts its effects on organs other than hypothalamus. These effects on peripheral targets are due to the distribution of Leptin receptors on various cell types. The various peripheral actions of leptin are:

1. Regulation of energy expenditure.

2. Harmonizing fetal and maternal metabolism.

3. Role in puberty.

4. Activation of immune cells and pancreatic beta islet cells.

5. Interaction with other energy regulatory hormones like insulin[44].

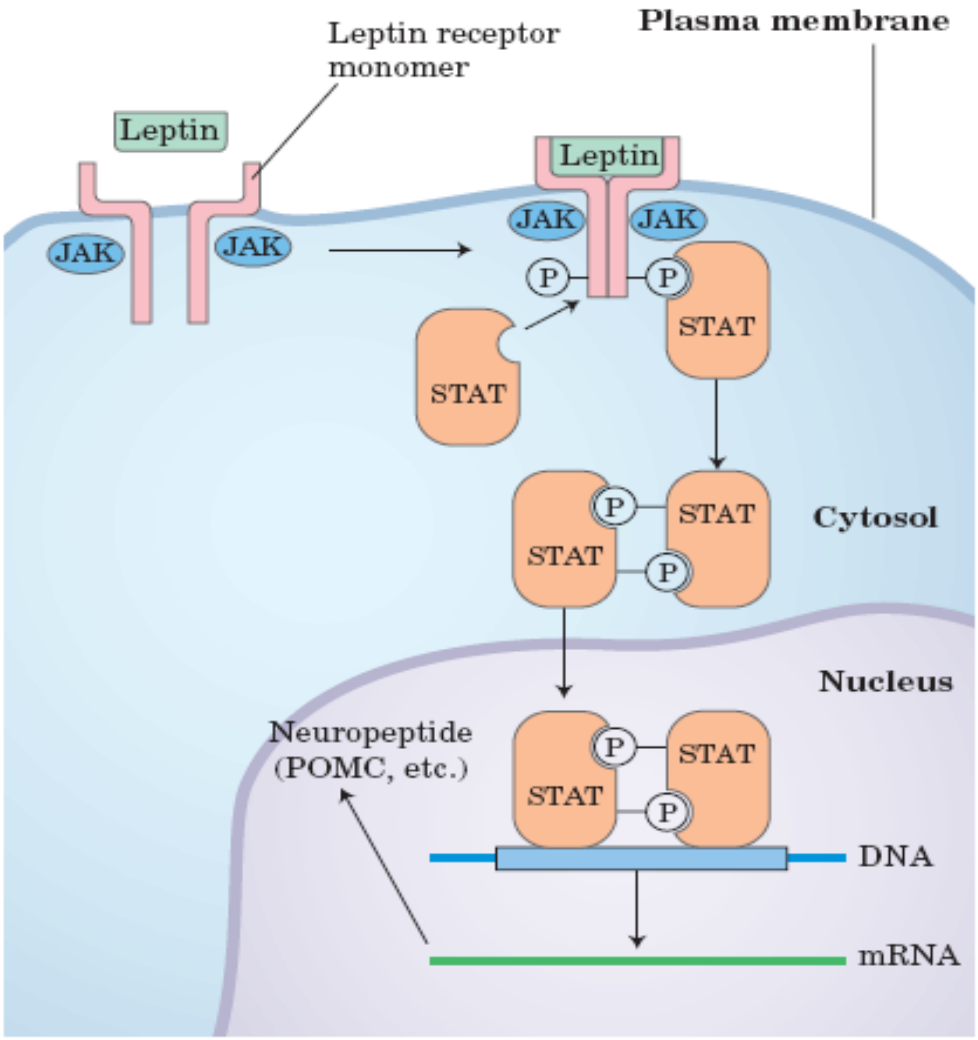
Mechanism of action:

The transduction of the Leptin signal is by the Janus kinase – signal transducers and activators of transcription pathway(JAK-STAT). The structure of Leptin receptor has a single transmembrane segment. This receptor has two monomers and when Leptin binds to the extracellular domain, it dimerizes. Janus kinase (JAK) acts on the intracellular domain by phosphorylating both the monomers on tyrosine residues. These

residues which are phosphorylated becomes the site of docking by STAT sometimes called fat-STATS. These three proteins serve as signal transducers and activators of transcription. Janus kinase (JAK) phosphorylates tyrosine residues of the docked STATs. Now the dimerized STAT shifts to nucleus. After shifting, they attach to the target DNA sequences and the activation of the genes expression occurs. This includes the gene for POMC (pro-opiomelanocortin) by which α – melanocyte stimulating hormone is secreted.

Leptin causes increased production of thermogenin, a product of UCP1 (uncoupling protein 1) gene. This is a mitochondrial uncoupling protein produced in adipocytes whose effects are increased catabolism and thermogenesis. The mechanism by which Leptin stimulates the formation of thermogenin is by changing the transmission of impulses from arcuate nucleus neurons to adipose tissue and others. In adipose tissue Leptin increases the secretion of norepinephrine and the action is exhibited through beta 3 adrenergic receptors, which leads to the UCP1 gene transcription. This paves the way for the uncoupling of transfer of electrons from oxidative phosphorylation. This utilizes fat and becomes thermogenic[45] .

Leptin signal transduction in the hypothalamus by JAK-STAT mechanism:



Source: Dave Nelson and Mike Cox. Lehninger Principles of Biochemistry 5th edition.chapter 23: Hormonal Regulation and Integration of mammalian Meatbolism. Page no 933.

Obesity and diabetes:

Obesity and diabetes, altogether known as diabesity, is the evolving metabolic disease of developed and developing countries. The risk of developing type 2 diabetes mellitus increases with the upper normal limit of body mass index. Both men and women carry equal risk. Another important risk factor is the duration of obesity. EPIC Potsdam cohort study showed that an increase in BMI of one unit between the age group of 25 to 40 years elevates the relative risk of diabetes by 25%[46]. The chance of developing metabolic syndrome rises even with low degree of overweight, upper normal range of body mass index and the pattern of fat distribution. Obesity encompasses increased insulin secretion and decreased hepatic insulin clearance. As a result of obesity, diabetes will be developed by obese people who had a genetic failure of pancreas to compensate for insulin resistance[47]

Developmental programming of obesity and diabetes:

Maternal hyperglycemia and long time over nutrition during pregnancy predisposes the fetus to develop many adverse consequences. It includes

hyperinsulinemia, hypercortisolemia and hyperleptinemia. As the hypothalamus is involved in energy homeostasis and metabolism, these consequences will result in malprogramming. This will lead to a increase in lifetime risk of developing diabetes. Due to these effects other adverse health effects may occur. Epigenetic processes like DNA methylation, histone modification and change in the microRNA pattern are all concerned with these mechanisms[48].

Environmental factors promoting obesity and diabetes:

Current worldwide epidemic of obesity and diabetes is caused by the vast alteration in the lifestyle and environmental factors. In the past 20-30 years a dramatic change has taken place in the eating habits, food choices and physical activity. Fast foods have taken the foremost role in promoting obesity and diabetes. The evidence for this comes from a study conducted by Rosenheck[49]. Olsen et al have highlighted the usage of high consumption of calorically sweetened beverages[50]. The high energy density of modern foods is also implicated in undesirable health effects.

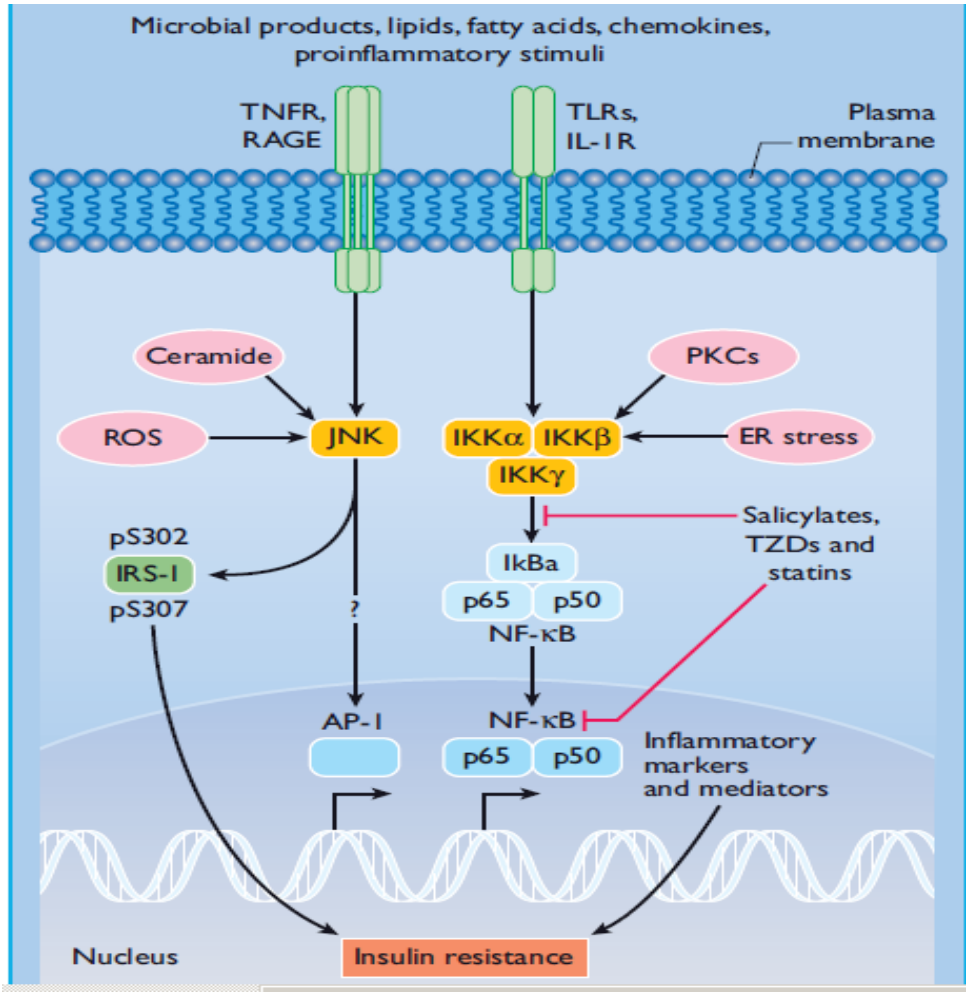
The study conducted by Prentice et all gives an alarming fact that average energy density of fast foods are 65% higher than the healthy diets. They also add that these energy dense foods challenges human appetite control systems with conditions for which they are never developed[51]. The final and a strong determinant of developing obesity and diabetes is the socioeconomic status.

Pathophysiological link involving obesity and type 2 diabetes:

In obesity there is a surplus production of non-esterified fatty acids from adipocytes. Glucose consumption is competed and there is a blockade of glycolytic enzymes pyruvate dehydrogenase, phosphofructokinase and glucokinase. Due to this, blood glucose levels increase and glucose oxidation is declined. Glycerol is formed from the fatty acids which are produced in excess amount and utilizes for hepatic glucose production. This will enhance the imbalance of carbohydrate metabolism. In obese diabetic patients insulin resistance favours elevated intramyocellular lipid accumulation. Ceramide, diacylglycerol and coenzyme A accumulates in the muscle because of too much delivery of fatty acids. This leads to the impairment of insulin mediated serine-threonine kinase cascade finally ensuing the serine-threonine phosphorylation of insulin receptor substrate 1 and 2 (IRS -1 &2). Thus, insulin signaling is destructed which ends up in insulin resistance[52]. When glucose levels rise due to raised fatty acids, impairment

of insulin secretion is mediated through the increased expression levels of Uncoupling protein 2 (UCP-2) in beta cells of pancreas[53].

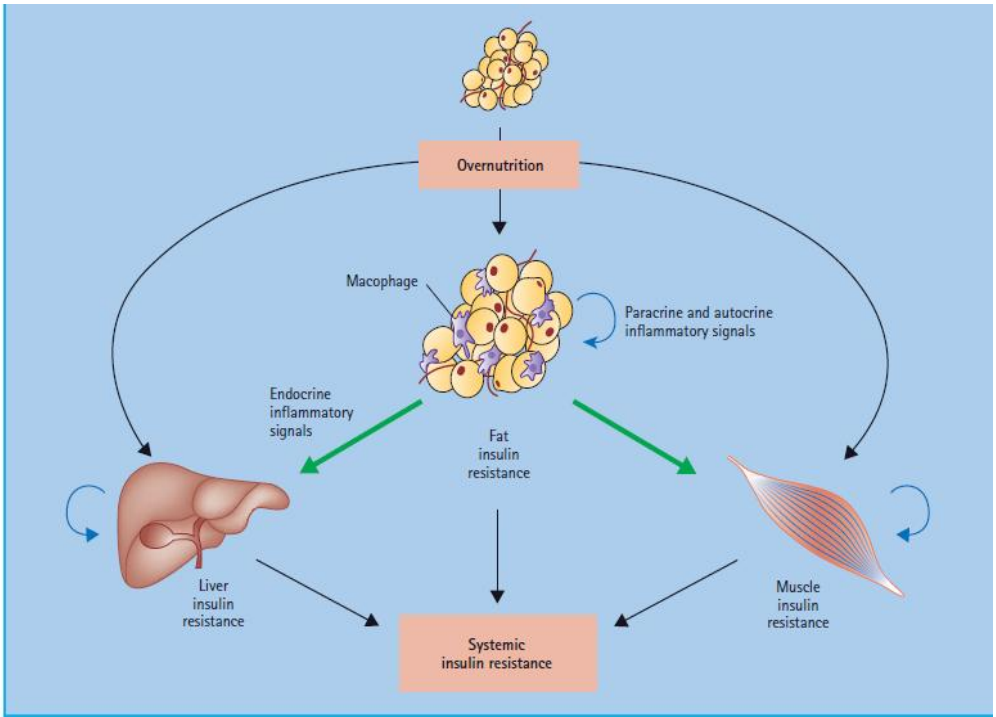
CELLULAR MECHANISMS FOR INFLAMMATION AND DEVELOPMENT OF INSULIN RESISTANCE:



Source: Shoelsen SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; 116**:** 1793 – 801.

Lipids stimulate the c-Jun N-terminal kinase (JNK) and IKK β – NF –kβ pathway which serves to mediate the inflammation in adipose tissue. In this pathway IRS-1 gets phosphorylated leading to insulin resistance. JNK activity is elevated in adipose tissue, liver and muscle[54]. It was recently highlighted that hypertropic fat cells leads to proinflammatory state relating with type 2 diabetes[55,56]. In a state of excess glucose, stress was created in endoplasmic reticulum interfering insulin signaling. This was with inositol requiring kinase-1α (IRE-1α) activating c-Jun N-terminal kinase pathway[57]. Pre-adipocytes and adipocytes secrets many chemokines attracting monocytes and macrophages through stromal cell derived factor 1 (SDF-1). This process mediates inflammation ending up in metabolic and cardiovascular complications of obesity[58,59].

OBESITY AND INSULIN RESISTANCE:



Source: de Luca C, Olefsky JM.Stressed out about obesity and insulin resistance.

Nat Med. 2006 Jan; 12(1):41-2

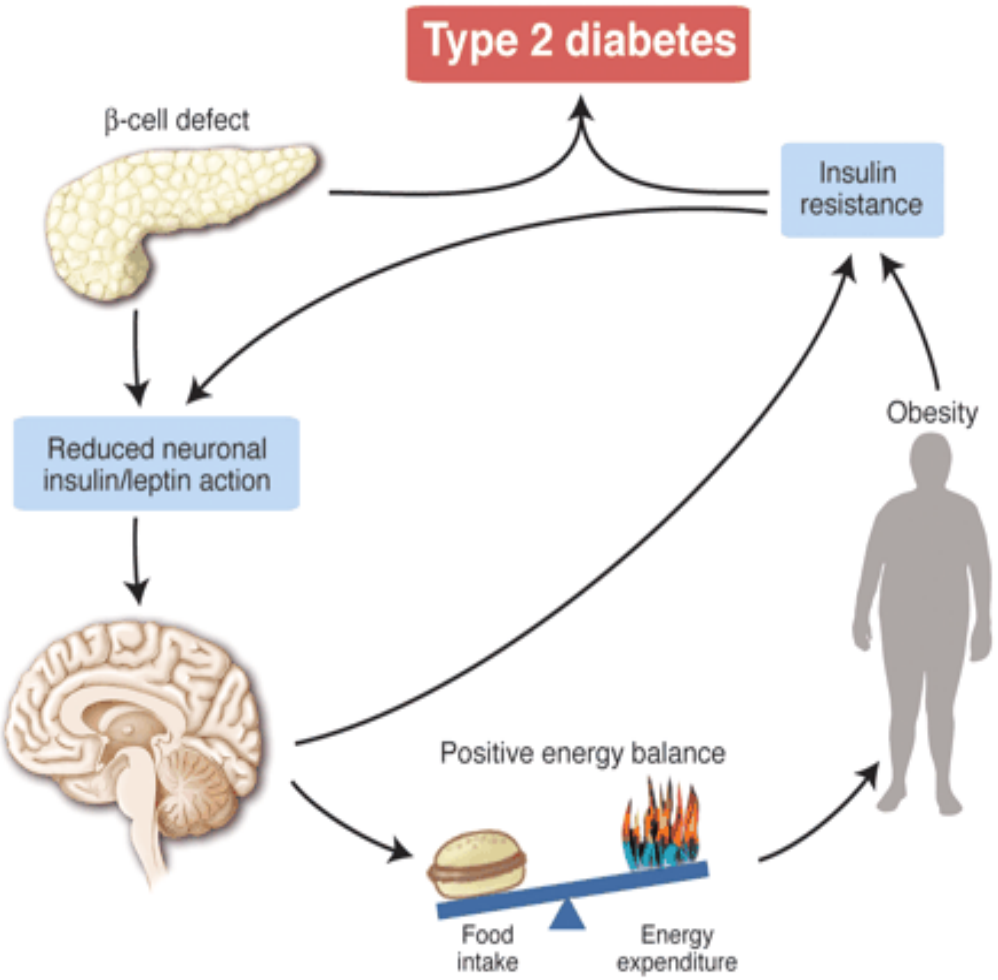
Leptin and diabetes:

The expression of Leptin can be harmonized by insulin. This is supported by many studies and the effect is exerted by insulin mainly by stimulating mRNA expression. These studies were conducted in isolated adipocytes[60-64]. Leptin levels are increased for a longer period when hyperinsulinemia was induced by clamp techniques[62,65-67]. Leptin receptors are also found on pancreatic beta cells which secrete insulin[68]. The insulin secretion is lowered by the action of Leptin mediated through these receptors. The way by which insulin secretion is synchronised is through phospholipase C (PLC)/ protein kinase C (PKC) mediated pathways. The first phase of this pathway involves the lowering of intracellular calcium levels which is linked with the activation of ATP-sensitive potassium channels[69,70]. The second phase in the pathway includes the inhibition of calcium dependent mediator, protein kinase C[71,72]. Another suppressive function of insulin by Leptin is by activating phosphodiesterase 3B[73]. This ends up in the suppression of cyclic AMP and inhibition of GLP-1 stimulated insulin secretion. Recent advancement in studying the action of Leptin in lowering insulin secretion is the consequence of Leptin

action on transcription of insulin gene in beta cells of pancreas. Due to this, Preproinsulin mRNA levels are reduced to half of its level[74,75].

There exist two hypotheses for the progression of insulin resistance and type 2 diabetes mellitus in obese persons. In obesity, high Leptin levels are noted. Hyperleptinemia causes decrease of insulin secretion caused by hyperglycemia. The next hypothesis states that there is a desentisation of Leptin receptors in beta cells of pancreas. The consequence of this is defective Leptin receptor signaling and resulting in chronic hyperinsulinemia. This paves way for the pathogenesis of diabetes[76].

ASSOCIATION OF LEPTIN WITH TYPE 2 DIABETES MELLITUS:



Source: Seufert J, Kieffer TJ, Leech CA, Holz GG, MoritzW, Ricordi C et al. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 1999; 84: 670-676.

MATERIALS AND METHODS:

The study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore. Ethical clearance was obtained from the Institutional Human Ethics Committee. An informed consent was taken from the patients before sample collection.

The study design is case control study in which obese type 2 diabetics (n =40) and thin type 2 diabetics (n =40) are selected as cases. The diagnosis of Type 2 diabetes is based on case record description which is based on insulin level and other factors at the time of diagnosis. Cases were selected from diabetic patients attending Endocrinology and Medicine OPD. Patients satisfying the diagnosis, inclusion criteria and BMI (measured by the investigator), and not coming under exclusion criteria, were given explanation about this study. If they were willing to participate, the consent forms were filled and the samples were collected. The samples were processed and stored for analysis.

Control individuals were mainly selected from the master health check up. For master health check up patients, weight, height and BMI were available in the case sheet. Person fulfilling the control group criteria was requested to participate in the study. If they were willing, the consent forms were filled and sample were collected from them. Their samples were also processed and stored for analysis. Patient data were retrieved from their case sheet.

INCLUSION CRITERIA:

GROUP 1:

Thin built, diabetic – BMI below 25

Both males and females

Age: 30-60 yrs.

Duration of diabetes - minimum period of 6 months from the date of diagnosis.

GROUP 2:

Obese, diabetic – BMI 30 and above

Males & females

Age: 30-60 yrs.

GROUP 3:

Thin built, non-diabetic – BMI below 25

Males & females

Age: 30-60 yrs.

GROUP 4:

Obese, non-diabetic - BMI 30 and above

Age: 30-60yrs.

Males & Females.

EXCLUSION CRITERIA:

Subjects with:

1. Acute ill patients.

2. Malignant neoplasm.

3. End stage renal disease.

4. Visual/motor/sensory defect.

5. On treatment for above conditions.

Collection of blood samples:

As cases are diabetics and controls are patients attending master health check up, fasting sample – fluoride vacutainer for fasting plasma glucose, EDTA vacutainer for HbA1c and clot activator vacutainer for lipid profile were collected routinely. The 2^{nd} hour post prandial plasma glucose sample was also collected routinely. The left over serum in the clot activator vacutainer tube used for lipid profile was transferred to

secondary labelled plain tube. This sample was stored at -20 degree celcius for Leptin assay.

The methods used for estimation of biochemical parameters were given below.

LEPTIN ELISA:

To quantify Leptin in serum by an enzyme immunoassay method[77].

PRINCIPLE OF THE TEST:

This is a sandwich type ELISA assay. The assay utilises two monoclonal antibodies which are highly specific. One monoclonal antibody is for Leptin which is immobilized in the microtiter plate and the second monoclonal antibody is specific for another epitope of Leptin which is biotin conjugated. Initially, sandwich complex is formed by Leptin present in the samples and standards bound to the immobilized antibody and to the biotinylated antibody. Then the microtiter plate is washed to remove the excess and unbound biotinylated antibody. Following which streptavidin conjugated horse radish peroxidase (HRP) is added. This binds to the bound biotinylated antibody specifically. Onceagain microtiter plate is washed to remove the unbound streptavidin – HRP. Then enzyme substrate tetramethylbenzidine (TMB) is added. Yellow colour is produced and the absorbance is measured at 450nm.

PROCEDURE:

Add 20 µl of sample per well.

Add 80 µl of biotinylated antibody per well. Incubate 1 hour.

Wash, then add 100 µl of Streptavidin-HRP conjugate per well. Incubate 30 minutes.

Wash, then add 100 µl of TMB Substrate per well. Incubate 10 minutes.

Add 50 µl of Stop Solution per well. Read at 450 nm immediately.

PLASMA GLUCOSE

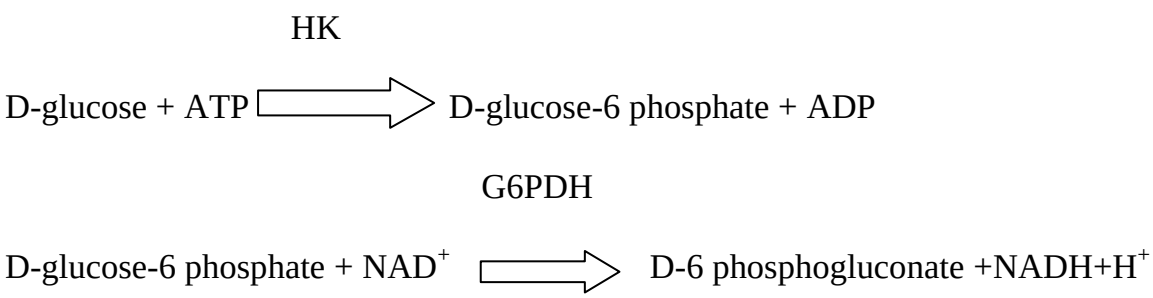
Method:

Enzymatic reference method with Hexokinase.

Principle:

The phosphorylation of glucose by ATP to form glucose-6 phosphate and ADP is catalysed by Hexokinase (HK). This is followed by the action of another enzyme, glucose-6 phosphate dehydrogenase (G6PDH). This enzyme catalyzes oxidation of glucose-6 phosphate by NAD^+ to form NADH.

Reaction:



The NADH concentration is directly proportional to the concentration of glucose and the increase in absorbance is measured at 340nm.

Test definition:

Mode of measurement	Absorbance
Mode of calculation of absorbance	Endpoint
Mode of reaction	R-S
Direction of Reaction	Increase
Wavelength A/B	340/409 nm
Test value	0-40 mmol/L
Unit	mmol/L

SERUM TOTAL CHOLESTEROL

Method:

Enzymatic colorimetric test.

The method involves use of enzymes cholesterol esterase, cholesterol oxidase and peroxidase.

Principle:

Cholesterol esters are cleaved by the action of cholesterol esterase (CE) to yield free cholesterol and fatty acids. Cholesterol oxidase (CHOD) in a second reaction helps in the formation of cholest-4-en-3-one and hydrogen peroxide by the oxidation of cholesterol. Peroxidase (POD) converts the hydrogen peroxide that is formed and helps in the oxidative coupling of phenol and 4-aminoantipyrine (AAP) forming a quinone-imine(red).

Reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{Cholesterol} + \text{R-COOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{Cholest-4-ens-3-one} + H_2$$

$$2\,H_2O_2 + 4\,\text{AAP} + \text{Phenol} \xrightarrow{POD} \text{Quinone-imine dye} + H_2O$$

The intensity of the red colour produced is directly proportional to the serum cholesterol level and is determined by the increase in absorbance measured at 512 nm.

Test definition:

Mode of measurement	Absorbance
Mode of calculation of absorbance	Endpoint
Mode of reaction	R-S
Direction of Reaction	Increase
Wavelength A/B	512/659 nm
Test value	0-20.7 mmol/L
Unit	mmol/L

SERUM TRIGLYCERIDES:

Method:

Enzymatic colorimetric method.

Involves enzymes glycerol phosphate oxidase and 4-aminophenazone.

Principle:

Lipoprotein lipase (LPL) hydrolyzes the triglycerides to glycerol and fatty acids. Glycerol kinase (GK) catalyses the conversion of glycerol to glycerol-3-phosphate with the help of ATP phosphorylation. Glycerol phosphate oxidase (GPO) catalyses the oxidation of glycerol-3-phosphate to form dihydroxyacetone phosphate and hydrogen peroxide. Peroxidase (POD) converts hydrogen peroxide, 4-chlorophenol and 4-aminophenazone to form a red-coloured quinoneimine dye. The intensity of color is measured at 512 nm and the concentration of triglyceride is assessed in the sample.

Reaction:

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3 phosphate + ADP}$$

$$\text{Glycerol -3 phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxy acetone Phosphate+} H_2O_2$$

$$2\ H_2O_2 + \text{4 aminophenazone} \xrightarrow{\text{POD}} \text{Quinoneimine dye+}4H_2O\text{+4 Chlorophenol}$$

Test definition:

Mode of measurement	Absorbance
Mode of calculation of absorbance	Endpoint
Mode of reaction	R-S

Direction of Reaction	Increase
Wavelength A/B	512/659 nm
Test range	0-10 mmol/L (0-885 mg/dL)
Unit	mmol/L

SERUM HDL CHOLESTEROL:

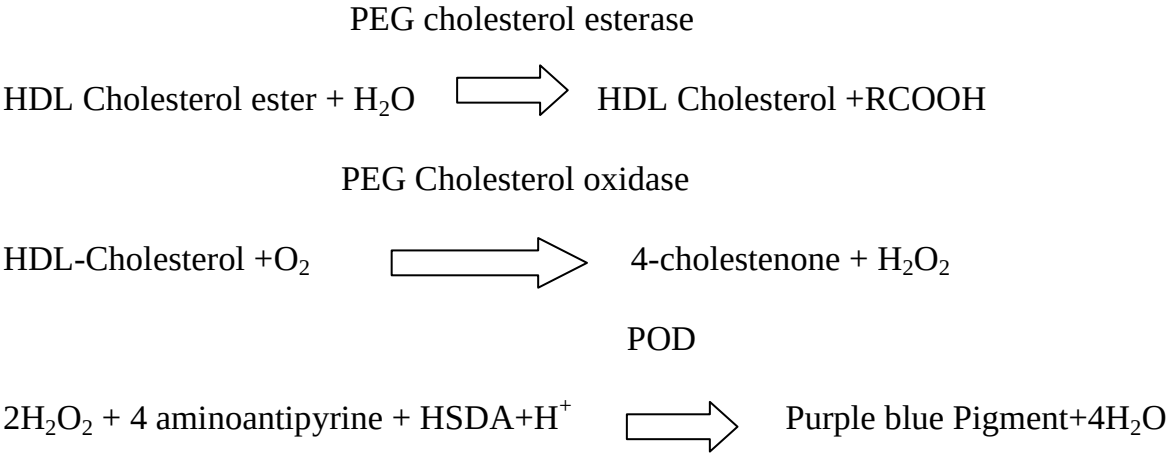
Method:

Homogenous enzymatic colorimetric assay.

Principle:

With magnesium ions, dextran sulphate complexes with LDL, VLDL and chylomicrons which are water soluble and resistant to polyethylene glycol (PEG) modified enzymes. Cholesterol esterase and cholesterol oxidase associated with PEG to the amino groups is used to determine the cholesterol level using enzymatic methods. Cholesterol ester is partitioned into free cholesterol and fatty acids with the help of cholesterol esterase. Oxidation of cholesterol by cholesterol oxidase is done in the presence of oxygen to Δ4-cholestenone and hydrogen peroxide. Hydrogen peroxide which is generated reacts with 4-aminoantipyrine and HSDA in the presence of peroxidase, forms a purple-blue dye. The cholesterol concentration is directly proportional to the intensity of blue colour and it is measured photometrically.

Reaction:



Test definition:

Mode of measurement	Absorbance
Mode of calculation of absorbance	Endpoint
Mode of reaction	R1-S-SR
Direction of Reaction	Increase
Wavelength A/B	583/659 nm
Test range	0-3.12 mmol/L (0-120 mg/dL)
Unit	mmol/L

SERUM LDL CHOLESTEROL:

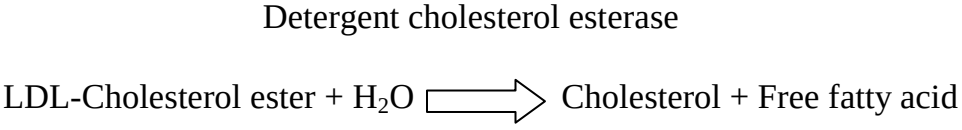
Method:

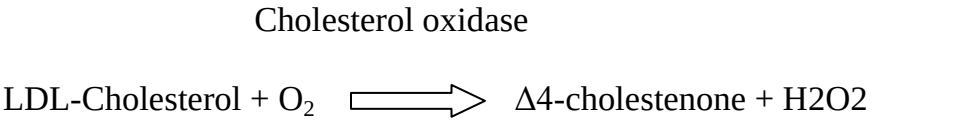
Homogeneous enzymatic colorimetric assay.

Principle:

This method is based on the property of selective micellary solubilization of LDL-cholesterol by a non-ionic detergent. It also utilizes the interaction of a sugar compound with lipoproteins such as VLDL and chylomicrons. Addition of a detergent to the enzymes used for cholesterol estimation (cholesterol esterase - cholesterol oxidase coupling reaction), increases the relative reactivities of cholesterol in the lipoprotein fractions in the following order: HDL < chylomicrons < VLDL < LDL. The activity of the enzymes used in the cholesterol estimation is reduced in the presence of magnesium ions and a sugar. The selective estimation of LDL-cholesterol in serum is enabled by the reaction of a sugar molecule with a detergent. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide. The intensity of the blue quinoneimine dye formed is directly proportional to the concentration of LDL cholesterol. It is determined by monitoring the increase in absorbance at 583 nm.

Reaction:





$2H_2O_2$ + 4 aminoantipyrine + HSDA+H^+ ⟹ Purple blue pigment+$4H_2O$

Test definition:

Mode of measurement	Absorbance
Mode of calculation of absorbance	Endpoint
Mode of reaction	R1-S-SR
Direction of Reaction	Increase
Wavelength A/B	583/659 nm
Test range	0-14.2 mmol/L (0-550 mg/dL)
Unit	mmol/L

Glycated haemoglobin: (HbA1c)

Method:

Turbidimetric inhibition immunoassay

Principle:

EDTA tube collected whole blood specimen is hemolysed after collection. Total hemoglobin and HbA1c concentrations are determined. Total hemoglobin is measured colorimetrically. The percentage of HbA1c is determined immunoturbidimetrically. The ratio of both these levels gives the final percent HbA1c result.

EDTA collected blood is hemolysed with hemolysis reagent in the predilution cuvette. This leads to reduction in osmotic pressure which lyses the

erythrocytes. Lysis of erythrocytes releases the haemoglobin. It is degraded by the proteolytic action of the enzyme pepsin. This renders the beta-N terminal structures more available for the immunoassay. In the hemolysate, a colorimetric method helps in the determination of total haemoglobin. This is done on the basis of production of a brownish-green chromophore. This occurs in alkaline detergent solution using a cyanide free method. The intensity of color relates to the level of total hemoglobin in the sample. The concentration is calculated by sensing the raise in the absorbance at 552nm. A fixed factor that is obtained from the primary calibrator chlorohemin calculates the test results.

Turbidimetric method to measure HbA1c is done using monoclonal antibodies which are present attached to latex particles. The monoclonal antibodies bind the amino terminal fragments of HbA1c. The unbound free antibodies agglutinate with an artificial polymer. The change in turbidity is inversely related to the quantity of bound glycated proteins. This is measured turbidimetrically at 552nm. HbA1c result is given as percentage of HbA1c. It is evaluated from the ratio of HbA1c/Hb as given below:

According to IFCC

HbA1c (%) = (HbA1c / Hb) x 100

According to DCCT/NGSP

HbA1c(%) = (HbA1c/Hb) x 87.6 + 2.27

Test definition:

Mode of measurement	Absorbance
Mode of calculation of absorbance	Endpoint
Mode of reaction	D-R1-S-SR
Direction of Reaction	Increase
Wavelength A/B	552nm
Test range	1.4-4.7μmol/L (2.4-75.8mg/dL)
Unit	μmol/L

STATISTICAL ANALYSIS:

The data obtained after the estimation of Leptin, Body Mass Index, Fasting Glucose, Post Prandial Glucose, Cholesterol, Triglycerides, HDL, LDL and HbA1c were statistically analyzed using IBM SPSS software version 19.

The data distribution was displayed by histogram, dot plot and scatter plot. The data summary was represented by bar diagram.

The dependence of categorical variable was tested with Chi Square test. For comparing two groups t test was used. For comparing multiple group means one way ANOVA (Analysis Of Variance) was done. For post hoc testing, Tukey and Games Howel were used according to the group n and equivalence of variance. Significance was assessed at 5% level.

The correlation between quantitative parameters were done using Pearson and further checked with scatter plot. For correction of right side skewed data, natural logarithm transformation was used.

RESULTS:

The age, sex and BMI distribution of the study groups are given in the figures 1, 2 & 3 respectively. This shows the even matching of cases and controls. But the BMI of obese subjects in both cases and controls were more clustered around 30. The glucose values in fasting and postprandial states of the cases and controls are given in the bar diagram (figure 4) is consistent with diabetic and non diabetic groups.

The distribution of values of various parameters in the four groups is given in figures 5 to 11. Fasting glucose, Post prandial glucose and HbA1c are low in control group as expected. The distribution of Cholesterol, HDL cholesterol and LDL cholesterol are similar in all groups. The triglycerides distribution is towards lower side in Thin-non-diabetic group.

Leptin distribution was confined to lower side both in thin diabetic and thin non diabetic groups while there is some dispersion to higher side in obese groups. The Leptin distribution in obese diabetic group shows a right skewing. So the natural logarithm and log10 transformations were applied to the distribution. After the transformation the distribution was showing slight right skewing in all groups except the obese diabetic group. Anyhow the natural logarithm of Leptin was used for examining the correlation between quantitative variables. The correlation coefficients are presented in Table 14 and the corresponding scatter plots are given in the figure 16.

FIGURE:1 DISTRIBUTION OF AGE IN STUDY GROUPS

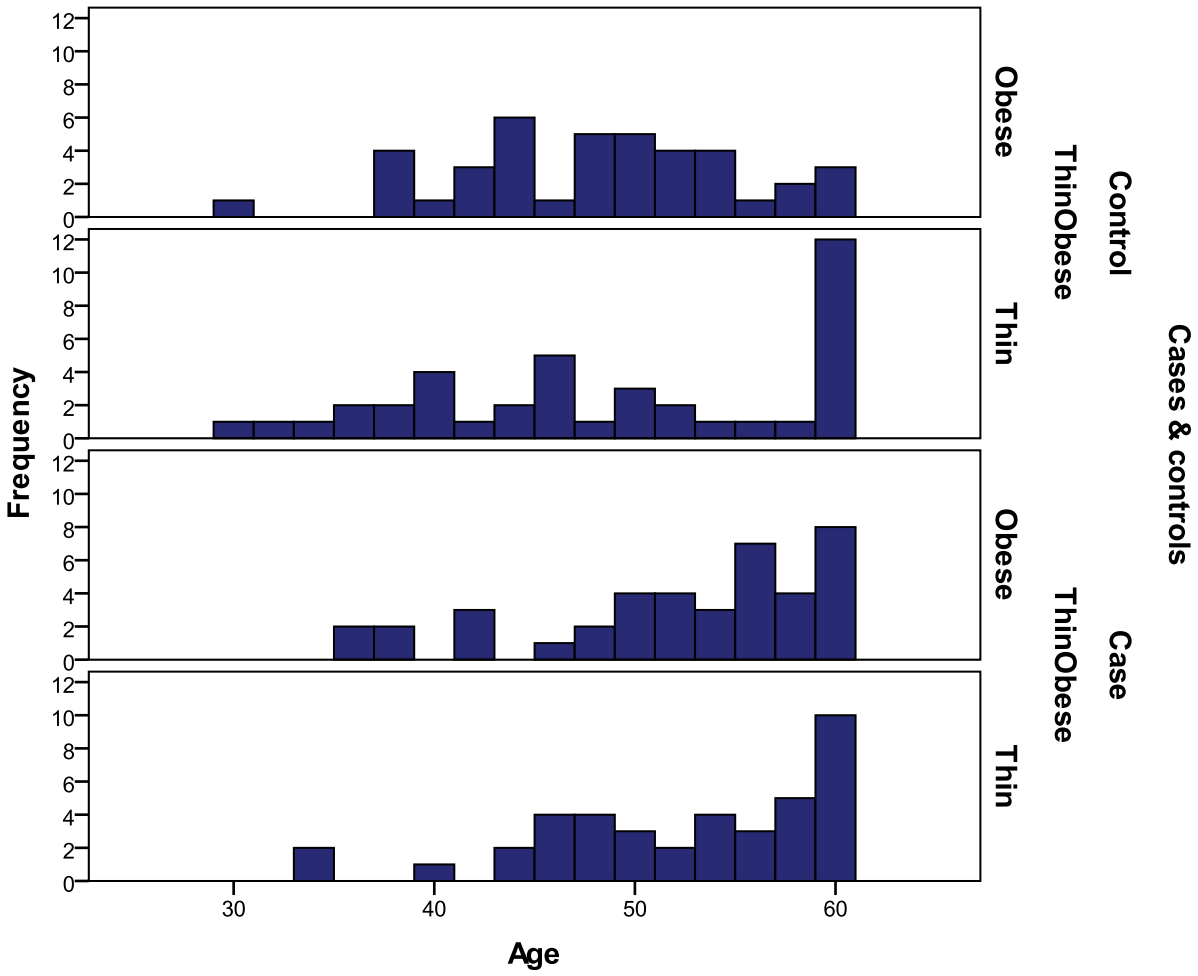


TABLE 1: COMPARISON OF AGE AMONG STUDY GROUPS

ANOVA

Age

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	627.769	3	209.256	3.428	.019
Within Groups	9522.725	156	61.043		
Total	10150.494	159			

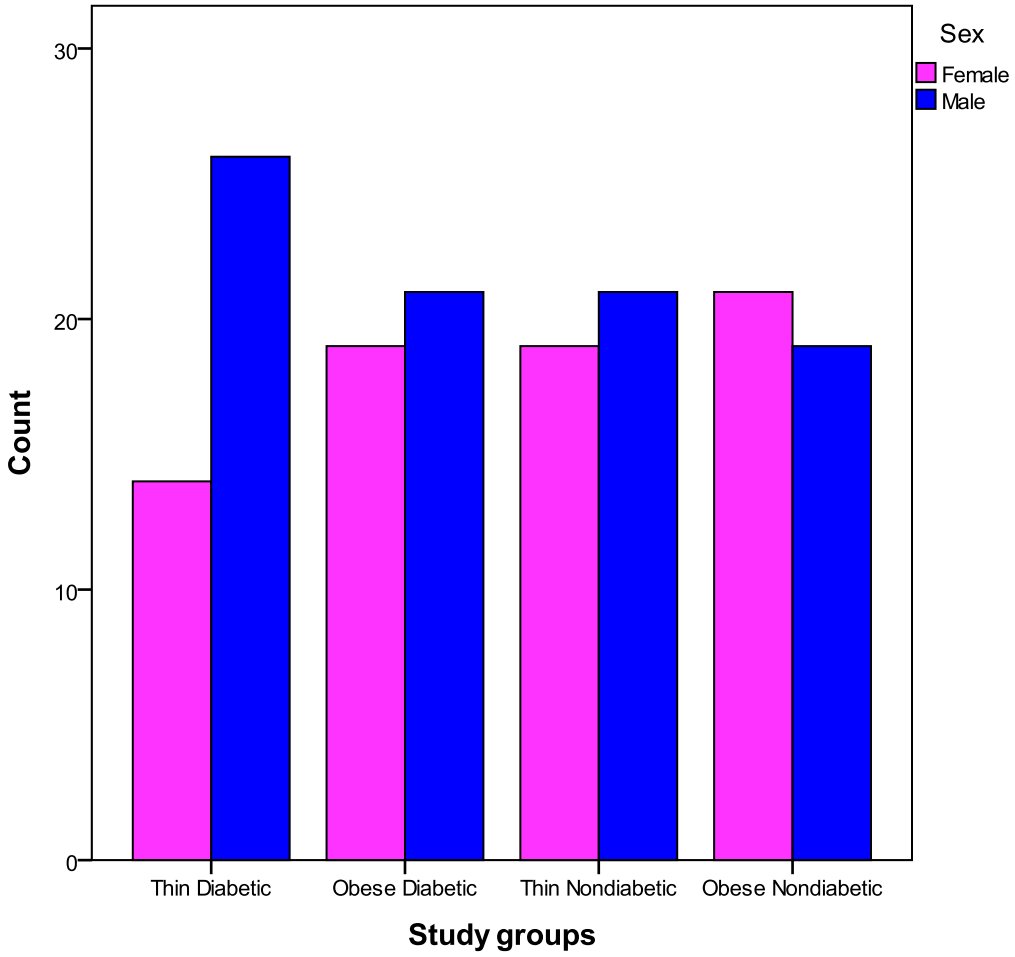
Multiple Comparisons

Age

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	.225	1.747	.999	-4.31	4.76
	Thin Nondiabetic	3.600	1.747	.171	-.94	8.14
	Obese Nondiabetic	4.450	1.747	.057	-.09	8.99
Obese Diabetic	Thin Diabetic	-.225	1.747	.999	-4.76	4.31
	Thin Nondiabetic	3.375	1.747	.219	-1.16	7.91
	Obese Nondiabetic	4.225	1.747	.078	-.31	8.76
Thin Nondiabetic	Thin Diabetic	-3.600	1.747	.171	-8.14	.94
	Obese Diabetic	-3.375	1.747	.219	-7.91	1.16
	Obese Nondiabetic	.850	1.747	.962	-3.69	5.39
Obese Nondiabetic	Thin Diabetic	-4.450	1.747	.057	-8.99	.09
	Obese Diabetic	-4.225	1.747	.078	-8.76	.31
	Thin Nondiabetic	-.850	1.747	.962	-5.39	3.69

FIGURE:2 DISTRIBUTION OF SEX IN STUDY GROUPS



According to CHI-SQUARE test, p value was found to be 0.266 (Not Significant)

FIGURE:3 DISTRIBUTION OF BODY MASS INDEX IN STUDY GROUPS

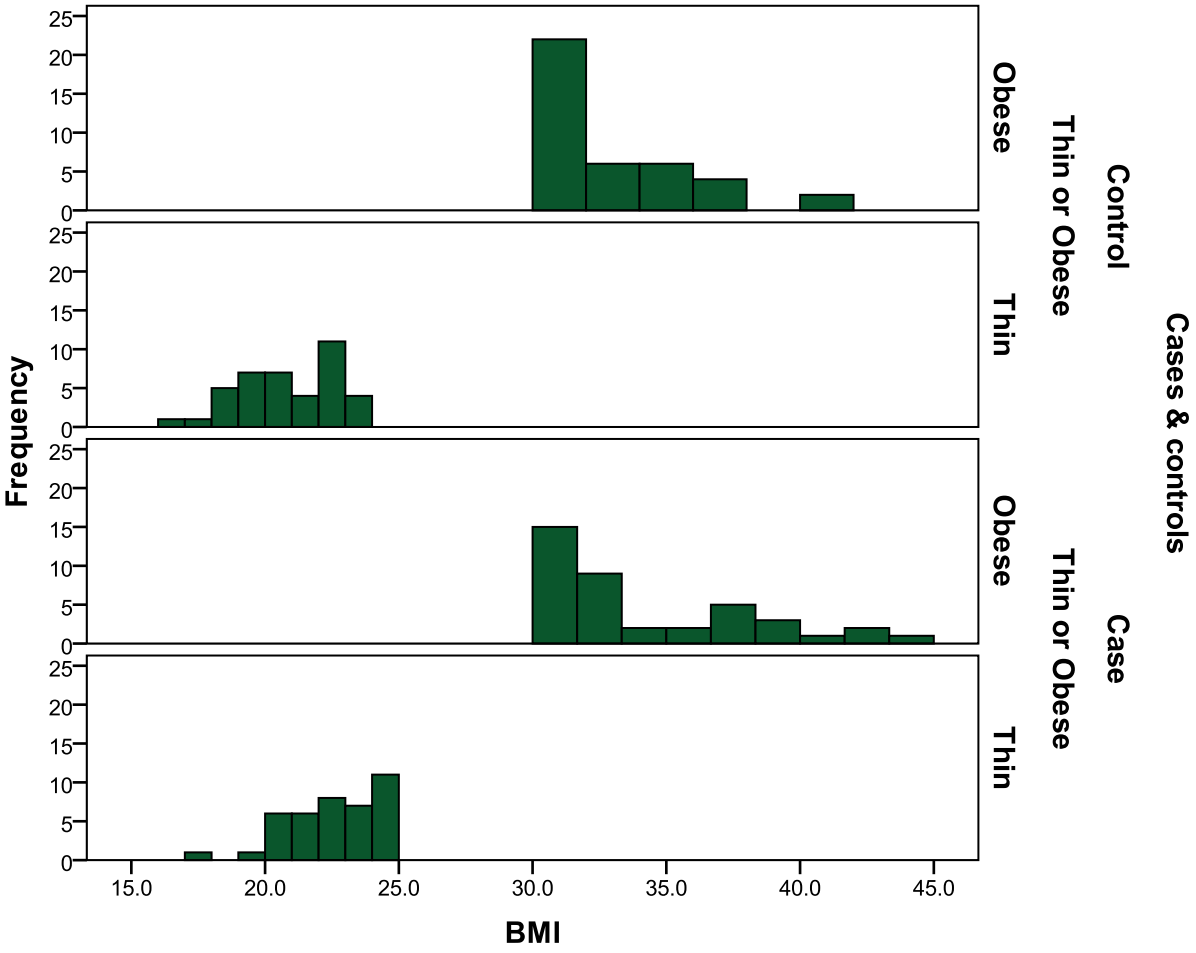


TABLE 2: COMPARISON OF BMI AMONG STUDY GROUPS

ANOVA

BMI

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5747.804	3	1915.935	243.922	.000
Within Groups	1225.336	156	7.855		
Total	6973.139	159			

Multiple Comparisons

BMI

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	-11.7255*	.6267	.000	-13.353	-10.098
	Thin Nondiabetic	1.7827*	.6267	.026	.155	3.410
	Obese Nondiabetic	-10.2408*	.6267	.000	-11.868	-8.613
Obese Diabetic	Thin Diabetic	11.7255*	.6267	.000	10.098	13.353
	Thin Nondiabetic	13.5083*	.6267	.000	11.881	15.136
	Obese Nondiabetic	1.4848	.6267	.087	-.143	3.112
Thin Nondiabetic	Thin Diabetic	-1.7827*	.6267	.026	-3.410	-.155
	Obese Diabetic	-13.5083*	.6267	.000	-15.136	-11.881
	Obese Nondiabetic	-12.0235*	.6267	.000	-13.651	-10.396
Obese Nondiabetic	Thin Diabetic	10.2408*	.6267	.000	8.613	11.868
	Obese Diabetic	-1.4848	.6267	.087	-3.112	.143
	Thin Nondiabetic	12.0235*	.6267	.000	10.396	13.651

*. The mean difference is significant at the 0.05 level.

FIGURE 4: FASTING AND POST PRANDIAL GLUCOSE LEVELS IN STUDY GROUPS

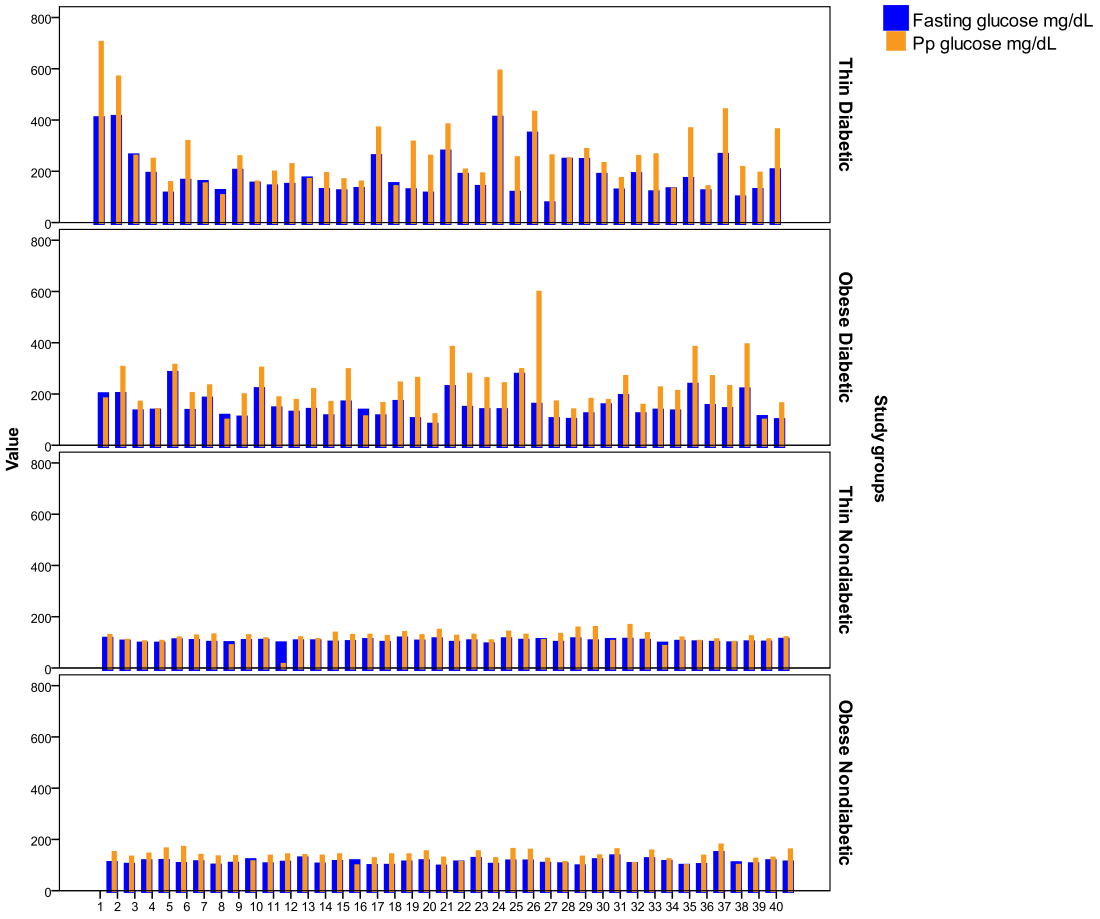
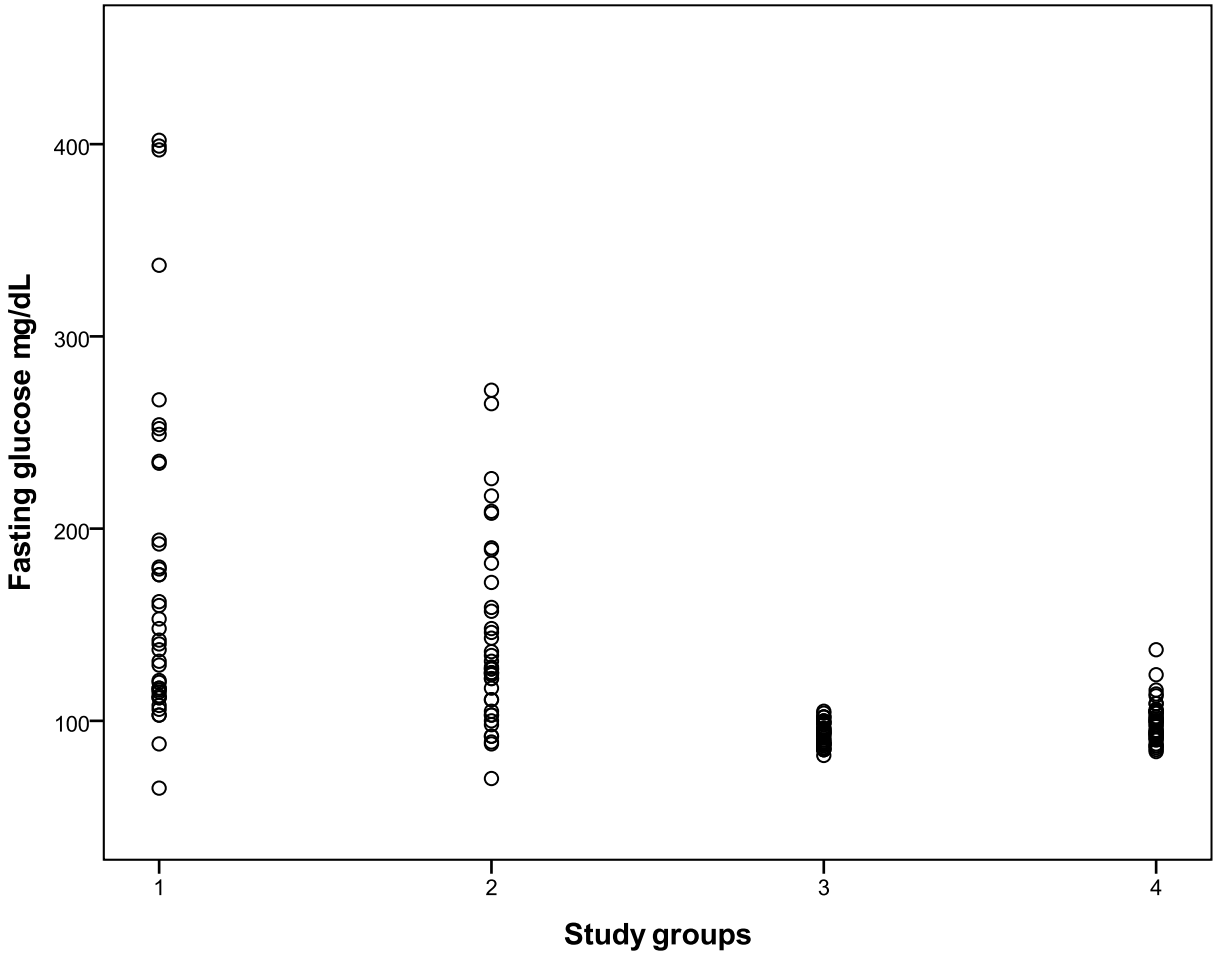


FIGURE 5: FASTING GLUCOSE LEVEL IN STUDY GROUPS



1 - THIN DIABETIC
2 - OBESE DIABETIC
3 - THIN NON- DIABETIC
4 - OBESE NON - DIABETIC

TABLE 3: COMPARISON OF FASTING GLUCOSE LEVEL AMONG STUDY GROUPS

ANOVA

Fasting glucose mg/dL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	180580.069	3	60193.356	24.205	.000
Within Groups	387944.925	156	2486.826		
Total	568524.994	159			

Multiple Comparisons

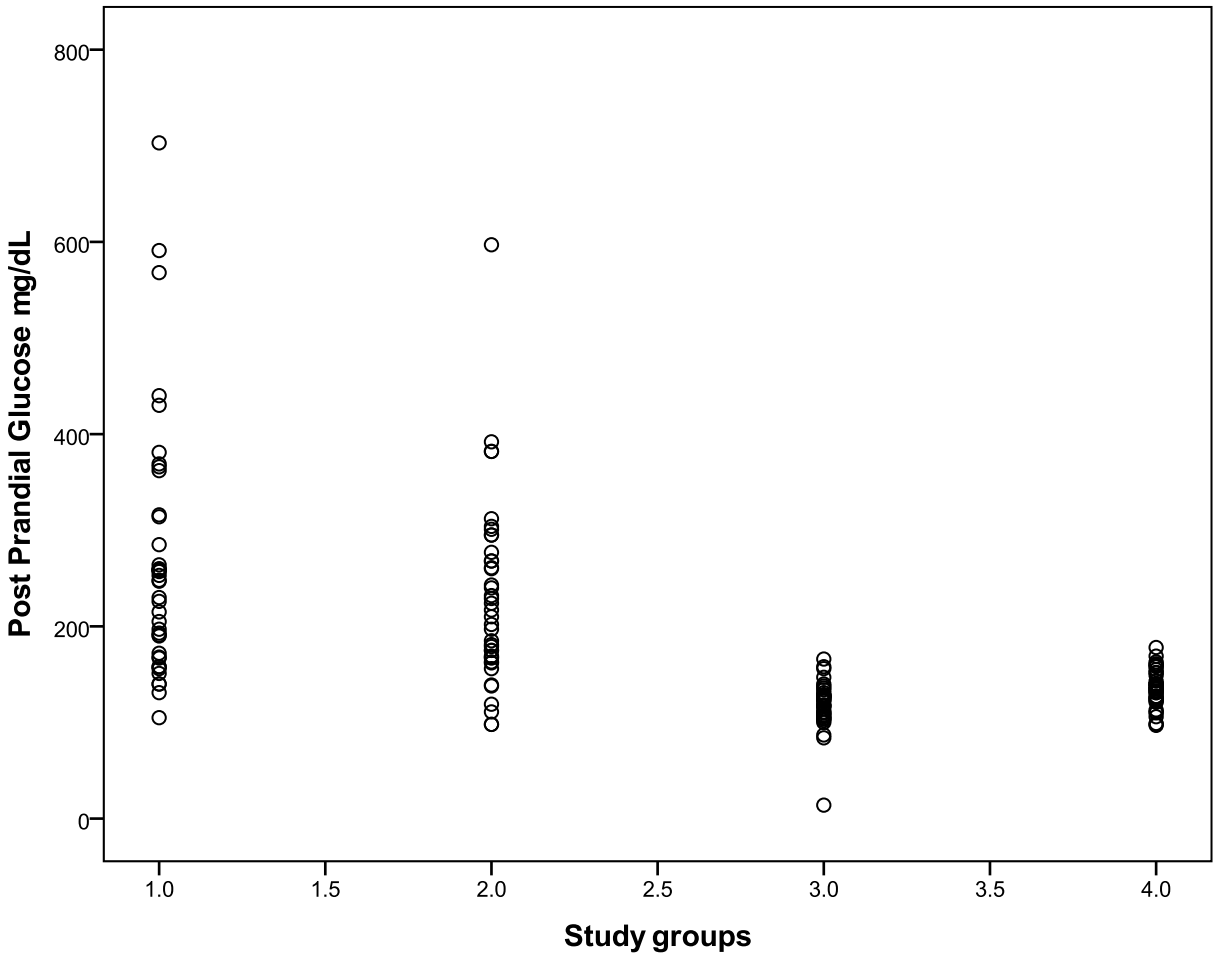
Fasting glucose mg/dL

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	33.825*	11.151	.015	4.87	62.78
	Thin Nondiabetic	82.850*	11.151	.000	53.89	111.81
	Obese Nondiabetic	76.200*	11.151	.000	47.24	105.16
Obese Diabetic	Thin Diabetic	-33.825*	11.151	.015	-62.78	-4.87
	Thin Nondiabetic	49.025*	11.151	.000	20.07	77.98
	Obese Nondiabetic	42.375*	11.151	.001	13.42	71.33
Thin Nondiabetic	Thin Diabetic	-82.850*	11.151	.000	-111.81	-53.89
	Obese Diabetic	-49.025*	11.151	.000	-77.98	-20.07
	Obese Nondiabetic	-6.650	11.151	.933	-35.61	22.31
Obese Nondiabetic	Thin Diabetic	-76.200*	11.151	.000	-105.16	-47.24
	Obese Diabetic	-42.375*	11.151	.001	-71.33	-13.42
	Thin Nondiabetic	6.650	11.151	.933	-22.31	35.61

*. The mean difference is significant at the 0.05 level.

FIGURE 6: POST PRANDIAL GLUCOSE IN STUDY GROUPS



1 - THIN DIABETIC
2 - OBESE DIABETIC
3 - THIN NON- DIABETIC
4 - OBESE NON - DIABETIC

TABLE 4: COMPARISON OF POST PRANDIAL GLUCOSE LEVEL AMONG STUDY GROUPS

ANOVA

Pp glucose mg/dL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	627509.469	3	209169.823	30.332	.000
Within Groups	1075775.625	156	6895.998		
Total	1703285.094	159			

Multiple Comparisons

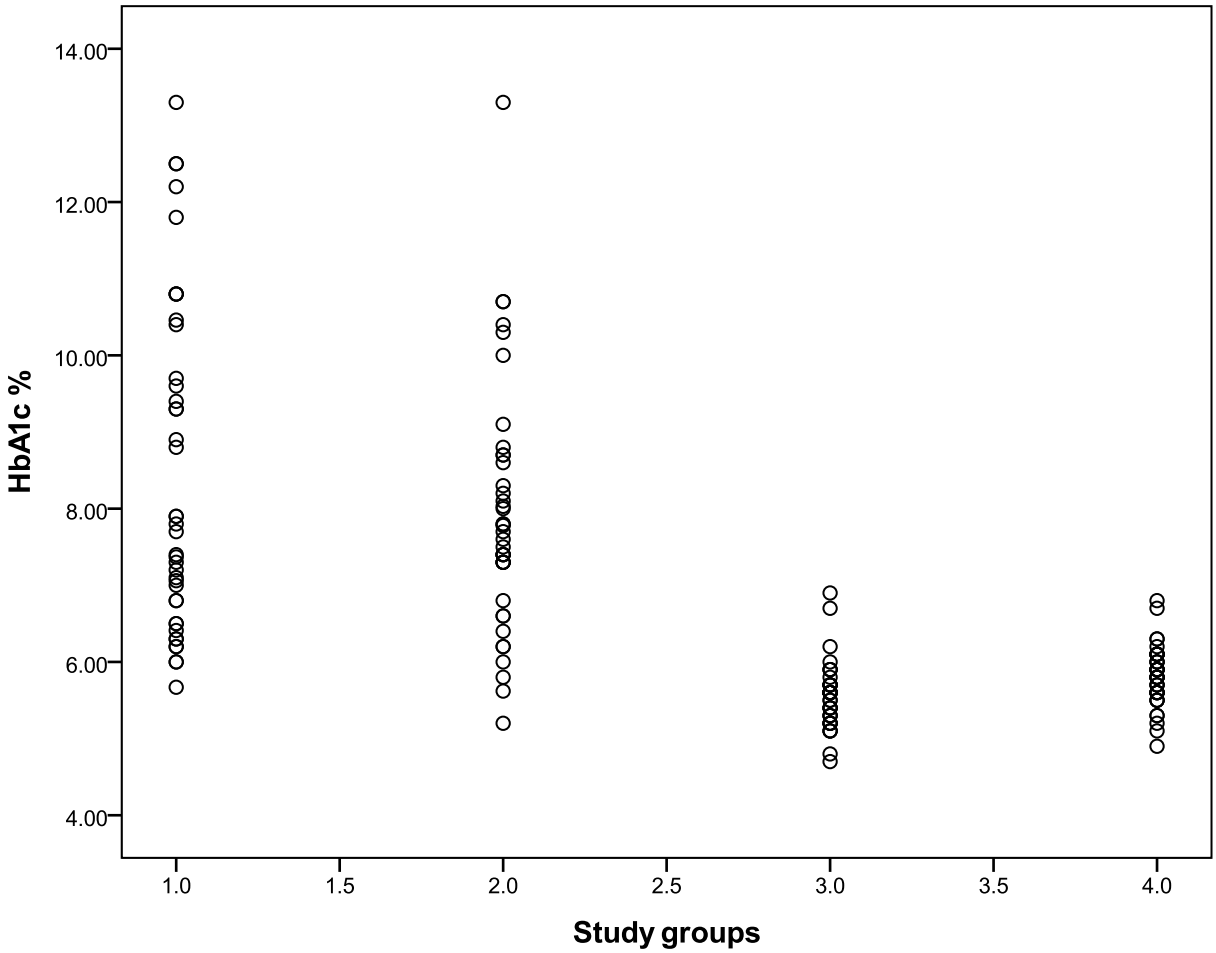
Pp glucose mg/dL

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	38.800	18.569	.161	-9.42	87.02
	Thin Nondiabetic	149.225*	18.569	.000	101.00	197.45
	Obese Nondiabetic	132.900*	18.569	.000	84.68	181.12
Obese Diabetic	Thin Diabetic	-38.800	18.569	.161	-87.02	9.42
	Thin Nondiabetic	110.425*	18.569	.000	62.20	158.65
	Obese Nondiabetic	94.100*	18.569	.000	45.88	142.32
Thin Nondiabetic	Thin Diabetic	-149.225*	18.569	.000	-197.45	-101.00
	Obese Diabetic	-110.425*	18.569	.000	-158.65	-62.20
	Obese Nondiabetic	-16.325	18.569	.816	-64.55	31.90
Obese Nondiabetic	Thin Diabetic	-132.900*	18.569	.000	-181.12	-84.68
	Obese Diabetic	-94.100*	18.569	.000	-142.32	-45.88
	Thin Nondiabetic	16.325	18.569	.816	-31.90	64.55

*. The mean difference is significant at the 0.05 level.

FIGURE 7: HbA1c LEVEL IN STUDY GROUPS



1 - THIN DIABETIC
2 - OBESE DIABETIC
3 - THIN NON- DIABETIC
4 - OBESE NON - DIABETIC

TABLE 5: COMPARISON OF HbA1c LEVEL AMONG STUDY GROUPS

ANOVA

A1c %

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	255.236	3	85.079	45.020	.000
Within Groups	294.805	156	1.890		
Total	550.041	159			

Multiple Comparisons

A1c %

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	.54100	.30739	.297	-.2573	1.3393
	Thin Nondiabetic	2.89175*	.30739	.000	2.0935	3.6900
	Obese Nondiabetic	2.62925*	.30739	.000	1.8310	3.4275
Obese Diabetic	Thin Diabetic	-.54100	.30739	.297	-1.3393	.2573
	Thin Nondiabetic	2.35075*	.30739	.000	1.5525	3.1490
	Obese Nondiabetic	2.08825*	.30739	.000	1.2900	2.8865
Thin Nondiabetic	Thin Diabetic	-2.89175*	.30739	.000	-3.6900	-2.0935
	Obese Diabetic	-2.35075*	.30739	.000	-3.1490	-1.5525
	Obese Nondiabetic	-.26250	.30739	.828	-1.0608	.5358
Obese Nondiabetic	Thin Diabetic	-2.62925*	.30739	.000	-3.4275	-1.8310
	Obese Diabetic	-2.08825*	.30739	.000	-2.8865	-1.2900
	Thin Nondiabetic	.26250	.30739	.828	-.5358	1.0608

*. The mean difference is significant at the 0.05 level.

FIGURE 8: CHOLESTEROL LEVEL IN STUDY GROUPS

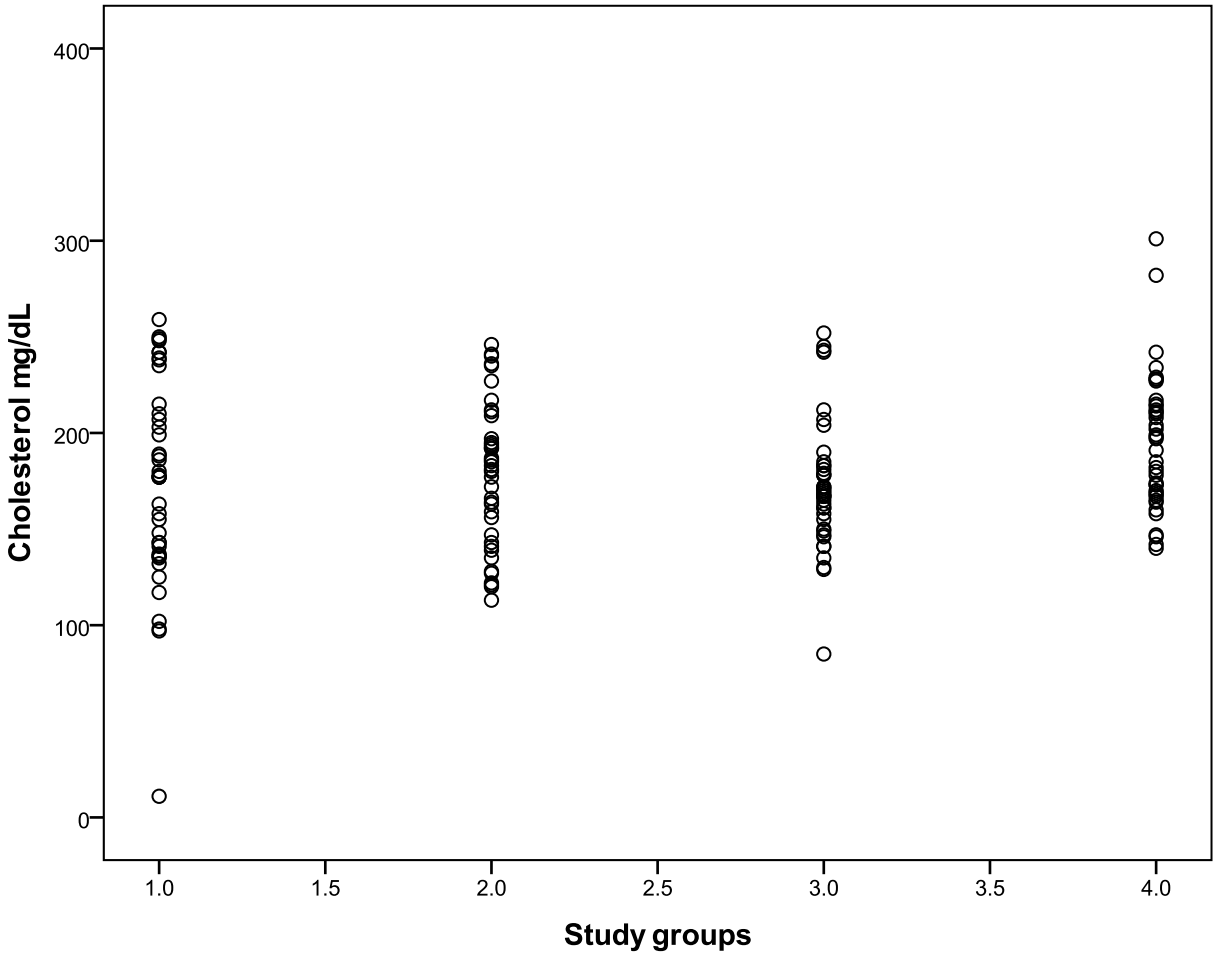


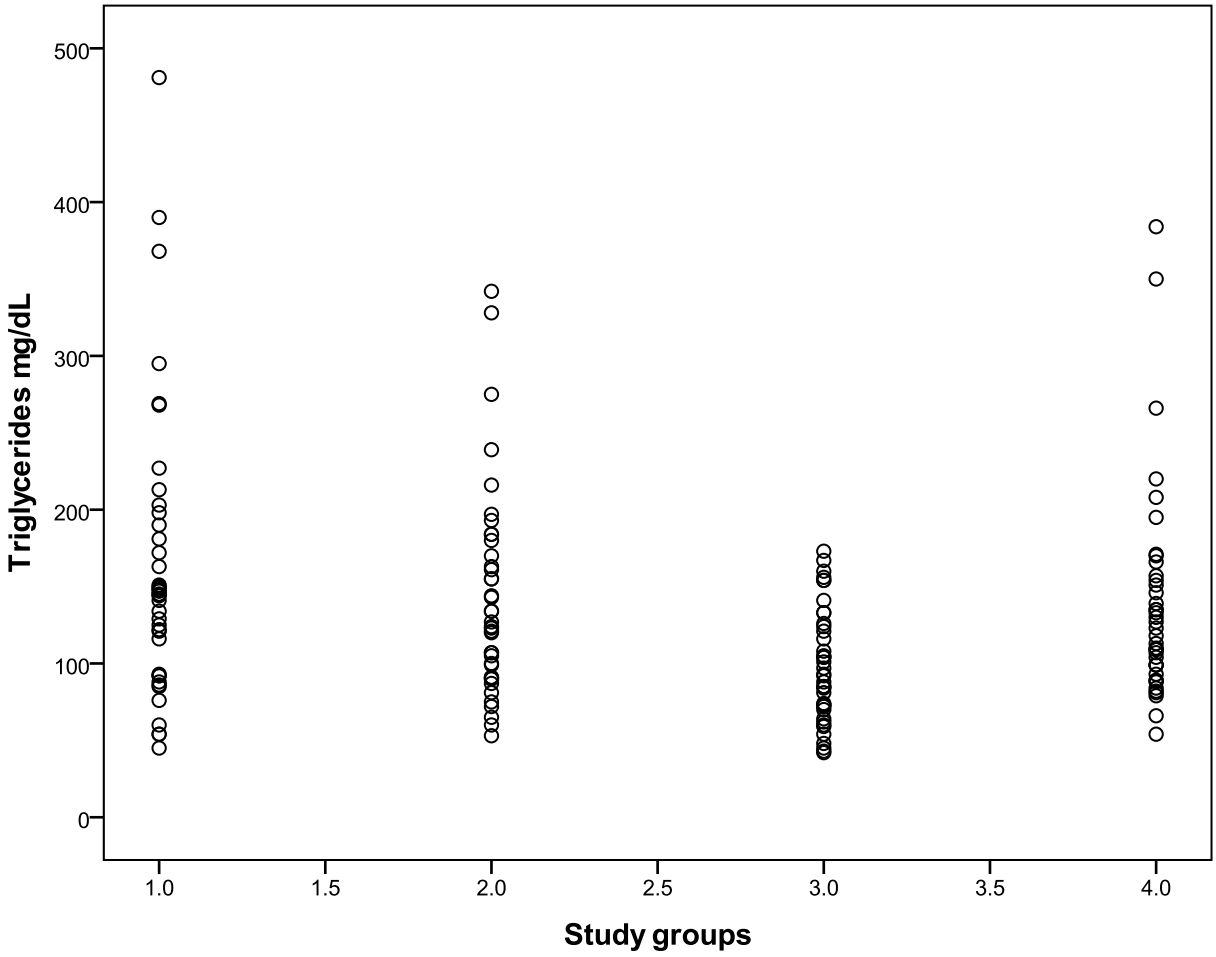
TABLE 6: COMPARISON OF CHOLESTEROL LEVEL AMONG STUDY GROUPS

ANOVA

Cholesterol mg/dL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12432.850	3	4144.283	2.473	.064
Within Groups	261462.250	156	1676.040		
Total	273895.100	159			

FIGURE 9: TRIGLYCERIDE LEVEL IN STUDY GROUPS



1 - THIN DIABETIC
2 - OBESE DIABETIC
3 - THIN NON- DIABETIC
4 - OBESE NON - DIABETIC

TABLE 7: COMPARISON OF TRIGLYCERIDE LEVEL AMONG STUDY GROUPS

ANOVA

Triglycerides mg/dL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	87716.869	3	29238.956	5.997	.001
Within Groups	760623.875	156	4875.794		
Total	848340.744	159			

Multiple Comparisons

Triglycerides mg/dL

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	20.625	15.614	.551	-19.92	61.17
	Thin Nondiabetic	64.650*	15.614	.000	24.10	105.20
	Obese Nondiabetic	24.450	15.614	.401	-16.10	65.00
Obese Diabetic	Thin Diabetic	-20.625	15.614	.551	-61.17	19.92
	Thin Nondiabetic	44.025*	15.614	.028	3.48	84.57
	Obese Nondiabetic	3.825	15.614	.995	-36.72	44.37
Thin Nondiabetic	Thin Diabetic	-64.650*	15.614	.000	-105.20	-24.10
	Obese Diabetic	-44.025*	15.614	.028	-84.57	-3.48
	Obese Nondiabetic	-40.200	15.614	.053	-80.75	.35
Obese Nondiabetic	Thin Diabetic	-24.450	15.614	.401	-65.00	16.10
	Obese Diabetic	-3.825	15.614	.995	-44.37	36.72
	Thin Nondiabetic	40.200	15.614	.053	-.35	80.75

*. The mean difference is significant at the 0.05 level.

FIGURE 10: HDL LEVEL IN STUDY GROUPS

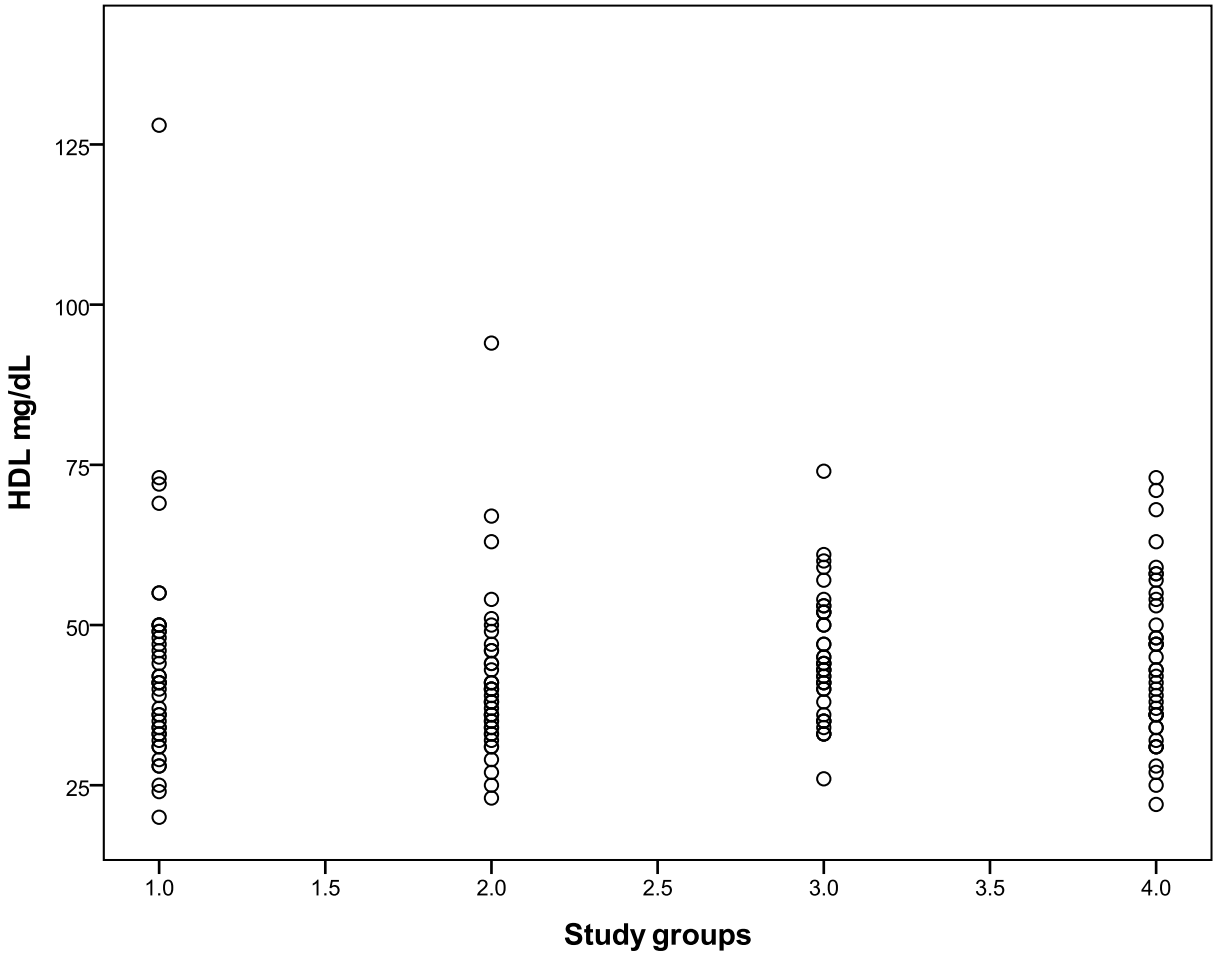


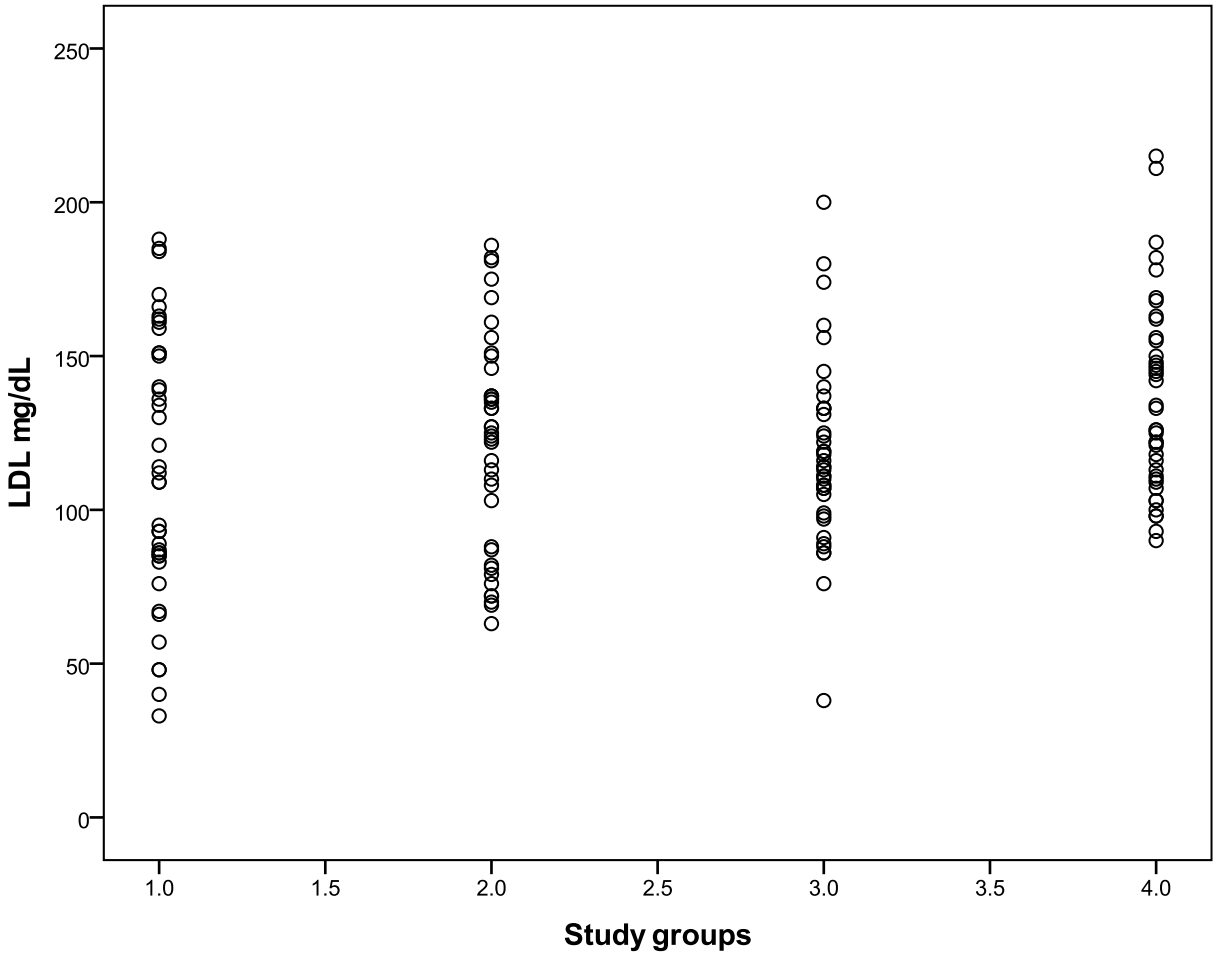
TABLE 8: COMPARISON OF HDL LEVEL AMONG STUDY GROUPS

ANOVA

Hdl mg/dL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	337.769	3	112.590	.605	.613
Within Groups	29054.425	156	186.246		
Total	29392.194	159			

FIGURE 11: LDL LEVEL IN STUDY GROUPS



1 - THIN DIABETIC
2 - OBESE DIABETIC
3 - THIN NON- DIABETIC
4 - OBESE NON - DIABETIC

TABLE 9: COMPARISON OF LDL LEVEL AMONG STUDY GROUPS

ANOVA

Ldl mg/dL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11310.950	3	3770.317	3.039	.031
Within Groups	193508.950	156	1240.442		
Total	204819.900	159			

Multiple Comparisons

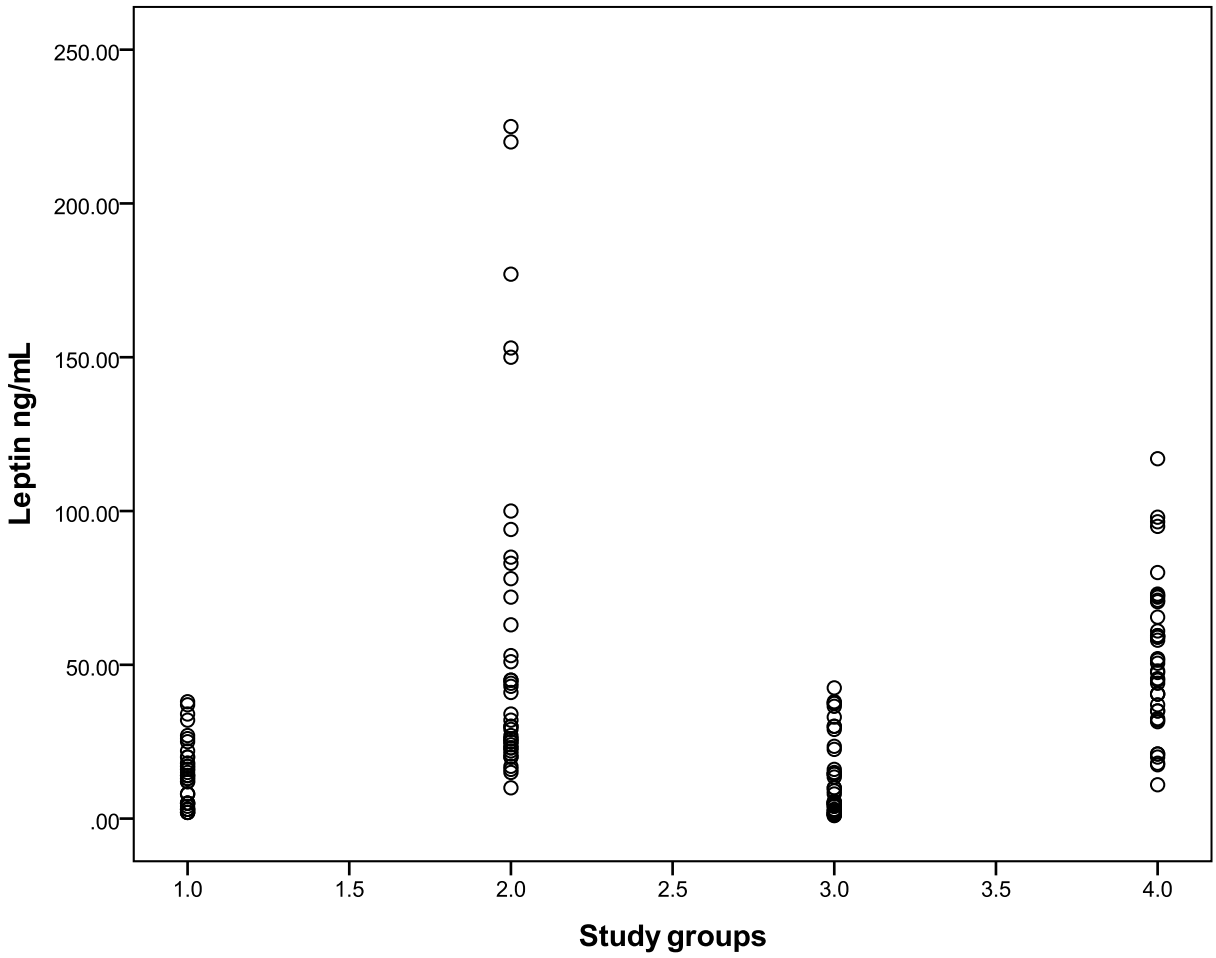
Ldl mg/dL

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	-8.175	7.875	.727	-28.63	12.28
	Thin Nondiabetic	-4.250	7.875	.949	-24.70	16.20
	Obese Nondiabetic	-22.375*	7.875	.026	-42.83	-1.92
Obese Diabetic	Thin Diabetic	8.175	7.875	.727	-12.28	28.63
	Thin Nondiabetic	3.925	7.875	.959	-16.53	24.38
	Obese Nondiabetic	-14.200	7.875	.276	-34.65	6.25
Thin Nondiabetic	Thin Diabetic	4.250	7.875	.949	-16.20	24.70
	Obese Diabetic	-3.925	7.875	.959	-24.38	16.53
	Obese Nondiabetic	-18.125	7.875	.102	-38.58	2.33
Obese Nondiabetic	Thin Diabetic	22.375*	7.875	.026	1.92	42.83
	Obese Diabetic	14.200	7.875	.276	-6.25	34.65
	Thin Nondiabetic	18.125	7.875	.102	-2.33	38.58

*. The mean difference is significant at the 0.05 level.

FIGURE 12: LEPTIN LEVEL IN STUDY GROUPS



1 - THIN DIABETIC
2 - OBESE DIABETIC
3 - THIN NON- DIABETIC
4 - OBESE NON - DIABETIC

TABLE 10: COMPARISON OF LEPTIN LEVEL AMONG STUDY GROUPS

ANOVA

Leptin value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	68598.467	3	22866.156	23.591	.000
Within Groups	151205.469	156	969.266		
Total	219803.936	159			

Multiple Comparisons

Leptin value

Tukey HSD

(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic	Obese Diabetic	-43.97500*	6.96156	.000	-62.0537	-25.8963
	Thin Nondiabetic	.28750	6.96156	1.000	-17.7912	18.3662
	Obese Nondiabetic	-38.15000*	6.96156	.000	-56.2287	-20.0713
Obese Diabetic	Thin Diabetic	43.97500*	6.96156	.000	25.8963	62.0537
	Thin Nondiabetic	44.26250*	6.96156	.000	26.1838	62.3412
	Obese Nondiabetic	5.82500	6.96156	.837	-12.2537	23.9037
Thin Nondiabetic	Thin Diabetic	-.28750	6.96156	1.000	-18.3662	17.7912
	Obese Diabetic	-44.26250*	6.96156	.000	-62.3412	-26.1838
	Obese Nondiabetic	-38.43750*	6.96156	.000	-56.5162	-20.3588
Obese Nondiabetic	Thin Diabetic	38.15000*	6.96156	.000	20.0713	56.2287
	Obese Diabetic	-5.82500	6.96156	.837	-23.9037	12.2537
	Thin Nondiabetic	38.43750*	6.96156	.000	20.3588	56.5162

*. The mean difference is significant at the 0.05 level.

FIGURE 13: LEPTIN LEVEL IN STUDY SUB GROUPS

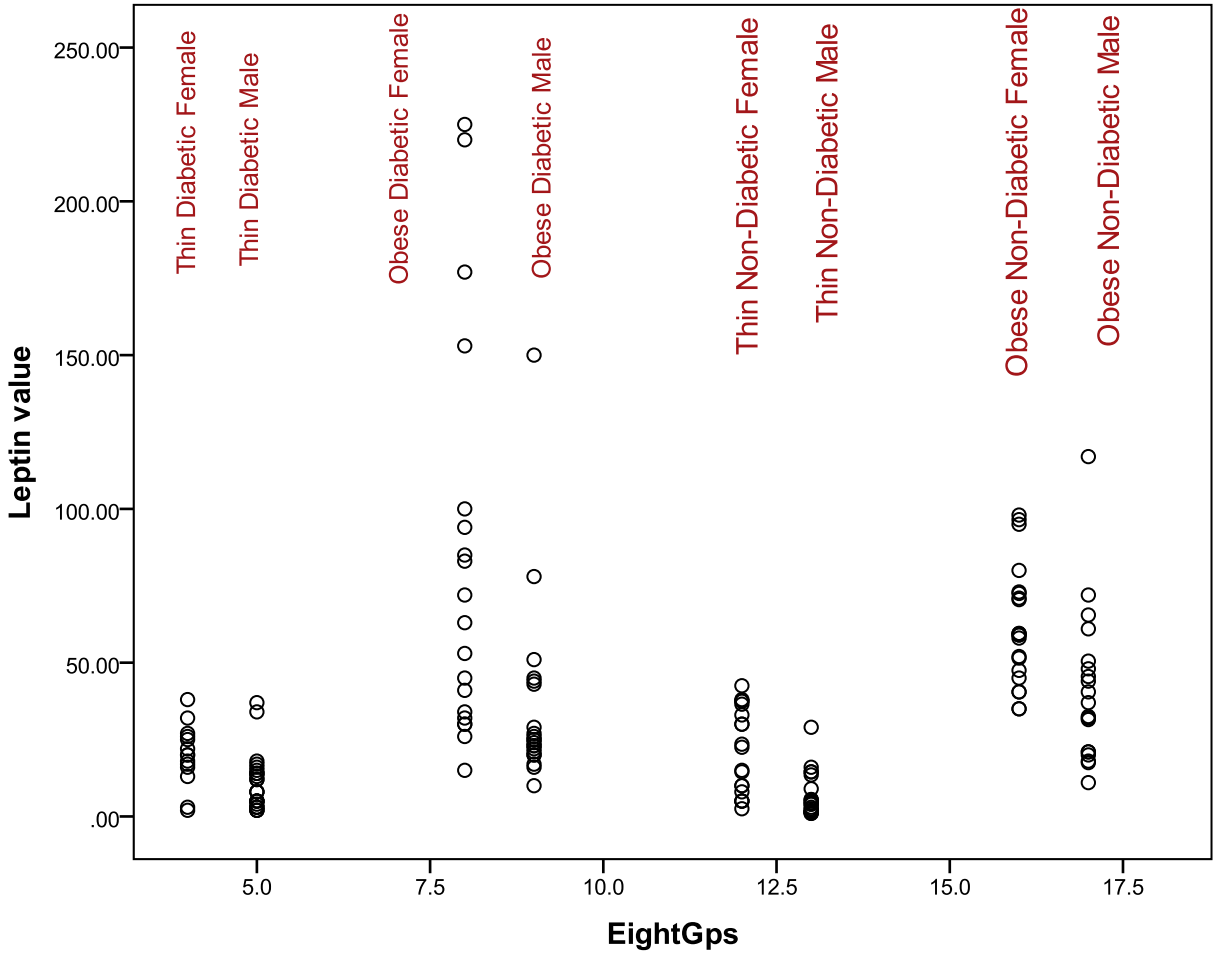


TABLE 11: COMPARISON OF LEPTIN LEVEL AMONG STUDY SUB GROUPS

ANOVA

Leptin value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	98556.354	7	14079.479	17.651	.000
Within Groups	121247.582	152	797.681		
Total	219803.936	159			

TABLE 12: COMPARISON OF LEPTIN LEVEL AMONG STUDY SUB GROUPS BASED ON GENDER

Multiple Comparisons - Leptin value

Games-Howell

(I) EightGps	(J) EightGps	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Thin Diabetic Female	ThinDiabeticMale	9.19780	3.17764	.117	-1.3043	19.6999
	ObeseDiabeticFemale	-63.12406*	15.15967	.010	-114.4808	-11.7674
	ObeseDiabeticMale	-15.26190	7.14256	.419	-38.7673	8.2435
	ThinNonDiabeticFemale	-1.43985	4.13210	1.000	-14.8541	11.9744
	ThinNonDiabeticMale	13.23810*	3.06527	.006	2.9945	23.4817
	ObeseNonDiabeticFemale	-41.90476*	4.96636	.000	-58.0128	-25.7967
	ObeseNonDiabeticMale	-21.41353*	6.34706	.043	-42.3715	-.4556
Thin Diabetic Male	ThinDiabeticFemale	-9.19780	3.17764	.117	-19.6999	1.3043
	ObeseDiabeticFemale	-72.32186*	15.03079	.003	-123.4321	-21.2116
	ObeseDiabeticMale	-24.45971*	6.86478	.030	-47.2990	-1.6204
	ThinNonDiabeticFemale	-10.63765	3.63090	.103	-22.4858	1.2105
	ThinNonDiabeticMale	4.04029	2.34628	.673	-3.4155	11.4961
	ObeseNonDiabeticFemale	-51.10256*	4.55784	.000	-66.0508	-36.1544
	ObeseNonDiabeticMale	-30.61134*	6.03277	.001	-50.8098	-10.4128
Obese Diabetic Female	ThinDiabeticFemale	63.12406*	15.15967	.010	11.7674	114.4808
	ThinDiabeticMale	72.32186*	15.03079	.003	21.2116	123.4321
	ObeseDiabeticMale	47.86216	16.33534	.109	-6.0588	101.7831
	ThinNonDiabeticFemale	61.68421*	15.26112	.012	10.1314	113.2370
	ThinNonDiabeticMale	76.36216*	15.00744	.001	25.2948	127.4295
	ObeseNonDiabeticFemale	21.21930	15.50781	.861	-30.8341	73.2727
	ObeseNonDiabeticMale	41.71053	16.00352	.203	-11.4412	94.8623

Obese Diabetic Male	ThinDiabeticFemale	15.26190	7.14256	.419	-8.2435	38.7673
	ThinDiabeticMale	24.45971*	6.86478	.030	1.6204	47.2990
	ObeseDiabeticFemale	-47.86216	16.33534	.109	-101.7831	6.0588
	ThinNonDiabeticFemale	13.82206	7.35542	.575	-10.1969	37.8410
	ThinNonDiabeticMale	28.50000*	6.81350	.008	5.7691	51.2309
	ObeseNonDiabeticFemale	-26.64286*	7.85447	.034	-51.9945	-1.2912
	ObeseNonDiabeticMale	-6.15163	8.79278	.997	-34.3483	22.0451
Thin NonDiabetic Female	ThinDiabeticFemale	1.43985	4.13210	1.000	-11.9744	14.8541
	ThinDiabeticMale	10.63765	3.63090	.103	-1.2105	22.4858
	ObeseDiabeticFemale	-61.68421*	15.26112	.012	-113.2370	-10.1314
	ObeseDiabeticMale	-13.82206	7.35542	.575	-37.8410	10.1969
	ThinNonDiabeticMale	14.67794*	3.53298	.006	3.0659	26.2900
	ObeseNonDiabeticFemale	-40.46491*	5.26790	.000	-57.3973	-23.5325
	ObeseNonDiabeticMale	-19.97368	6.58568	.084	-41.5112	1.5638
Thin NonDiabetic Male	ThinDiabeticFemale	-13.23810*	3.06527	.006	-23.4817	-2.9945
	ThinDiabeticMale	-4.04029	2.34628	.673	-11.4961	3.4155
	ObeseDiabeticFemale	-76.36216*	15.00744	.001	-127.4295	-25.2948
	ObeseDiabeticMale	-28.50000*	6.81350	.008	-51.2309	-5.7691
	ThinNonDiabeticFemale	-14.67794*	3.53298	.006	-26.2900	-3.0659
	ObeseNonDiabeticFemale	-55.14286*	4.48022	.000	-69.9116	-40.3741
	ObeseNonDiabeticMale	-34.65163*	5.97434	.000	-54.7305	-14.5728
Obese NonDiabetic Female	ThinDiabeticFemale	41.90476*	4.96636	.000	25.7967	58.0128
	ThinDiabeticMale	51.10256*	4.55784	.000	36.1544	66.0508
	ObeseDiabeticFemale	-21.21930	15.50781	.861	-73.2727	30.8341
	ObeseDiabeticMale	26.64286*	7.85447	.034	1.2912	51.9945
	ThinNonDiabeticFemale	40.46491*	5.26790	.000	23.5325	57.3973
	ThinNonDiabeticMale	55.14286*	4.48022	.000	40.3741	69.9116
	ObeseNonDiabeticMale	20.49123	7.13874	.111	-2.5581	43.5406
Obese NonDiabetic Male	ThinDiabeticFemale	21.41353*	6.34706	.043	.4556	42.3715
	ThinDiabeticMale	30.61134*	6.03277	.001	10.4128	50.8098
	ObeseDiabeticFemale	-41.71053	16.00352	.203	-94.8623	11.4412
	ObeseDiabeticMale	6.15163	8.79278	.997	-22.0451	34.3483
	ThinNonDiabeticFemale	19.97368	6.58568	.084	-1.5638	41.5112
	ThinNonDiabeticMale	34.65163*	5.97434	.000	14.5728	54.7305
	ObeseNonDiabeticFemale	-20.49123	7.13874	.111	-43.5406	2.5581

FIGURE 14: LEPTIN LEVEL IN MALE & FEMALE SUBJECTS

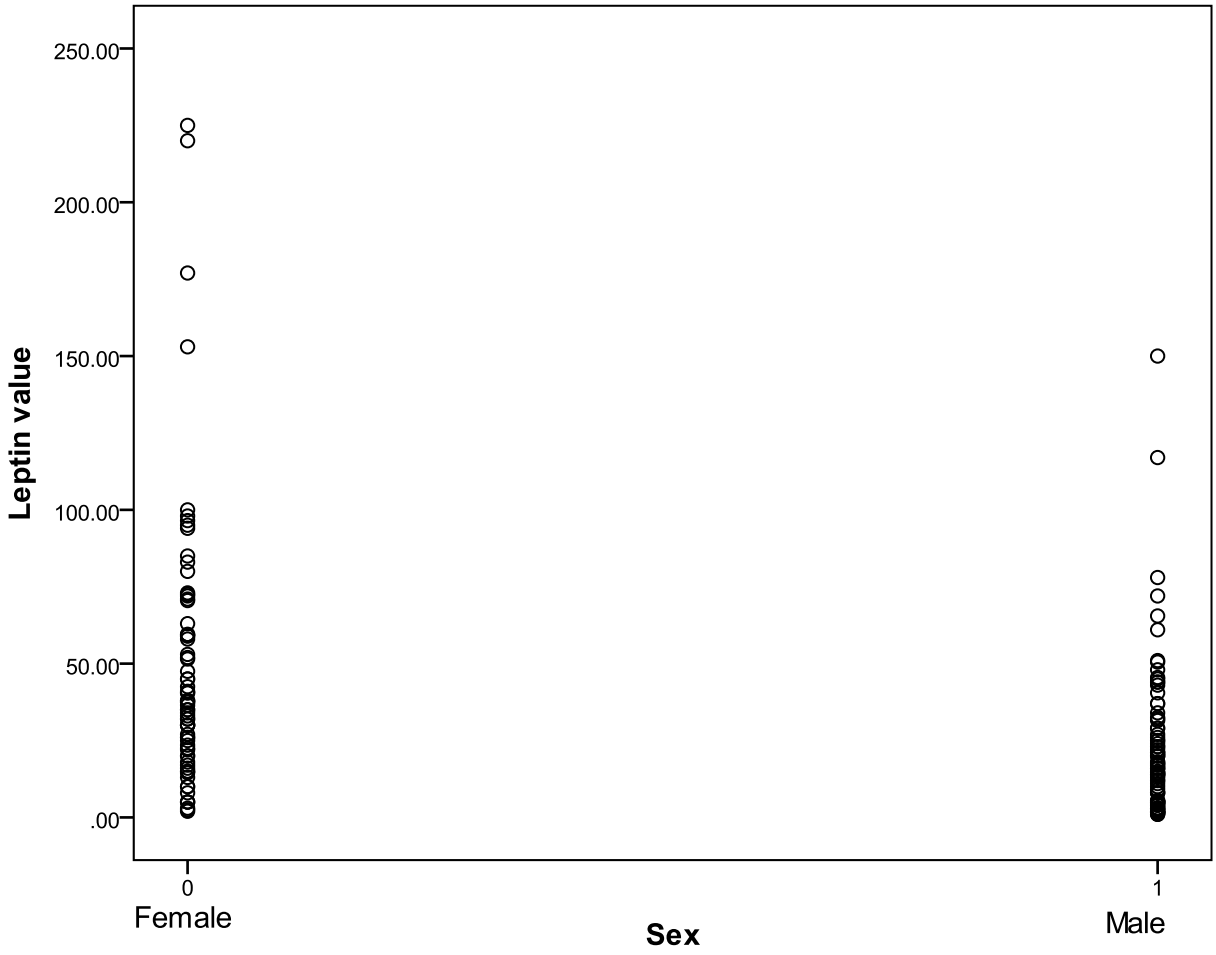


TABLE 13: LEPTIN LEVEL IN MALE & FEMALE SUBJECTS

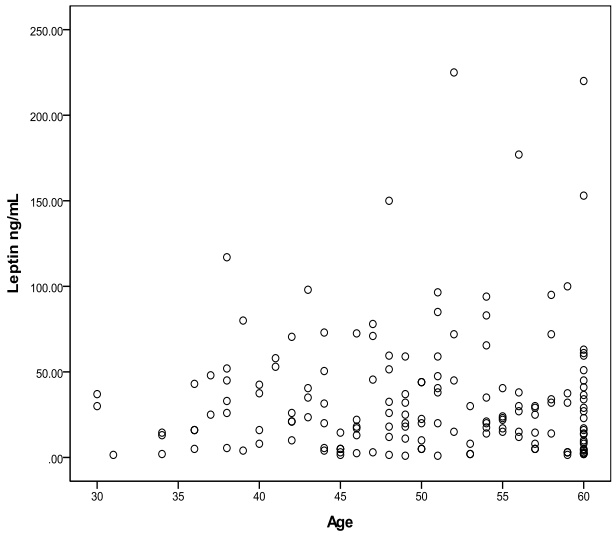
Group Statistics

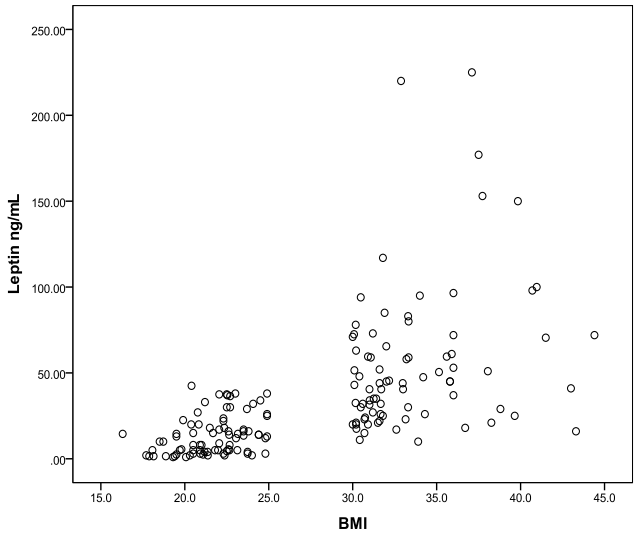
	VAR00001	N	Mean	Std. Deviation	Std. Error Mean
Leptin value	Male	87	22.3448	24.56036	2.63315
	Female	73	48.7877	44.12309	5.16422

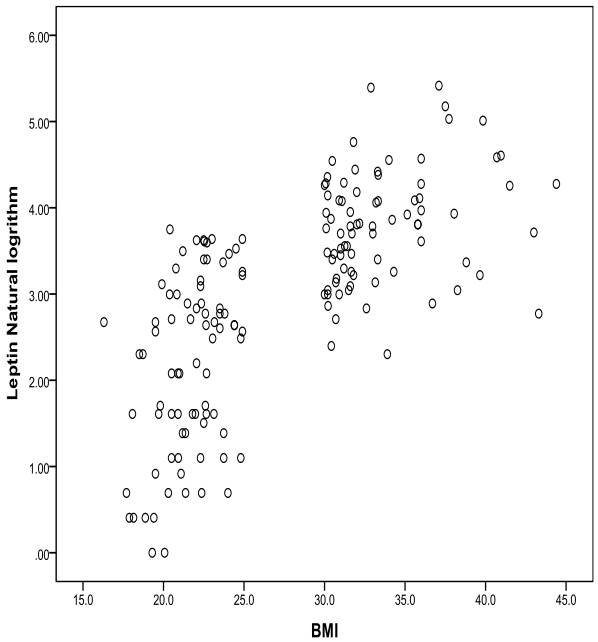
Independent Samples Test

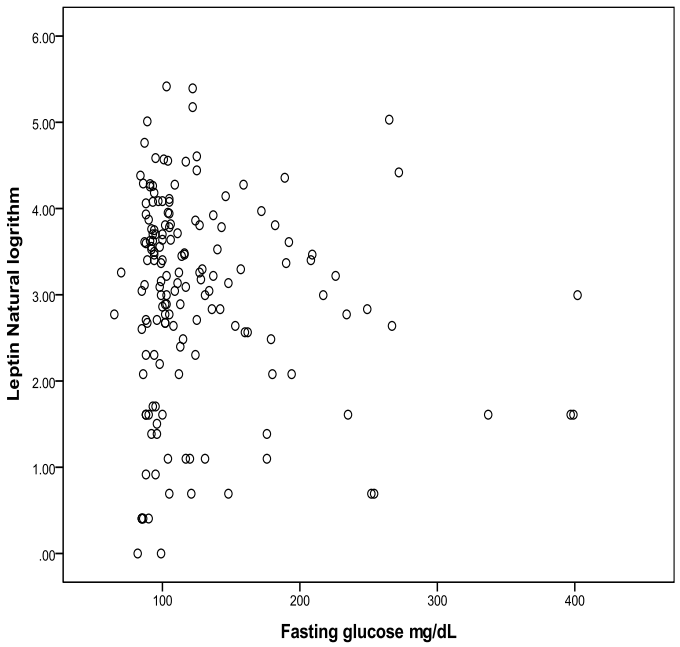
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Leptin value	Equal variances assumed	12.07	.001	-4.78	158	.000	-26.44	5.53	-37.37	-15.51
	Equal variances not assumed			-4.56	108.18	.000	-26.44	5.79	-37.93	-14.95

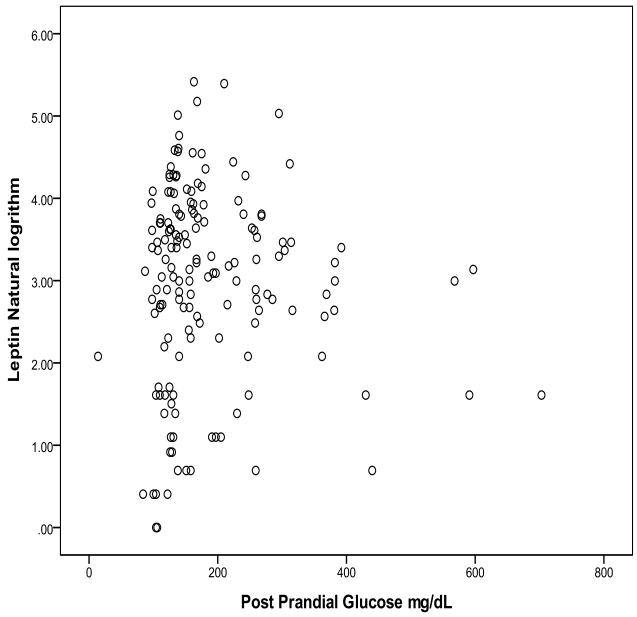
FIGURE 15: SCATTER PLOTS OF LEPTIN WITH VARIOUS PARAMETERS

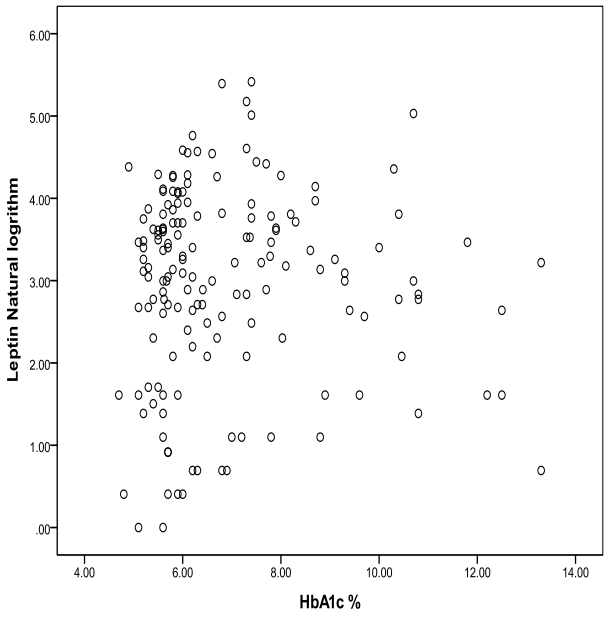


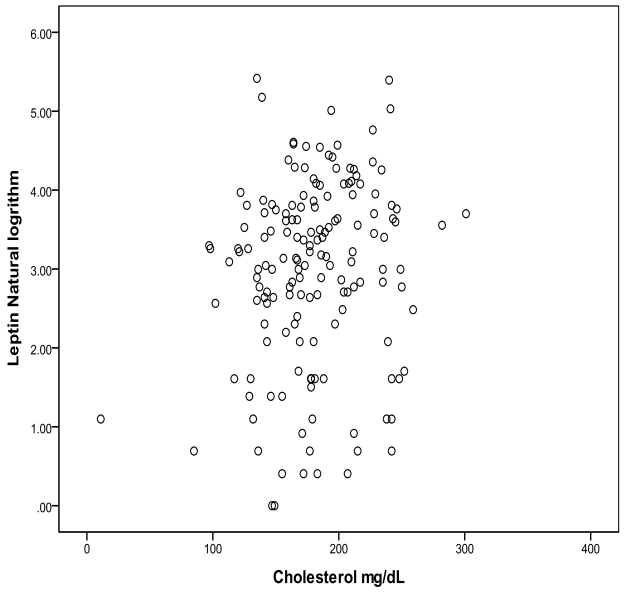


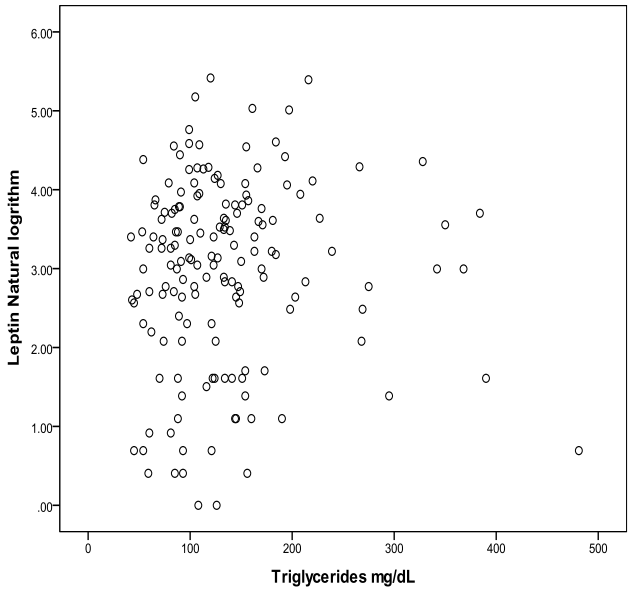


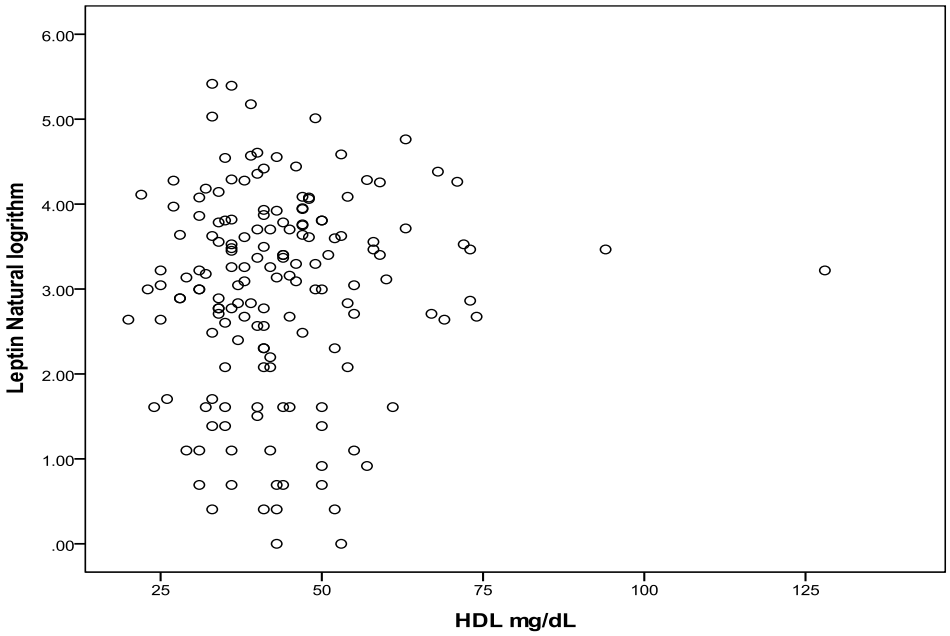



Leptin Natural logrithm
6.00
5.00
4.00
3.00
2.00
1.00
.00
0
200
400
600
800
Post Prandial Glucose mg/dL


Leptin Natural logrithm
6.00
5.00
4.00
3.00
2.00
1.00
.00
4.00
6.00
8.00
10.00
12.00
14.00
HbA1c %


Leptin Natural logrithm
6.00
5.00
4.00
3.00
2.00
1.00
.00
0
100
200
300
400
Cholesterol mg/dL


Leptin Natural logrithm
6.00
5.00
4.00
3.00
2.00
1.00
.00
0
100
200
300
400
500
Triglycerides mg/dL


Leptin Natural logrithm
6.00
5.00
4.00
3.00
2.00
1.00
.00
25
50
75
100
125
HDL mg/dL

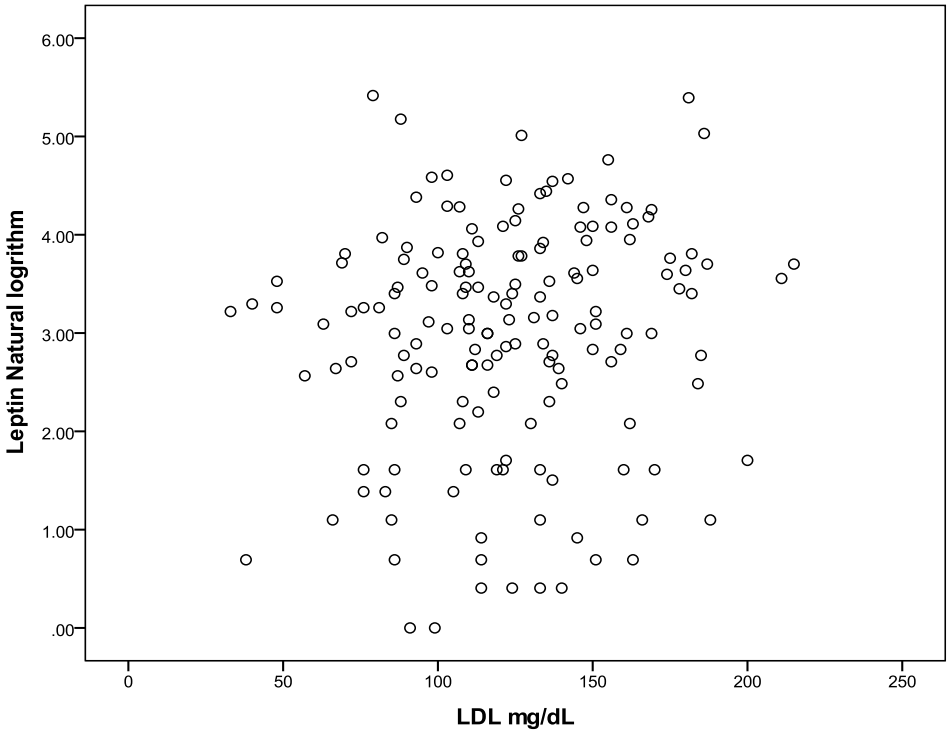
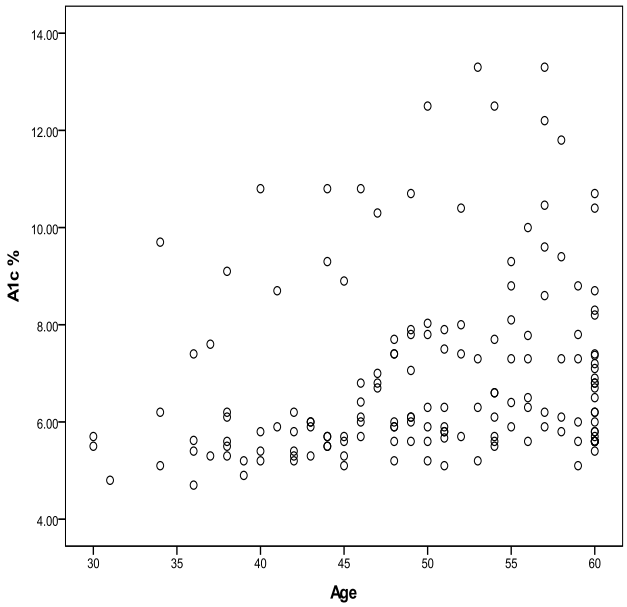
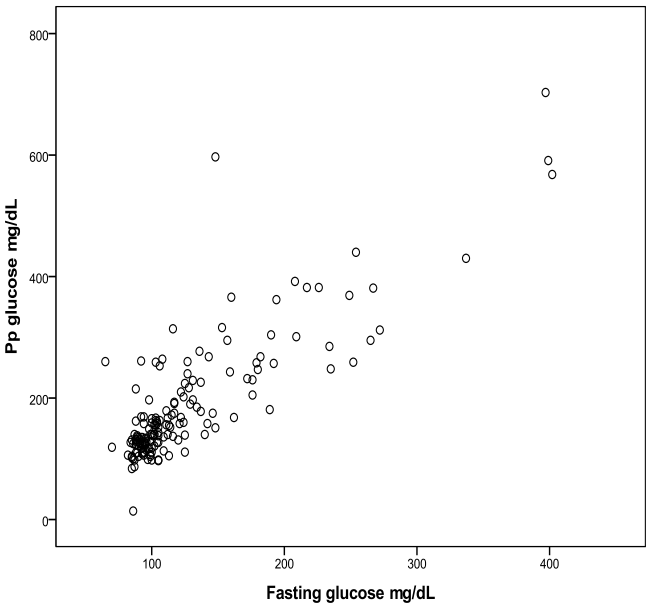

Leptin Natural logrithm
6.00
5.00
4.00
3.00
2.00
1.00
.00
0
50
100
150
200
250
LDL mg/dL

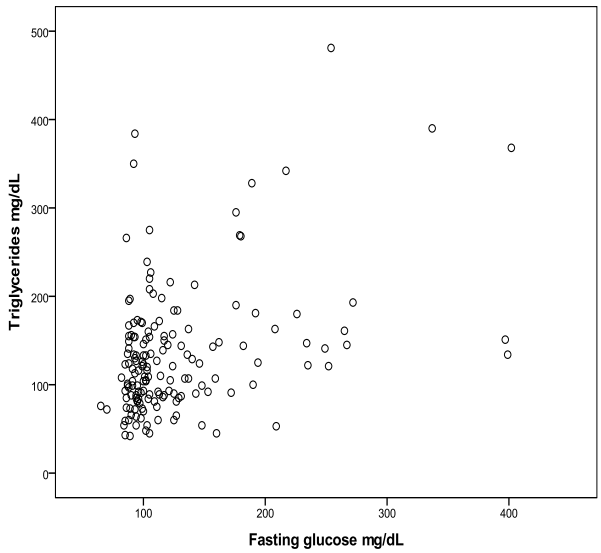
TABLE 14: CORRELATION OF VARIOUS BIOCHEMICAL PARAMETERS AMONG STUDY POPULATION.

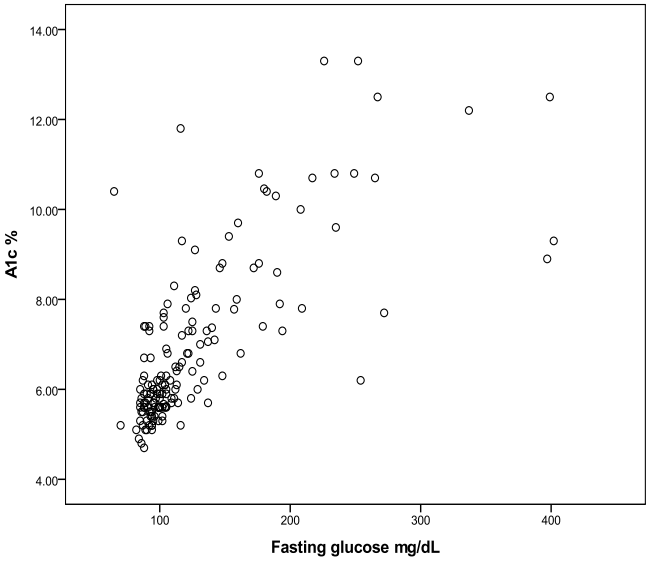
	Age	BMI	Glufas	Glupp	Chol	Tgl	Hdl	Ldl	A1c	Leptin	LeptinLn
Age	1.00	0.01	0.06	0.08	-0.03	-0.03	-0.02	-0.03	0.22	0.06	0.00
BMI	0.01	1.00	-0.08	-0.03	0.13	0.09	-0.08	0.15	0.02	0.60	0.70
Glufas	0.06	-0.08	1.00	0.84	0.14	0.39	-0.08	0.09	0.72	-0.08	-0.11
Glupp	0.08	-0.03	0.84	1.00	0.06	0.30	-0.06	0.02	0.72	-0.08	-0.04
Chol	-0.03	0.13	0.14	0.06	1.00	0.51	0.07	0.92	0.01	0.13	0.16
Tgl	-0.03	0.09	0.39	0.30	0.51	1.00	-0.26	0.44	0.26	0.05	0.03
Hdl	-0.02	-0.08	-0.08	-0.06	0.07	-0.26	1.00	-0.21	-0.10	0.00	0.06
Ldl	-0.03	0.15	0.09	0.02	0.92	0.44	-0.21	1.00	-0.03	0.12	0.12
A1c	0.22	0.02	0.72	0.72	0.01	0.26	-0.10	-0.03	1.00	0.00	-0.01
Leptin	0.06	0.60	-0.08	-0.08	0.13	0.05	0.00	0.12	0.00	1.00	0.79
LeptinLn	0.00	0.70	-0.11	-0.04	0.16	0.03	0.06	0.12	-0.01	0.79	1.00

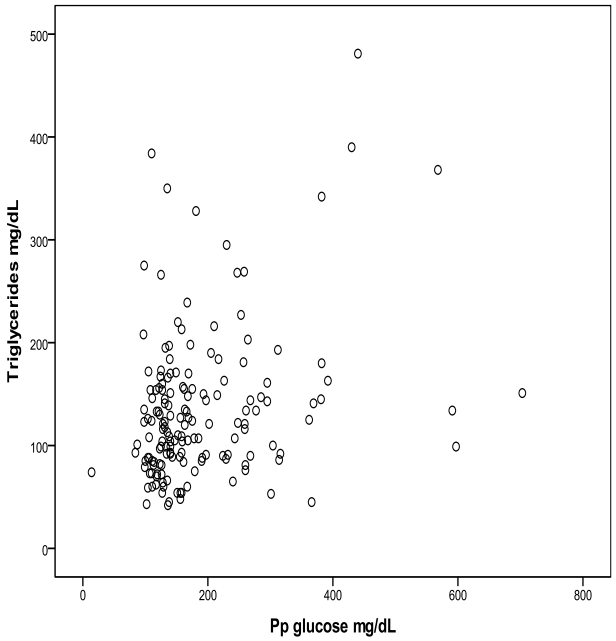
FIGURE 16: SCATTER PLOTS OF SIGNIFICANT CORRELATIONS

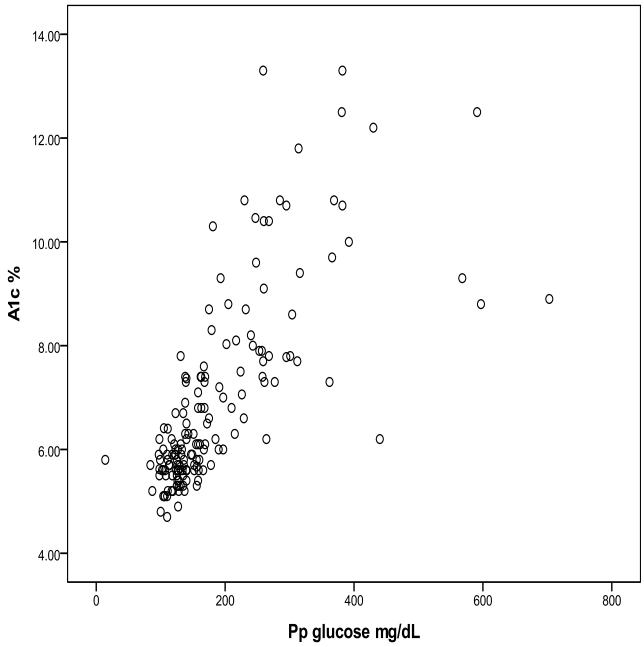


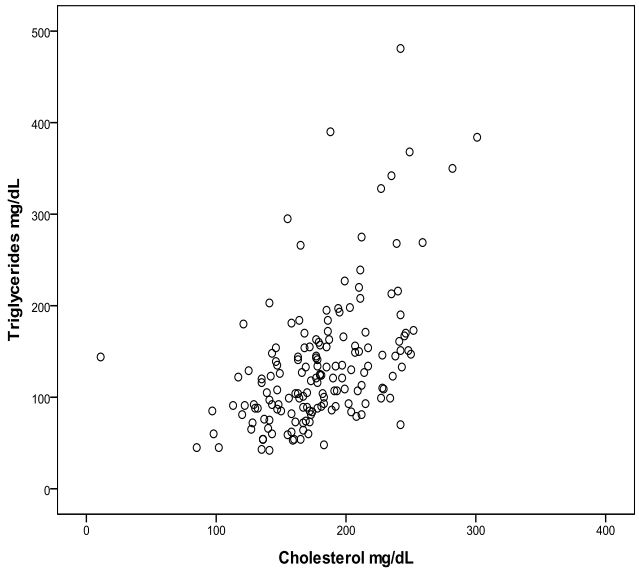


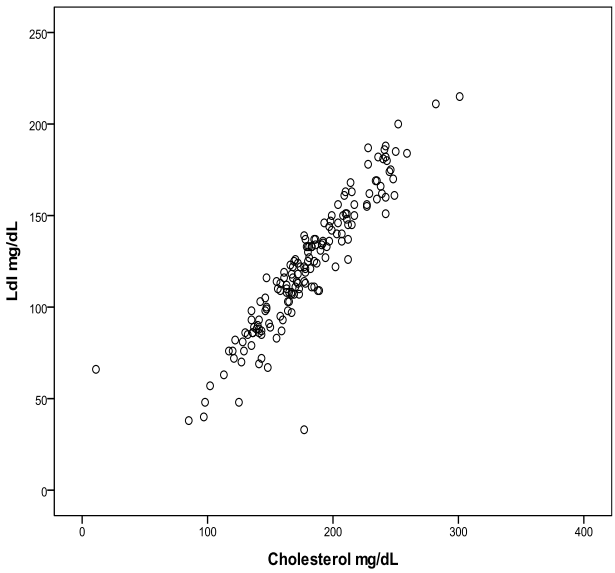


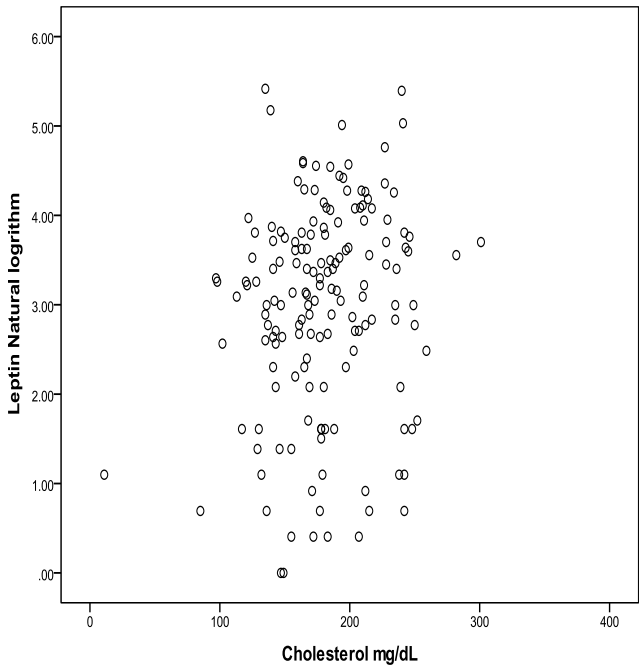


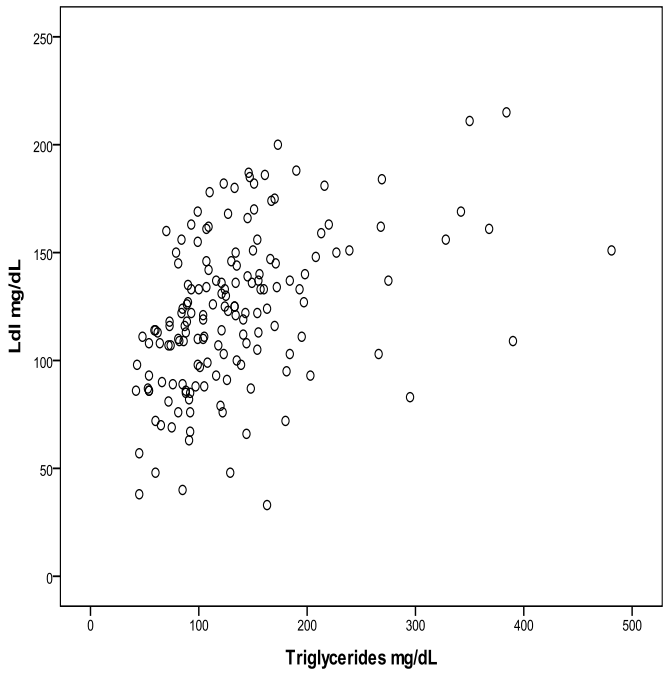

Triglycerides mg/dL
500
400
300
200
100
0
0
200
400
600
800
Pp glucose mg/dL

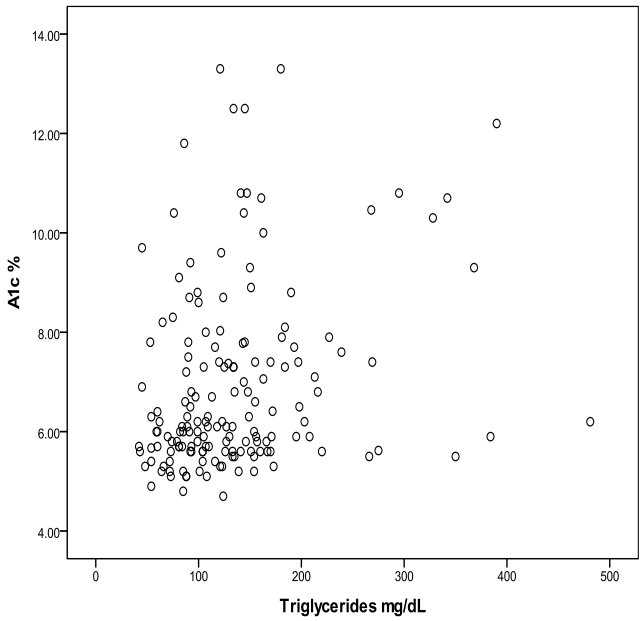

A1c %
14.00
12.00
10.00
8.00
6.00
4.00
0
200
400
600
800
Pp glucose mg/dL

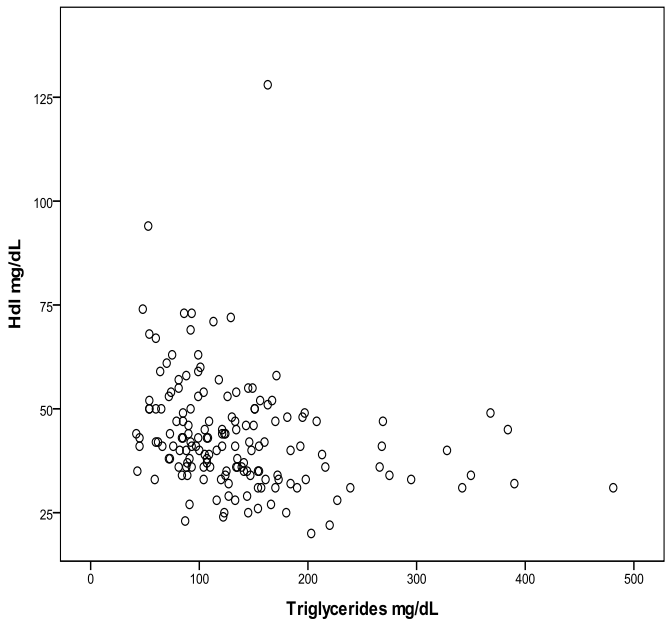

Triglycerides mg/dL
500
400
300
200
100
0
0
100
200
300
400
Cholesterol mg/dL

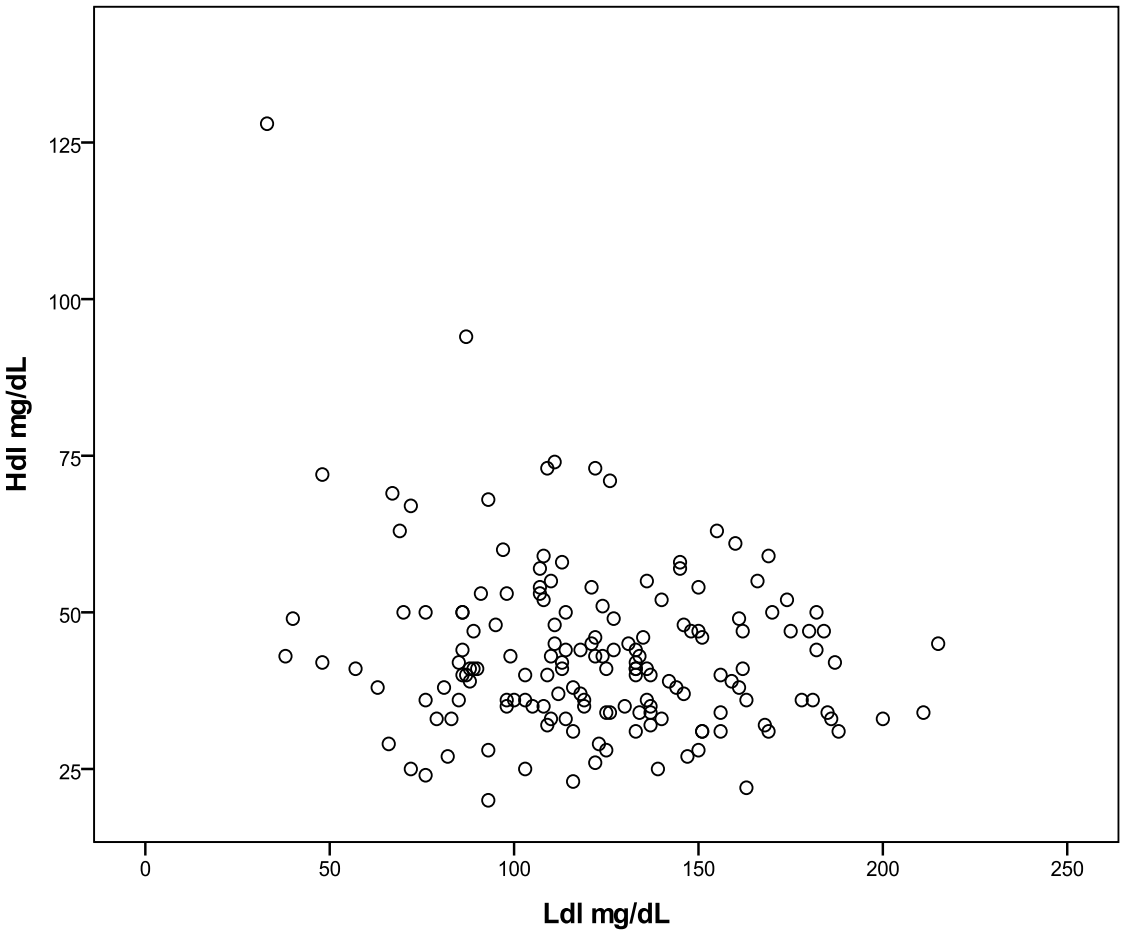

Ldl mg/dL
250
200
150
100
50
0
0
100
200
300
400
Cholesterol mg/dL


Leptin Natural logrithm
6.00
5.00
4.00
3.00
2.00
1.00
.00
0
100
200
300
400
Cholesterol mg/dL


Ldl mg/dL
250
200
150
100
50
0
0
100
200
300
400
500
Triglycerides mg/dL


A1c %
14.00
12.00
10.00
8.00
6.00
4.00
0
100
200
300
400
500
Triglycerides mg/dL


Hdl mg/dL
125
100
75
50
25
0
100
200
300
400
500
Triglycerides mg/dL


Hdl mg/dL
125
100
75
50
25
0
50
100
150
200
250
Ldl mg/dL

DISCUSSION:

The age distribution of the 4 study groups were between 30 and 60 years. But in the diabetic groups and thin non-diabetic group the distribution was more towards 60 years. In the obese non diabetic group the distribution was uniform throughout the age range. Though this difference was slightly reflected in ANOVA with a significance of 0.019, the post hoc Tukey test didn't show difference between these groups.

The sex distribution was even and there was no significant difference in Chi Square test. The distribution of BMI was according to the inclusion criteria used to separate the groups.

The fasting and corresponding post prandial plasma glucose levels were shown in the figures 4 & 5. As expected the non diabetic groups were having lower values while the diabetic groups were having higher values. Interestingly, the thin diabetic group was having higher plasma glucose level than the obese diabetic. The distribution of fasting plasma glucose values, shown as dot plot of different groups, also illustrates the same. In the between group ANOVA, the thin diabetic group have a significant higher mean than the obese diabetic. But this was not observed in post prandial plasma glucose levels. Otherwise, the anticipated significant differences between diabetic and non diabetic groups were observed in dot plots and ANOVA.

HbA1c data was clearly divided between diabetic and non diabetic groups. There was no difference between obese and thin. This was in accordance with a study performed by Ghorban Mohammadzadeh et al[77].

Among the lipid profile parameters, total cholesterol and HDL cholesterol showed no significant differences between the four groups. LDL cholesterol of obese non diabetic was significantly higher than the thin diabetic. Triglyceride levels were significantly lower in thin non diabetic group than other groups.

Leptin distribution was also clearly divided between obese and thin, irrespective of diabetic status and this result was consistent with the studies conducted by Piyali Das et al[78]. We examined the influence of sex. The female group mean was significantly different from male group mean. This mandates sex wise sub grouping and comparison. The sub group wise Leptin level distribution was given in the Table 12. The comparison of the sub group was done by ANOVA and Games Howel post hoc test as the numbers of data in each group were not equal and the equivalence of the variance cannot be assumed.

The eight sub groups were compared with each other and 56 combinations were possible. Significant difference in Leptin level was observed between obese and thin in 26 combinations (coloured yellow). Obesity was associated with higher level of Leptin. In 19 combinations (green) there was no difference and here the uniting factor among the comparisons was obese versus obese or thin versus thin. Hence, in 45 out of 56 combinations the deciding factor was obese/thin.

Among the remaining groups, 6 combinations (orange) have no significant difference between the groups. Here the obesity/thin and male/female effects were acting in opposite direction, resulting in no significant difference. There was a significant difference in 5 combinations (blue) and here the deciding factor was sex. Females have higher Leptin level. Our result was consistent with the finding of Couillard et al and many other studies which showed that Leptin level increases in females[79]. This was due to the fact that women have higher percentage of fat in the body. Females have increased ratio of subcutaneous to visceral fat. This was consistent with the finding that Leptin strongly correlates with the body fat mass. Few studies illustrate that there was an unconstrained production of Leptin by adipocytes in women than men[80]. Casabiel et al have demonstrated that females have higher percentage of subcutaneous to omental Leptin mRNA expression[81]. Estrogens stimulate Leptin secretion in women and androgens have an inhibitory effect[80].

The correlation between the quantitative parameters was assessed by correlation coefficient and scatter plots. BMI–Leptin correlation had an r value of 0.6 and the scatter plot also showed a linear positive correlation. Log transformed Leptin levels showed even better correlation with r value 0.7. Ahsan et al have showed the same finding[82].

Positive correlation was also observed between following parameters:

Fasting glucose and post prandial glucose,

Fasting glucose and HbA1c,

Post prandial glucose and HbA1c &

Total cholesterol and LDL cholesterol .

These correlations were well established relations. There were negative correlations in Triglyceride-HDL cholesterol and HDL cholesterol-LDL cholesterol pairs, which were not supported in their respective scatter plot. These negative correlations were also expected ones only.

Conclusion:

1. Leptin is not elevated in thin diabetic when compared with thin non-diabetic. The development of type 2 diabetes mellitus in thin individuals may not be related to Leptin level.
2. Obesity and Female sex are associated with increased Leptin level and not with type 2 diabetes mellitus.
3. BMI and Leptin have a linear positive correlation.

Summary:

The possibility of evolving Diabetes Mellitus rises with the upper limit of body mass index. Genetic, lifestyle and environmental factors combined together promotes obesity. Diabetes mellitus is a multifactorial disorder characterized by chronic hyperglycemia. It is associated with alterations in carbohydrate, protein and fat metabolism due to defective insulin secretion or action or both.

Leptin the product of ob gene is a protein hormone secreted by adipocytes and also by placenta, bone marrow, muscle, etc. It has a major role in maintaining body weight and in energy metabolism. The hypothalamus is the predominant site of action of Leptin. Receptors for Leptin is present in various sites like brain and peripheral tissues like pancreas, liver, lungs, adipose tissues, T-lymphocytes and epithelial cells. This gives the evidence for the role of Leptin in carbohydrate metabolism, reproduction and many other functions. Leptin exhibits its insulin lowering action through the receptors present in beta cells of pancreas.

This study was an attempt to know about the Leptin levels in four groups namely thin diabetic, obese diabetic, thin non-diabetic and obese non-diabetic populations. Other parameters like Fasting and Post Prandial Glucose, HbA1c, Cholesterol, Triglycerides, High Density Lipoprotein and Low Density Lipoprotein were also considered.

In each group 40 subjects were recruited. Their samples were collected and Leptin was assayed using Leptin ELISA kit. Fasting and Post Prandial Glucose, HbA1c,

Cholesterol, Triglycerides, High Density Lipoprotein and Low Density Lipoprotein were analyzed in autoanalyzer. There was no significant difference in Leptin levels in both obese diabetic and obese control groups. Leptin level was confined to the lower side in thin groups. There was a significant positive correlation between Leptin and body mass index. Female subjects have got increased Leptin value than male subjects. This confers that Leptin is a marker of obesity rather than Diabetes Mellitus.

FUTURE SCOPE OF THE STUDY:

As Leptin has no association with Type 2 Diabetes Mellitus, other adipocytokines (Ghrelin, Resistin, Serpin, Adiponectin, Intelectin, etc) can be looked for the Pathogenesis of Type 2 Diabetes Mellitus in thin individuals.

Metabolomics study comparing thin and obese type 2 diabetic may be carried out.

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ANNEXURE:

DATA COLLECTION TOOL (QUESTIONNAIRE)

1. Age:

2. Sex:

3. Height:

4. Weight:

5. BMI:

6. History of illness:

    6.1. Diabetes: Type 1 Type 2

    6.2. Duration of diabetes:

    6.3. Hypertension: 1. Yes 2. No

    6.4. Smoking history:

    6.5. History of myocardial infarction / Angina / LV dysfunction:

    1. Yes 2. No

    6.7. Motor/sensory functions:

    6.8. Duration of treatment:

7. Lab investigations: