

ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my supervisor, Prof. Dr. Güldal Mehmetcik for her supervision, advice, understanding and keeping encourage from the very early stage of the dissertation.

I would also like to express my sincere thanks to Mr. Ziya Salman. His wisdom, knowledge and commitment to the highest standards have inspired and motivated me and guided me to the right path. I am thankful for the other staff members in my department.

I owe lots of thanks to Prof. Dr. Nihal Salmayenli, Dr .Gaylany H. Abdullah, Faisal Sher and my class fellows, Muhammad. I. K. Afridi and Rameez Hassan.

As much as I would like to thank my best friends Majeed Jbraeel Saleem, Dr. Shirwan Baban, and Dr. Kawis Aziz Faraj for their support and continuous asking about my situation.

I wish to thank my family members and friends especially my parents who always supported and encouraged me in all aspects of my life.

In the end I would like to pay my regards gratitude and thanks to my loving wife Kazhal Z.Rasool and my sweet kids, without their inspiration and motivation it would be impossible for me to complete this study.

PSHTIWAN ABDULLAH YOUSIF

ABSTRACT

Yousif A. P., Leptin Values in Menopausal Women. Near East University, Institute of Health Sciences, Biochemistry, Master Thesis, Nicosia, 2014.

Menopausal status is related with weight gain, abnormal lipid and glucose metabolism. Leptin is a protein secreted by adipose tissues and has important endocrine functions. In previous studies, it has been shown that leptin concentrations are higher in menopausal women. The aim of this study was to determine the circulating leptin levels and to correlate these levels in relation with menopause.

Across-sectional study has been carried out for 85 subjects, the mean age of menopausal group (n = 50) was 58.16 ± 2.73 , and that of control group (n = 35) was 40.12 ± 3.46 .

Fasting serum leptin levels were increased considerably when compared to control group. Fasting blood sugar and triglyceride levels were higher in menopausal women when compared to the control group. Leptin was well correlated with BMI in both groups. Leptin also had significant correlation with fasting blood sugar in both groups.

High leptin levels are known to cause serious health problems and in order to investigate the relationship between menopause and leptin, further work must be carried out.

Keywords: Menopause, Leptin, BMI, Obesity

CONTENTS

ACKNOWLEDGMENTS.....	i
ABSTRACT.....	ii
CONTENTS.....	iii
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
LIST OF ABBREVIATIONS	ix
CHAPTER 1: INTRODUCTION.....	1
1.1 Adipose Tissues.....	1
1.1.1 Morphology and Development of Adipose Tissue.....	2
1.1.2 Adipose-Tissue Metabolism.....	5
1.1.2.1 Lipogenesis.....	5
1.1.2.2 Lipolysis.....	6
1.1.3 Adipose Tissue Distribution.....	7
1.1.4 Definition and Causes of Obesity.....	9
1.2Leptin.....	11
1.3 Leptin Structure.....	12
1.4 Leptin Function.....	16
1.5 Biosynthesis of Leptin.....	18

1.6 Leptin Production Regulation.....	18
1.7 Receptor of Leptin.....	21
1.8 Leptin Resistance.....	21
1.9 Insulin.....	22
1.9.1 Leptin and Insulin.....	23
1.10 Insulin Resistance.....	24
1.11 Glucose.....	25
1.11.1 Effects of Leptin on Glucose.....	26
1.11.2 Plasma Lipids.....	26
1.11.2.1 Cholesterol.....	26
1.11.2.2 Triglycerides.....	27
1.11.2.3 High Density Lipoprotein- Cholesterol (HDL-C).....	28
1.11.2.4 Low-Density Lipoprotein- Cholesterol (LDL-C).....	29
1.12 Menopause Effects on Leptin Level.....	30
1.13 Menopausal Woman.....	31
1.13.1 Pre-Menopausal.....	33
1.13.2 Differences between Menopause and Post-Menopause.....	34
1.14 Leptin with Menopause.....	35

CHAPTER 2: RELATED RESEARCH.....	36
2.1 Overview.....	36
2.2 Related Work.....	36
2.3 Summary.....	43
 CHAPTER 3: MATERIALS AND METHODS.....	 44
3.1 Overview.....	44
3.2 General Laboratory Equipment.....	44
3.3 Disposable Laboratory Equipment.....	45
3.4 Laboratory Analyses.....	45
3.5 Leptin Determination.....	46
3.6 Reagents.....	46
3.6.1 Reagents provided.....	46
3.6.2 Materials required but not provided	47
3.6.3 Storage Conditions.....	47
3.7 Specimen Collection and Preparation.....	48
3.7.1 Specimen Collection.....	48
3.7.2 Specimen Storage and Preparation.....	48
3.7.3 Specimen Dilution.....	48
3.8 Quality Control.....	48
3.9 Assay Procedure.....	49

3.9.1 General Remarks.....	50
3.9.2 Test Procedure.....	50
 CHAPTER 4: RESULTS.....	 51
4.1 Cholesterol Analysis.....	55
4.2 Triglycerides Analysis.....	56
4.3 HDL-C Analysis.....	57
4.4 Discussion.....	61
 CHAPTER 5: CONCLUSION	 62
5.1 Conclusion.....	62
 REFERENCES.....	 63

LIST OF TABLES

Table 1.1: Positioning of alpha helices in leptin.....	14
Table 4.1: Comparison of anthropometric and metabolic values in menopausal and control group.....	53
Table 4.2: Leptin value compared in patients and control groups.....	61

LIST OF FIGURES

Figure 1.1: White fat cell and brown fat cell.....	3
Figure 1.2: Diagrammatic representation of triglyceride storage.....	5
Figure 1.3: Adipose Tissue Distribution in Men and Women.....	7
Figure 1.4: Brown fat (A) and white fat (B) tissue distribution in adult.....	8
Figure 1.5: Interleukin-4.....	12
Figure 1.6: Primary structure of leptin.....	12
Figure 1.7: Energy scaffolds for IL-2 and a structural model for leptin.....	13
Figure 1.8: Proposed structure of leptin E100.....	13
Figure 1.9: The E helix acting as a hydrophobic cap.....	14
Figure 1.10: Disulfide bonding in leptin.....	15
Figure 1.11: Leptin effects on weight.....	17
Figure 1.12: Long-term signals regulating energy balance.....	19
Figure 1.13: Schematic view of the Regulation of Leptin Production.....	20
Figure 1.14: Mechanisms of Leptin Resistance.....	22
Figure 4.1: Histogram of age.....	54
Figure 4.2: Histogram of weight.....	55
Figure 4.3: Distribution histogram of BMI.....	55
Figure 4.4: Hip study.....	56
Figure 4.5: Waist diagram in cm.....	56
Figure 4.6: Leptin Levels.....	61

LIST OF ABBREVIATIONS

ADP:	Adenosine di phosphate
ATP:	Adenosine tri phosphate
AGRP:	Agouti-related peptide
AIDS:	Acquired immunodeficiency syndrome
alpha-MSH:	Alpha-melanocyte-stimulating hormone
BL:	Bound leptin
BMI:	Body mass index
CART:	Cocaine and amphetamine-regulated transcript
CHD:	Coronary heart disease
CHO:	Carbohydrates
CHOL:	Cholesterol
C.f.a.s:	Calibrator for automated systems
CRH:	Corticotropin releasing hormone
CVD:	Cardiovascular disease
df:	Degree of freedom
DHEA:	Dehydroepiandrosterone
DM:	Diabetesmellitus
FL:	Free leptin
FSH:	Follicle stimulating hormone

GH-IGF-I:	Growth hormone-insulin-like growth factor I
GLP-1:	Glucagon- like peptide-1
HDL:	High density lipoprotein
HDL-C:	High-density lipoprotein-cholesterol
HRT:	Hormone replacement therapy
HSDA:	N- (2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline
IDL:	Intermediate density lipoprotein
LDL:	Low density lipoprotein
LDL-C	: Low-density lipoprotein-cholesterol
LEP:	Leptin gene
LH:	Lutinising hormone
Lep R:	Leptin receptor
MCH:	Melanin-concentrating hormone
MC4R:	Melanocortin-4 receptor
MEIA:	Microparticle Enzyme Immunoassay
NPY:	Neuropeptide Y
NSB:	non specific binding
Ob-Ra:	Leptin receptor type a
Ob-Rb:	Leptin receptor type b
Ob-Rc:	Leptin receptor type c
Ob-Rd:	Leptin receptor type d

Ob-Re:	Leptin receptor type e
ob/ob mice:	mice homozygous for abnormalities in leptin gene
p:	2 tailed significance
PTA:	Pituitary-thyroid axis
QC:	Quality Control
r:	Pearson correlation coefficient
RV:	Reaction vessel
RIA:	Radioimmunoassay
SD	Standard deviation
SEK:	Standard error of kurtosis
SEM:	Standard error of mean
SES:	Standard error of skewness
SNS:	Sympathetic nervous system
TEAH:	Tetraethyl ammonium hydroxide
TG:	Triglycerides
VLDL:	Very low-density lipoprotein
WHO:	World health organisation
t²:	Chi square
HCG	Human Chorionic Groundotropin

INTRODUCTION

1.1 Adipose Tissues

Fat tissue may be found in warm blooded creatures by 50 percent mixed bags: white fat and tan fat tissue. The region, entirety, and scattering of each movements is based upon the species.

White greasy tissue serves three capacities: heat insurance, mechanical cushion, or more all, an origination of imperativeness. Subcutaneous oily tissue, found particularly inside the skin layers, is point of fact a particularly basically hotness encasing inside the body, as it leads warm one and only third as instantly as diverse tissues. Fat tissue similarly envelops body organs and supplies some protection for these organs from knocking(Barbara et al., 2004;Patricia et al., 2002).

Since the genuine sort of essentialness stockpiling, fat offers a support for imperativeness disproportionate qualities when essentialness permit really isn't proportional to imperativeness yield. It is a gainful strategy to store inexhaustible essentialness, which is secured with almost no water. Consequently, more imperativeness may be derived for each gram of fat (9 kcal.gm-1) than for each gram of starch (4 kcal.gm-1) or protein (4 kcal.gm-1). Moreover, if physical animals set away their excess essentialness as starch, the raised mass would intrude with compactness. The ordinary woman with 20% undesirable weight has something like 30 days of one's secured as fat. There are different commitments for the utilization of fat as fuel. erythrocytes) must make use of starch for essentialness and need an uncommon copious supply available. Likewise, under normal conditions the mind relies on upon glucose for essentialness and doesn't use fats. In peculiar metabolic circumstances, the psyche can use ketone bodies (a by-consequence of the divided fat assimilation framework) when they're inside sufficiently high sums. Finally, a standard consuming technique has a high degree of sugar, and likewise the vehicle of insoluble lipids through blood obliges a specific instrument, set up that it might

be "less complex" metabolically for tissues to work with glucose under normal dietary conditions(Barbara et al., 2004, and Jeong et al., 2010).

The tan oily tissue, which surmises its shade from rich vascularization and thickly stuffed mitochondria, is orchestrated in diverse territories, dependent upon the species and/or times of the animal. From the rat, tan fat tissue could be found crucial in the interscapular area and furthermore the axillae, minor totals are just on the thymus and the dorsal midline region with the thorax and tummy. All through advancement, in non-resting animals, tan fat tissue is metabolically less dynamic, though cool presentation can sanction it. In resting animals and neonates, tan fat tissue is incredibly basic for overseeing blood warm through non-shuddering thermogenesis (Barbara et al., 2004).

As opposed to serving as an issue, the lipid in tan fat tissue releases imperativeness direct as high temperature which may be, thusly utilized as a part of hotness handling for non-shuddering thermogenesis for make usage of excess calorie use by method for consuming regimen influenced thermogenesis. The arrangement of hotness period relates to one's absorption framework in the mitochondria(Barbara et al., 2004, and Patricia et al., 2002).

1.1.1 Morphology and Development of Adipose Tissue

In adult generally developed animals, the most central basically all oily tissues are routinely an isolates camaraderie of lipid-filled cells called adipocytes, which could be held in a skeleton of collagen strands(Mersminn et al., 2014).

Mitochondria are simply predominately inside the thicker district of the cytoplasmic edge near the center. Multilocular cells, consistently welcomed in tan oily tissue, hold various more humble lipid droplets. A cell in tan oily tissue may attain a separation crosswise over of 60 microns notwithstanding the lipid droplet from the cell may accomplish 25 microns in estimation(Barbara et al., 2004;Mersminn et al., 2014).

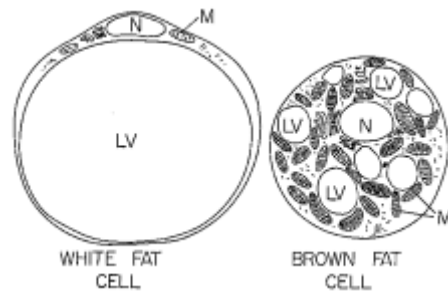


Figure 1.1: White fat cell and brown fat cell. Note the single large lipid vacuole in the white fat cell and the numerous smaller lipid vacuoles in the brown fat cell. LV: lipid vacuole; M: mitochondria; N: nucleus(Barbara Cannonet al., 2004)

Moving the bit of the consuming philosophy can vary the unsaturated fat profile in oily tissue. This course gives sufficient sponsorship to the element assimilation framework, which happens in the slim edge of cytoplasm including the lipid droplet(Barbara et al., 2004, and Mersminn et al., 2014).

How tremendous is oily tissue mass is regularly a limit of both adipocyte number and size. An assemble in oily tissue mass can happen by hyperplastic advancement, which may be a climb in the measure of adipocytes. This surge in number happens basically by mitotic activity in precursor cells. Fat tissue mass may moreover fantastically manufacture by hypertrophic advancement, which may be a stretched in the length of adipocytes. This surge in size happens essentially by lipid accumulating inside the telephone. Advancement of this tissue inside rat happens in general portrayed stages. From origination to 4 weeks of age, oily tissue advancement is hyperplastic. Over-burdening a rat all through this time can achieve never-ending grows in body volume and fat cell number. Imitating 14 weeks old, fat advancement happens fantastically by adipocyte hypertrophy. The formative progression of fat tissue in individuals is less for the most part portrayed. Rather than most neonates, the individual neonate arrives decently fat. Two times of hyperplastic advancement are as a general rule through the third trimester of childbearing and essentially before and all through pubescence. Not like earlier conviction, hyperplastic improvement can moreover happen in adulthood (both in individuals and rats). Right when adipocytes load with lipid and find with a fundamental

size, forerunner cells are stimulated to discrete, and a rising in adipocyte number results. This fundamental size probably does not happen with moderate over-burdening unless the over-burdening is of long compass. Also, you can undoubtedly find solitary assortments in the estimations which will achieve new adipocyte foundation. Once new adipocytes are organized, they stay all through life in maneuvering of a reducing in size the phone is extremely possible. This extended measure of adipocytes has broad conclusions to the pharmaceutical and suspicion of bulkiness(Barbara et al., 2004, and Mersminn et al., 2014).

1.1.2 Adipose-Tissue Metabolism

1.1.2.1 Lipogenesis

Essentialness ingested as fat past that needed for current imperativeness solicitations is kept in oily tissue. Starch may be changed over into triglycerides in a broad sense inside the liver and traded to oily tissue for limit(Pothoven, et al., 2013)

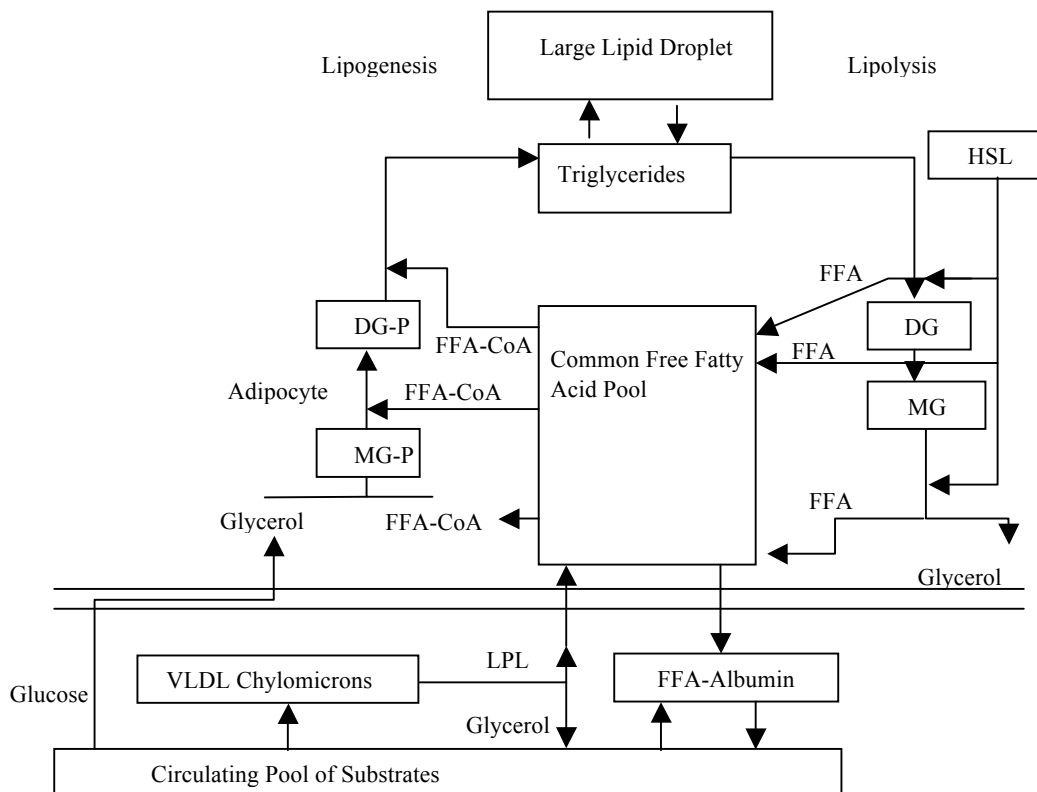


Figure 1.2: Diagrammatic representation of triglyceride storage (lipogenesis) and breakdown (lipolysis) in adipocytes. (Barbara et al., 2004)

Lipoprotein lipase is mixed in adipocytes and released into adjacent endothelial cells. The web effect of insulin should be to update stockpiling and square enactment and oxidation of efas. Finally, the change of glucose to key unsaturated fats is capable by insulin's incitation of multitudinous mixes(Pothoven et al., 2013, and Simon et al., 2013).

1.1.2.2 Lipolysis

Lipolysis is the substance crumbling and landing of unsaturated fats from fat tissue. This system beats lipogenesis when additional essentialness is required. Triglyceride lipase controls the pace of lipolysis, because its activity is low Hiroaki et al., (2002). This launch step changes over the unsaturated fats into a structure that is more pleasant to the dynamic biochemical changes that inevitably accomplish ATP game plan. Insulin decreases planning of fat from fat tissue via controlling triglyceride lipase. This disguise of lipolysis cuts down the event of essential unsaturated fat movement for the liver and periphery tissues. The outcome of less critical unsaturated fats towards the liver is a reducing in the ofketoacids (e.g., ketones). Insulin furthermore fortifies the by using ketoacids by periphery tissues, keeping a social affair of these acids from the blood(Hiroaki et al., 2002).

1.1.3 Adipose Tissue Distribution

Possibly the most simple observer will review that everyone does not pass on their fat tissue inside the same anatomical ranges. Oily tissue that is spotted dominantly on the abdominal area continues being termed android, male, central, abdominal area segment, or "products of the soil" movement as demonstrated in Figure 1.3.

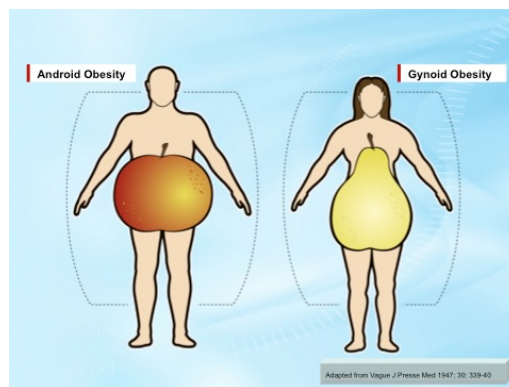


Figure 1.3:Adipose Tissue Distribution in Men and Women

This movement sample could be found more from time to time in male; hence the terms android and male. Exactly when fat assembles predominantly inside more level body, the terms gynoid, female, simpler body segment , or "pear" allotment are used as exhibited in Figure 1.4. As the terms gynoid and female close, this fat case could be found more routinely in women than in men.

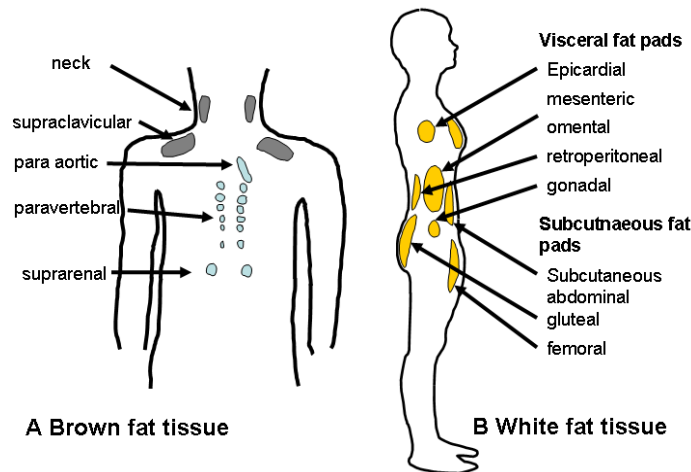


Figure 1.4: Brown fat (A) and white fat (B) tissue distribution in adult. Brown adipose tissue is involved in the regulation of the body.

Parts center fat spread, a key variable is genetic establishment, which may frequently certain you are seen by looking at the closeness in fat transport inside same-sex close relatives. Tagged long prior former, sexual introduction is known to impact undesirable weight territory. Women for the most part show more superb lower extra fat dispersal and men exhibit more midsection fat course Després et al. (2014). As an issue individual ages muscle to fat proportion proportions scattering may change. For example, succeeding the menopause a change towards center fat dissemination is viewed. This change are about related with a relative decreasing in LPL development in the less demanding body locale. Finally, convincing plenitude weight and weight cycling ("yo-yo consuming less") may manufacture center fat. Excess instinctual fat rather than wealth midsection muscles fat under the skin is a piece of extended peril. A quick test to center in the event that you be at risk for challenges joined with midsection fat movement is as an issue to figure the waist-to-hip-extent by measuring the edge of the waist and dividing it from the circuit of

one's hips. Women have landed at peril in the event the extent surpasses 0.85; for fellows the degree is 0.95 (Després et al., 2014).

1.1.4 Definition and Causes of Obesity

Weight truly isn't stand out issue. A rash of schedules and criteria are used diagnose the usage of weight. It doesn't take the measure of fat and not total body mass that describes weight Puska et al., (2003). Work from test animals shows clear genetic inconsistencies commonly basic reason behind bulkiness. Qualities for forcefulness may be detached, overpowering, or polygenic. Latent alleles that effect the generation of weight are recognized in a couple of rodents. Inside the occupants, bulkiness could be a quirk of multitudinous aloof disorders, including Bardet-Biedl, Lawrence-Moon, and Prader-Labhart-Willi, and moreover, triglyceride stockpiling sicknesses. These disorders are by and large extraordinary. One particular inherited conflict without different synchronous innate disorders has yet to be perceived in individuals. It will be possible that alleles at particular loci push a little effect that isn't adequately unmistakable without any other individual present. In case these alleles exist at a substantial number of loci, aggregately they will push a noteworthy impact heftiness. Qualities unmistakably effect body size and muscle to fat remainders dissemination, and in this way are subject to be joined with tweaking human weight (Puska et al., 2003, and McGarty et al., 2010).

In individuals, it is here and there convoluted to guard the lives inherited and regular components and dole out the relative responsibility of each on the change of weight. A bit familial, twin, and choice examination have given an effective methodology to study the responsibilities of heredity the earth. These studies give robust suggestive data to an inherited piece of heaviness, and likewise regulation of qualities by regular parts. Characteristic effects on weight, join food confirmation and doorway of action. Pigging out according to imperativeness needs may be a section of weight change. Nevertheless, from studies with exploratory animals it could be clear that various animals that make heftiness are overwhelmingly to an incredible degree capable than lean animals; which is, for a given number of sustenance, the strength slanted animals store more fat than do the weight safe animals. Really, when genetically vast rodents typically are not permitted to

enjoy from origination, these are still essentially fatter than their lean family. Study concerning sustenance permit in individuals is confounded from the prospect that some weighty individuals under-report the sum food they expend. The structure of the consuming system moreover can affect heaviness. Overweight individuals have been demonstrated to thoroughly enjoy more high-fat supports than conventional weight individuals (Puska et al., 2003, and McGarty et al., 2010).

The volume of imperativeness an individual debilitates will even effect the take off of heftiness. Extended level of development is a bit of leanness. Also, the vitality deficiency that makes with movement, metabolic movements with planning moreover impact fat utilization and limit. Arranged individuals have a brought lipolytic farthest point up in connection with inert persons. In rats that are robustness slanted, before the approaching of huskiness, catecholamine-energized lipolysis is gloomier inside their fat cells differentiated and fat cells from heaviness safe courses. A huge benefit of action all through fat disaster is reliably that it realizes preservation of without fat mass and licenses fat reduction that is similar to or higher than weight decrease. A couple of physiological segments could be incorporated in the advancement of heftiness. Included in this are balanced oily tissue absorption framework, hormonal changes, and movements at objectives inside the brain that control satiety, especially in the hypothalamus. There may be an unordinary pointer that impacts fat tissue metabolic process and changes fuel dividing, stretched stockpiling in fat as opposed to basic use in muscle. This surprising pointer is not perceived. Of course, lipoprotein lipase has been discovered to be stretched in heaviness, which compound can raise the limit of triglycerides in fat tissue. Hormonal changes that may impact huskiness consolidate hyperinsulinemia and adjustments in pituitary or adrenal limit. If fat expansion is absurd, it could conceivably end in dependable chubbiness. The piece of mental variables in the improvement and headway of weight is difficult to portray. There is really no uniting subject. Some strong individuals have dietary issues. Other bulky individuals may consume in a reaction to extend or wretchedness. Still other beefy individuals may devour considerable meals or may expend rapidly. It's similarly been proposed that some substantial individuals could

be controlled eaters, and once there is a set in their common calendar, they respond by unreasonable expending (McGarty et al., 2010).

1.2 Leptin

Leptin, is the central player to deal with the body weight which cuts down blood glucose and insulin level, to the degree that thought to be sufficient to turn around diabetes due to lipodystrophy. Moreover, leptin changes physiological strategies as running from angiogenesis, invulnerable limit, lavishness, and bone surrounding (Huanget al., 2000).

The particular adipocyte will be general seen to be a genuine endocrine cell and a couple of adipocyte induced hormones, or adipokines are truly not far in the past perceived. Leptin will be including these sorts of adipocyte decided hormones nearby are probably the afferent signs demonstrating mental execution connected with fat tissues force stores (fat stores). There is a positive relationship between the measure of weight cell muscle assess close by leptin release. The choosing results joined with leptin are all things considered recognized by basically their discourse close by special leptin receptors, that happen to be the two arranged encroach cells nearby from the central focused on framework. Leptin will be traded all through the blood-mind hindrance notwithstanding it is binds to help distinctive receptors on wanting for sustenances controlling neurons, especially and not by any stretch of the creative ability in the hypothalamic arcuate center. Exogenous leptin supervision switches not very good weight with the leptin nonappearance of. Regardless, leptin nonattendance of close by leptin receptor unlucky deficiency of being regularly a particularly eccentric principle driver of the human not very good weight. To be perfectly honest, a huge amount of bulky individuals gets high moving leptin obsessions which hyperleptinemic state will be nearby a comparably unimportant rate joined with leptin CSF to help serum levels when differentiated and low fat persons. Hence, it is offered, which rotund people are generally leptin adaptable. This kind of leptin wellbeing may possibly be a conclusion of deformities with transport, all through the blood-cerebrum snag or perhaps on account of hurt leptin showing. Therefore, alterations with leptin are generally associated with short of what incredible weight, which neuroendocrine irritation may be mixed up in age group or the genuine consistency on the fat state (Huanget al., 2000).

1.3 Leptin Structure

Helical cytokines join Interleukin-2, Interleukin-4 as demonstrated in Figure 1.5, granulocyte-macrophage state energizing variable, macrophage settlement enabling part, and advancement hormone (Imagawa et al., 1998).

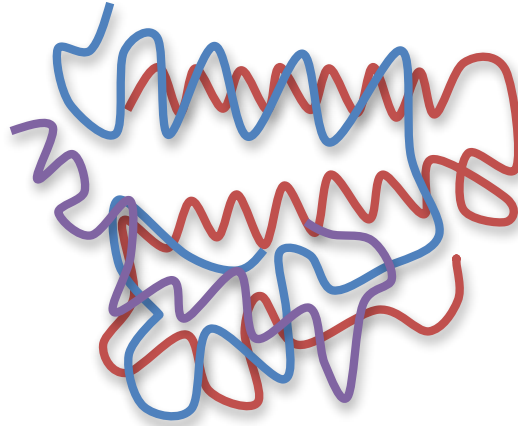


Figure 1.5: Interleukin-4 (<http://www.cs.stedwards.edu>)

The fundamental issue is that the vital structure, or amino destructive gathering of leptin showed in Figure 1.6 which exhibits no homology with whatever accessible protein in the data bank, making it troublesome to anticipate a three dimensional structure (Imagawa et al., 1998).

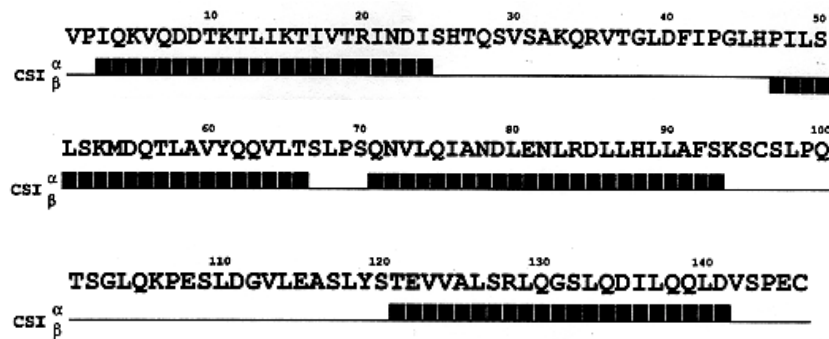


Figure 1.6: Primary structure of leptin (<http://www.cs.stedwards.edu>)

To get around this issue, researchers used a threading procedure of the proteins in the protein data bank, which is a methodology to find tertiary structure comparable qualities. Results from these examinations are demonstrated in Figure 1.7 underneath(Imagawa et al., 1998).

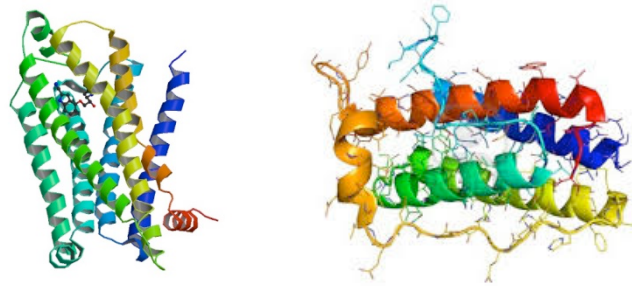


Figure 1.7Energy scaffolds for IL-2 and a structural model for leptin based on threading alignment with IL-2 (<http://www.cs.stedwards.edu/>)

This takes out the probability of using X-ray crystallography, a champion among the most skilled schedules for structure delineation. Masters could get a mutant sort of leptin to come to fruition, notwithstanding. Substitution at this moment with a tryptophan decreases hydrophobic correspondences and supports crystallization as shown in Figure 1.8(Imagawa et al., 1998;Al-Sulaimani, 2011).

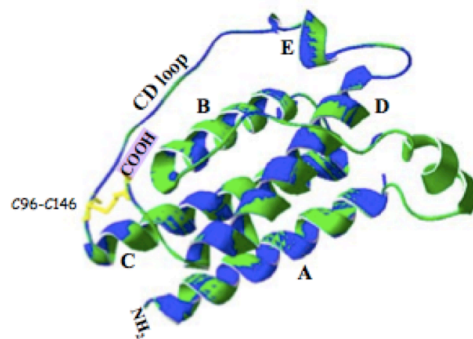


Figure 1.8:Proposed structure of leptin E100 (<http://www.cs.stedwards.edu/>)

The broad hydrophobic zone parallel to the helix pack is formed from the stores of the helices that face each other. These stores seem, by all accounts, to be basic for keeping up the structure as a result of their high security among diverse species. The interfacing rings wrap around the BD face of the helix bunch and the interhelical plot and eccentricities of the loops are similar to those found in the long chain helical cytokine team(Imagawa et al., 1998, and Jain et al., 2012).

Table 1.1: Positioning of alpha helices in leptin

Helix	Position
A	3-26
B	51-67
C	71-94
D	120-143

A captivating trick of leptin is the minimal helical section, E, that is found in the CD loop and is full hard against the helical pack. The E helix is ruined, bowing powerfully in the focal point, and is practically perpendicular (87 degrees) to the helix aggregate as shown in Figure 1.9.

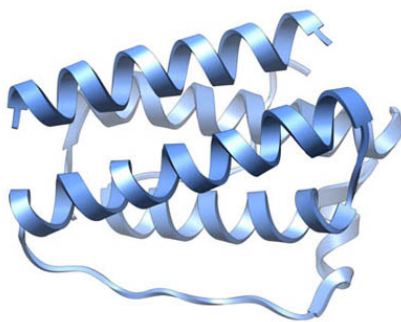


Figure 1.9: The E helix acting as a hydrophobic cap (<http://www.cs.stedwards.edu/>)

Despite the way that disulfide holding cases are not significantly apportioned inside the cytokine family, it creates the impression that leptin's single disulfide expansion is segregating for the headway of its helical cytokine caving in. Both of the cysteines in leptin are checked among species and change of either renders the protein biotically sit still, demonstrating that the disulfide platform and the pleat in the D helix are central for true blue falling and receptor tying as shown in Figure 1.10(Imagawa et al., 1998;Jain et al., 2012, and Fortufio et al., 2003).

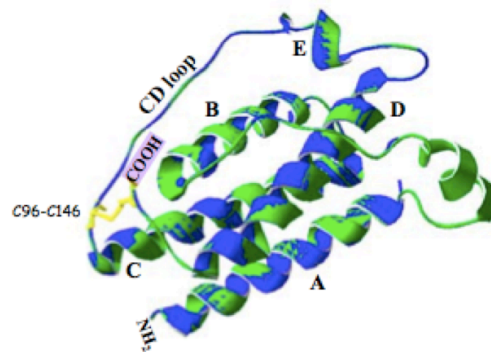


Figure 1.10: Disulfide bonding in leptin (<http://www.cs.stedwards.edu/>)

Understanding these contrasts and the other recognizing aspects of leptin will give knowledge into its particular capacity.

1.4 Function of Leptin

Leptin is changed by fat tissue where, the revolving around leptin overhauls the cerebrum of muscle to fat remainders. Leptin publicizes weight decrease by covering wanting and enabling absorption framework. Neuropeptide Y (NPY), no-limit in the hypothalamus, is responsible for the slant of longing for. The assertion and entry of NPY are limited by leptin. This stifles the limit of NPY making a response to strength. Sustenance affirmation is reduced, essentialness consumption is extended, and the astute activity is stretched. Of course, as weight is loosed, there is a lessening in leptin course, which constructs the effect of NPY making a response to starvation. The animals craving is

extended. The animal will diminish unnecessary imperativeness utilization, including regenerative limit. The general temperature of the animal is reduced, and there is a growing in parasympathetic activity. This complex is responsible for correspondence with the fat cell, which demonstrates the era or the stop the handling of leptin, dependent upon the gathering of leptin. On the off chance that the receptor is mutant, then it is hard to sign for the cell to stop creating and taking care of leptin. In case leptin is not producing the animal can then get huge. Leptin meets expectations nearby NPY to control longing. NPY lives up to expectations at the hypothalamus. In case an animal is over-creating leptin then the coupling areas in the hypothalamus are secured with leptin instead of NPY. This prompts a voracity that is never lessened, which accordingly causes weight. Leptin has been associated with other hormonal limits as well. Affirmation has exhibited that leptin appears to demonstrate the onset of youth. As leptin levels reduce, the starvation response decreases the development of unnecessary significant limits, for instance, duplication. Some work prescribes that they may provoke stretched activity of a basic controller of nervousness hormone era. They have watched that average leptin levels drenched down handling of a psyche peptide called corticotropin-releasing hormone that works through the pituitary organ to help era of adrenal steroids as showed in Figure 1.11.

Any responses, authoritative cycle having a couple of special steps has been perceived: (1) a sensor (Leptin yield by fundamentally fat cells) screens how huge is the fat tissue mass; (2) hypothalamic centers acknowledge and similarly adjust the energy of the leptin marker by method for leptin receptors (Lep R); (3) effector schemas, for instance, a mindful strained task, control every one critical determinant of imperativeness balance essentialness ingestion. Notwithstanding the way that this particular response, managerial cycle is dug in rodents, there are packs of unsolved solicitation concerning their suitability to body weight authorizations in individuals. After the thought ties with a particular finished objective to Lep R in the hypothalamus, leptin sways a picked showing a course that will accomplish the deterrent concerning different orexigenic neuropeptides, though revitalizing different anorexigenic peptides. (CRH) (Paul et al., 1998).

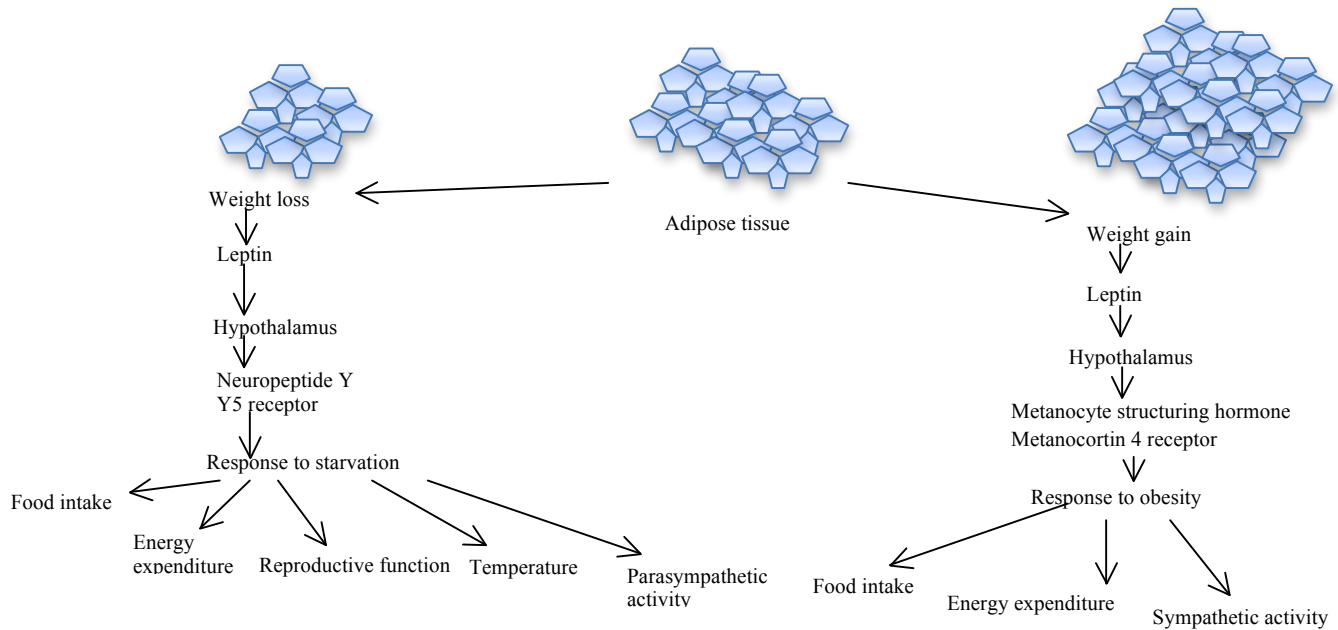


Figure 1.11: Leptin effects on weight (<http://www.cs.stedwards.edu>)

1.5 Biosynthesis of Leptin

This streams in our blood serum in both free (FL) close by robust proteins compelled (BL) sorts, with the dissolvable leptin receptor as an issue segregating presenting substance. Investigation have exhibited that will the sum getting more customary leptin is truly especially comparing to body to the exact entirety regarding weight science. Without a doubt thusly, different first sensible studies on exorbitantly overpowering people revealed unimportant leptin sums notwithstanding it was normal that will be overweight result from leptin nonattendance of. This particular made different master therapeutic trials using leptin to treat being overweight (Peter et al., 2000).

1.6 Leptin Production Regulation

The particular regulation, incorporating leptin readiness in standard subject will never be totally cleared up. Wholesome and similarly hormonal parts happen to be exhibited to impact leptin era. The vital issue is obviously weight, muscle size itself. Similarly, the real pituitary-thyroid turn (PTA), acting with the keen, concentrated on the schema (SNS), handles a focal point part in the regulation including fat cell rate of assimilation framework. In fasted or possibly malnourished condition PTA diversion is diminished, provoking diminished essentialness utilization, nearby inverse improvements inside the ate up condition and furthermore in chunky focuses. Leptin release additionally, is decreased nearby suppresses imprisonment recommending that will let in and what's more the PTA may well reply in parallel (Peter et al., 2000).

The more there's extra fat, the additionally stunning there's serum leptin focus. People who have a BMI in excess of 40 Kg/m² are in high threat identifying with diminished life compass and may have got around 11 circumstances the additional danger to life of individuals nearby a steady BMI mixture (Peter et al., 2000) The sum leptin greatly identifies with help the sum fat could be secured in body, together with greater levels indicate in those with more fat and diminished levels inside those that consumed less carbs. All things considered, the more amazing will be the entire body size and rate joined with undesirable weight, the more prominent the measures of leptin. Awful calories stops diminish serum leptin, so leptin's demonstrating utmost could help clear up the unnecessary costs connected with recover body weight ran crosswise over among men and women, since rapidly in the wake of consuming less carbs, the measures of leptin rots, suggesting that which a lesser measure of leptin may be made available to transmission serotonin levels as demonstrated in Figure 1.12.

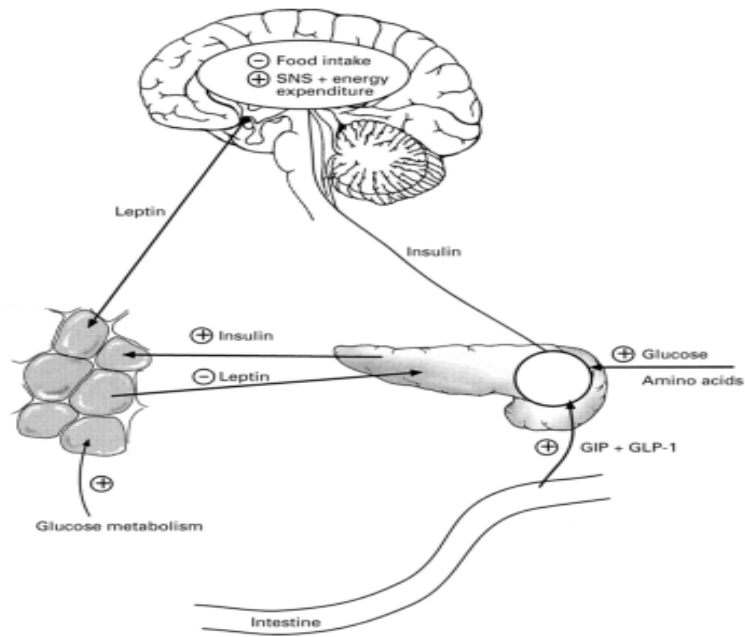


Figure 1.12:Long-term signals regulating energy balance (Peter et al., 2000)

This particular cutting down perhaps help with extended longings for sustenance and moderate fat seething utmost. On the off chance that this could be exact, leptin treatment could help persons oversee weight decrease rapidly in the wake of consuming less and leptin is a satiety viewpoint. Serum leptin centers conform more through weight lessening when differentiated and through bodyweight get. By technique for example, the affiliation including serum leptin centers and fat size truly isn't changed through fruitful bodyweight get inside individuals, on the other hand, serum leptin obsessions according to fat size regularly are baffled basically by in association with 50 % through weight decrease (Paul et al., 1998).

In any case, right away leptin seemed, by all accounts, to be found like a controller joined with support confirmation and imperativeness obliges, that happen later like an apheliotropic iota together with a degree of physiologic and pathologic assignments. It's been starting late ensured which leptin plays an essential limit not fundamentally inside the control connected with bodyweight stretch and furthermore in the advancement in the conceptive system capacities as demonstrated in Figure 1.13 (Paul et al., 1998).

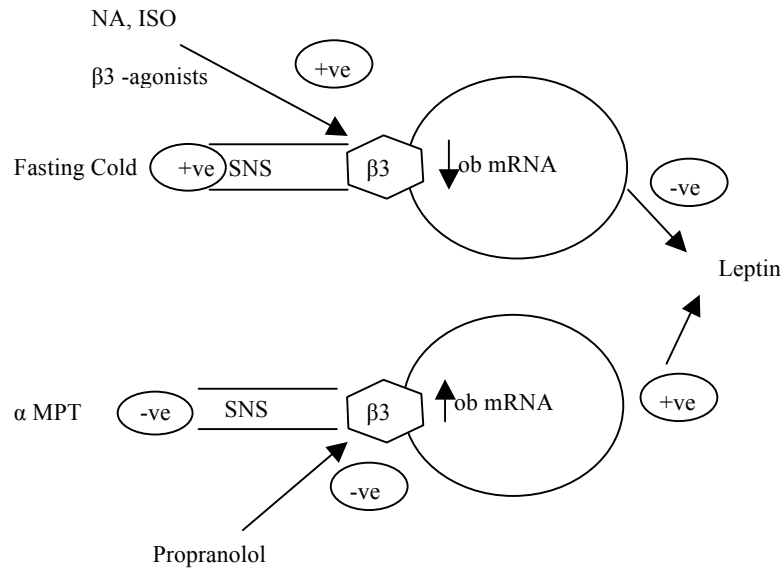


Figure 1.13: Schematic view of the Regulation of Leptin Production in White Adipose Tissue by the Sympathetic Nervous System (SNS) (Paul et al., 1998)

1.7 Receptor of Leptin

Ob-Rb is accessible in the showing, ventromedial, dorsomedial and moreover outside hypothalamus centers, in spite of the way that truly isn't recognizable inside diverse other mental work force zones. (Laura et al., 2007).

1.8 Leptin Resistance

It moreover helps the body to utilize fat as an issue source. Exactly when leptin is working truly in the body, it serves to control the sum is eaten up at suppers, which supports true blue weight organization and glucose regulation. As often as possible we wind up using fundamental sugars and other exceedingly took care of sustenance that smothers or usurps leptin's ability to diminishing our insatiability and this is the time when the detriment begins. As leptin wellbeing grows, so does insulin security. Glucose levels spike as over usage gets fair, making stretched insulin readiness which is a faultless setup for diabetes. Despite direct carbs and over usage, unending tension and stationary ways of life furthermore manufacture impenetrability to leptin as shown in Figure 1.14 (Cardiol et al., 2008).

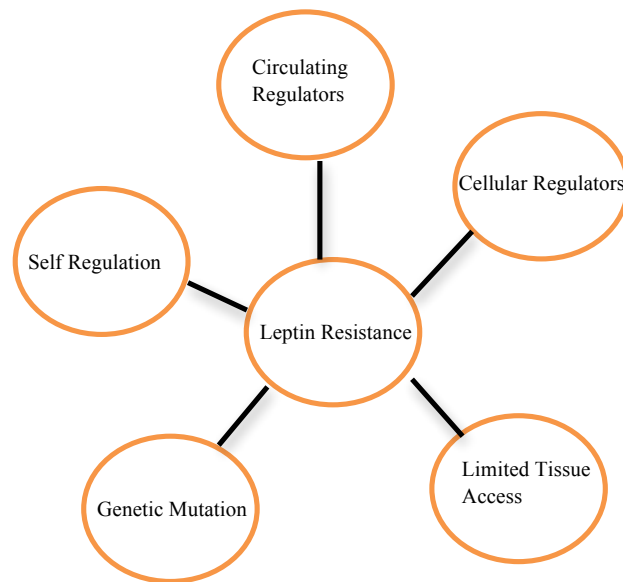


Figure 1.14:Mechanisms of Leptin Resistance (Cardiol et al., 2008)

1.9 Insulin

Insulin is genuinely a polypeptide hormone (MW 6000) embodying a few non-unclear restaurants, and N, enrolled together essentially by a few disulphide bonds. Insulin emanation assumes control over a few main things, tonic release and biphasic release. The genuine basal or possibly tonic emanation may be fair of arousal essentially by exogenous glucose, yet could be balanced through the fluctuations inside physiological levels of glucose. The genuine avoid release may be for the most part a crucial reply by method for arousal basically by exogenous glucose. Affection of insulin outflow could be brought on by a couple of fragments which join hyperglycemia, glucagon, amino acids, and fundamentally by exceptional things with respect to HGH or perhaps catecholamines. Additional stunning levels of insulin are regularly associated with not very good weight, Cushing's signs, mouth contraceptives, acromegaly, insulinoma and hyperthyroidism. Reduced levels of insulin are commonly found in a couple of arrangements of diabetes mellitus (DM) and they are allocated something to that impact of cutting edge structure as to catecholamines (Zimmet et al., 1998).

Diabetes mellitus may be a gaggle of metabolic illnesses of carbon absorption framework where glucose could be underutilized, creating hyperglycemia. You will find several key blended packs of diabetes mellitus: sort 1 diabetes mellitus, which much of the time is called insulin subordinate diabetes mellitus. The other sort could be sort 3 diabetes mellitus and various people who have this specific kind consolidate decreased insulin development. Weight issues are consistently joined with restorative plans and fat adversity to its habitually enhances the hyperglycemia. Commonly, additionally astounding overweight one is, more spectacular these people are obligated to demonstrate any evidences called "insulin measure of security. Slithers nevertheless, the measure of insulin measure of wellbeing offers a gathering inside overweight people (Michael et al., 2006, and Goodpaster et al., 2001).

1.9.1 Leptin and Insulin

Leptin modulates pancreatic B-cell perform in five. Elevated leptin amounts join large serum insulin amounts (Goodpaster et al., 2001).

Thusly, body stretched leptin level saw in bleak chunkiness could maybe delayed consequence of body never-ending hyperinsulinemia and extended cortisol turnover. As per this kind of speculation, recorded that assistance with the ob/ob rodents, which show any tender, non-insulin-subordinate diabetic phenotype, having leptin quality meds completed up in body institutionalization as to serum insulin entireties and sugar and carb resistance. These researches publish that leptin quality prescriptions may be utilized each for the organization regarding body weight and from the operations with respect to non-insulin-subordinate diabetes mellitus. Leptin relates having insulin security and diverse markers concerning metabolic evidences, it could transform into a key focus on as for brand-new solution courses of action as for diabetes, especially inside full people.(Goodpaster et al., 2001; Burén et al., 2012, and Bonen et al., 2006)

1.10 Insulin Resistance

Impenetrability to insulin-strengthened glucose uptake is accessible in the prevailing piece of patients with incapacitated glucose resistance (IGT) or non-insulin-subordinate diabetes mellitus (NIDDM) and in ~25% of no hefty individuals with normal oral glucose resilience. Right when this target can't be achieved, horrible decompensating of glucose homeostasis happens. The relationship between insulin wellbeing, plasma insulin level, and glucose bias is interceded to an essential degree by movements in enveloping plasma free-unsaturated fat (FFA) center. Patients with NIDDM are moreover impenetrable to insulin disguise of plasma FFA concentrate, however plasma FFA obsessions may be diminished by reasonably little increments in insulin fixation. Therefore, statures of coursing plasma FFA obsession could be foreseen if a ton of insulin may be emitted. In case hyperinsulinemia can't be kept up, plasma FFA obsession won't be covered regularly, and the resulting extend in plasma FFA center will provoke extended hepatic glucose era. Since these events happen in individuals who are really

impenetrable to insulin fortified glucose uptake, it is obvious that even little forms in hepatic glucose creation are inclined to incite critical fasting hyperglycemia under these conditions. Moreover, a quick relationship between plasma insulin center and beat has been noted. Hypertension can moreover be changed in run of the mill rats when they are fed a fructose-progressed consuming regimen, a mediation that in like manner prompts the change of insulin security and hyperinsulinemia. Then again, paying little heed to the likelihood that insulin security and hyperinsulinemia are excluded in the etiology of hypertension, it is likely that the extended threat of coronary channel disease (CAD) in patients with hypertension and the way that this peril if not diminished with antihypertensive solution are a direct result of the gathering of risk segments for CAD, despite hypertension, joined with insulin wellbeing. These consolidate hyperinsulinemia, IGT, extended plasma triglyceride obsession, and reduced high-thickness lipoprotein cholesterol concentrate, all of which are associated with stretched threat for CAD. It is likely that the same threat variables accept an immense part in the genesis of CAD in the masses all things considered. In perspective of these thoughts the probability is raised that impenetrability to insulin-invigorated glucose uptake and hyperinsulinemia are incorporated in the etiology and clinical course of three genuine related contaminations NIDDM, hypertension, and CAD (Reaven, 1988).

1.11 Glucose

Glucose, a separating monosaccharide, is resolved from this brokenness concerning glucose inside the consuming regimen plan (grains, starchy greens, and also vegetables) and similarly skeleton stores (glycogen), and furthermore essentially by endogenous activity by method for amino acids or perhaps in the glycerol moiety as to triglycerides. Right when power ingestion surpasses cost, the sugar and carbs may be changed over to muscle to fat proportion, degrees and also glycogen as to storage space with fat structure and in like manner liver or perhaps muscle tissue, separately. Right when force expense surpasses caloric affirmation, endogenous sugar and sugars creation happens in the brokenness as for carb stores and moreover by method for non-starch resources (amino

acids, lactate, and also glycerol) by technique for procedure termed gluconeogenesis (Clarke et al., 2012).

Glucose will be the essential vitality source with respect to our bodies. This metabolic methodology brings about (1) force preparation essentially by adjustment with a specific end goal to carbon dioxide and additionally mineral water, (2) storage room seeing that glycogen inside the liver or maybe triglycerides (TG) with fat structure, or maybe (3) change so as to keto acids, amino acids or maybe solid proteins (Clarke et al., 2012).

1.11.1 Effects of Leptin on Glucose

Leptin might maybe play an administrative part all through glucose metabolic rate in adipocyte cells and may figure out how to lessen blood glucose amounts. The examination noted which glucose imbuement has an impact on plasma leptin amounts (Lam et al., 2004).

1.11.2 Plasma Lipids

The major lipids of the blood plasma are cholesterol, triglycerides, and phospholipids. These are transported in the blood attached to proteins forming lipoproteins (Ríos-Lugo et al., 2010, and Howell et al., 1997).

1.11.2.1 Cholesterol

Esterified cholesterol from the consuming regimen will be hydrolyzed quickly from the passage to have the ability to unesterified cholesterol and moreover totally free unsaturated fats by cholesterol esters released from the pancreas and also littler vessel. Cholesterol rings from the stream by technique for lipoproteins, generally low-thickness lipoprotein (VLDL). Esterification associated with cholesterol is significant in this particular framework accommodated it fabricates body lipid-pass on potential from the lipoprotein inside plasma and moreover helps prevent intracellular noxious quality by unesterified cholesterol. Females drop their close insurance towards coronary heart

disease in menopause in light of changed over lipid cover record of estrogen need. Estrogen attributes effective responses of the lipid report, stretching high-thickness lipoprotein cholesterol levels and also decreasing low-thickness lipoprotein cholesterol levels each and every by pretty about 10%. Menopause fuses a telecasted effect on body going around degrees of lipids and furthermore lipoproteins. Inside postmenopausal ladies, the dietary reducing by and large wealth fat, thick excess fat, and in like manner cholesterol diminishments body volume, general cholesterol, and moreover LDL-C and also fat adversity and furthermore tally calories changes are regularly connected with a prevalent plasma lipid report inside powerful postmenopausal ladies (Lam et al., 2004; All About Cholesterol., 2009, and Howell et al., 1997).

1.11.2.2 Triglycerides

Triglycerides generally are oily corrosive triesters connected with glycerol. With man nourishment, triglycerides translated as 95% connected with cells protection weight and are the real dominating sort of glycerol ester acquired in plasma. Triglycerides normally are squanders in the duodenum and likewise proximal ileum. All through the movement connected with pancreatic and likewise stomach lipases and additionally in the profile connected with bile acids, they're hydrolyzed to help glycerol, monoglycerides, and additionally unsaturated fats. Emulating ingestion, the genuine unsaturated fats and additionally glycerol moiety resynthesizes triglycerides in the stomach epithelial cells and likewise supplement cholesterol and likewise APO B-48 so as to make chylomicrons. Chylomicrons normally are emitted for the lymphatic procedure, going over the thoracic conduit, lastly get to the genuine framework over the jugular unusual vein. From the going on a quick condition about all plasma triglycerides happen inside low-thickness lipoprotein (VLDL) (Howell et al., 1997, and McDermott et al., 2008).

1.11.2.3 High Density Lipoprotein- Cholesterol (HDL-C)

Move associated with lipids in plasma is normally as a result of a few gathered conjugated proteins named lipoproteins. Lipoproteins are roundabout particles utilizing non-polar lipids into their middle and significantly more polar lipids concentrated in the region. Also, they consolidate more than one particular protein, named Apolipoproteins which may be arranged on their specific sorts of surface. They get amped up for (1) starting critical digestive proteins in the lipoprotein metabolic trails, (2) maintaining the specific structural truthfulness of the lipoprotein modern, and (3) supporting the specific client base joined with lipoprotein into sun oriented cells by the method for their specific notoriety essentially by particular cell range receptors. Lipoproteins are classified as chylomicrons, very low- density lipoproteins (VLDL), intermediate- density lipoproteins (IDL), low- density lipoproteins (LDL), and high- density lipoproteins (HDL) (Zimmet et al., 1998).

HDL is responsible for the specific inverse exchange associated with cholesterol in the fringe sun based cells for the lean proteins. HDL cholesterol esters are transported to the specific lean proteins basically by one of a few succeeding things:

1. Cholesteryl esters are specifically assimilated through HDL, perhaps essentially by hepatic HDL receptors, alongside the HDL particles are returning for body to stream for furthermore exchange.
2. Cholesteryl esters are moved through HDL for you to APO B-100-holding lipoprotein, an action interceded essentially by cholesterol ester intervened wellbeing proteins, then assimilated through the lean proteins by method for receptors for these sorts of lipoproteins.
3. High-thickness lipoprotein APO Age could be perceived by hepatic remainder receptors.

The specific plasma degrees of HDL are dead set halfway basically by catabolism of lean proteins, however, it truly is normally uncertain the way that hepatic catabolism associated with radiance HDL is typically overseen or maybe intervened. An

extraordinary backwards association wins concerning serum HDL-C levels alongside the likelihood associated with atherosclerotic sickness. Expanded HDL-C levels are defensive against coronary illness, however diminished HDL-C consideration, particularly alongside upgraded triglycerides, supports the cardiovascular probability. Expanded HDL-cholesterol degrees are really in regards to diminished coronary illness plausibility. Cardiovascular infection may be the real explanation for mortality in postmenopausal females in creating countries as far and wide as possible. Another conceivable cardioprotective position joined with hormone substitution treatments (HRT) is generally prompted essentially by epidemiologic exploratory tests associated with HRT and diminished plausibility associated with coronary illness, together with basically by randomized demos joined with HRT and lipid subfractions (Castelli et al., 2009).

1.11.2.4 Low-Density Lipoprotein- Cholesterol (LDL-C)

Low- density lipoprotein could be the key organization with respect to cholesterol in the circulation system. It has another focal of around 1500 esterified cholesterol components. A spread with respect to phospholipids alongside unesterified cholesterol encompasses this specific extremely hydrophobic focal. The specific blanket what's more has B-100, a greatly significant essential protein (514 kd) (Kuklina et al., 2013).

The specific LDL receptors exhibit in the imprint tissues, cells are vital relating to LDL uptake. The specific preparation in regards to LDL receptors will be controlled through the cell's will need with respect to cholesterol. An ascent in BMI will be connected with unfriendly changes inside the plasma fats alongside lipoprotein record, bringing about enhanced complete cholesterol, LDL-C, alongside TG levels in addition to a decrease in HDL lipoprotein levels. The specific menopausal move will be connected with denser LDL, alongside more amazing LDL-C levels when contrasted and premenopausal ladies of all ages. Little doubt remains that in spite of the fact that LDL-C may well change late inside the menopausal move, the generation in regards to denser LDL contaminants looks at an early stage inside the menopausal move, both similarly carrying on for you to compound the lipoprotein account. More terrific triglycerides alongside diminished HDL seemed to consider the move toward little, thick LDL, possibly identified with upgrade

BMI. The specific alter in LDL robustness may well push the more costly risk in regards to atherosclerosis in postmenopausal ladies of all ages (Kuklina et al., 2013).

1.12 Menopause Effects on Leptin Level

Being overweight, especially with focal weight syndication, not with standing the casualty rate through pretty much all prompts generally areconnected straight with time especially all through center matured females. Numerous studies have shown that ladies in their midlife tend to addition pounds, which has a move to help instinctive weight syndication. Getting more established all through females might be connected to continuous decreases inside amounts of very much a couple of testosterone and additionally estrogens, dehydroepiandrosterone (DHEA) notwithstanding development hormone-insulin-like development segment When I (GH-IGF-I). These endocrine annoyances may bring about changed framework structure notwithstanding pounds attains. Being overweight all through postmenopausal females might be coupled with truly a couple of metabolic disturbances chief oftentimes to help upgrade casualty rate Postmenopausal females assemble a focal example including weight syndication studies have got endeavored to search for the result including menopausal changeover upon leptin sums. In the investigate made succeeding averting relating to unwanted fat, postmenopausal females, however obtained more amazing leptin sums than guys including similar period; their sums are not completely not the same as people including energetic females. Besides, asserted in which leptin sums typically are practically identical all through prein expansion to postmenopausal no twith standing all through postmenopausal females who're either accepting or surely not getting hormone substitution medications, inferring that it is unthinkable that this sex contrast is because of an impact including ladies the regenerative framework testosterone. It is conceivable that this sex distinction is on account of an inhibitory result including androgens and/or contrast all through unwanted fat syndication among male and females. Indeed in this way, succeeding menopause, there is an extension all through the body volume; framework, muscle size rundown (BMI) not with standing weight, muscle size with centralization including weight syndication and this may impact leptin degree. However

the unwanted fat syndication isn't going to aid being an indicator including leptin sums all through postmenopausal females (Nishat et al., 2006; Abdul et al., 2010).

1.13 Menopausal Woman

Menopause is truly on the grounds that the ovaries quit handling the endocrine framework estrogen and progesterone. When the lady is presently through menopause, she can't get pregnant any longer. A few people call numerous years preceding a lady's last period menopause, yet that point really is the menopausal move, or perimenopause (PER-ee-MEN-goodness pawz) (Rouen et al., 2009; Ayub et al., 2006; U.S. Department of Health and Human Services., 2005; Goodman et al., 2011; www.healthpromotion.ie., 2011, and Gass et al., 2012).

Menopause average age is 51, additionally for some ladies it happens when 40 or as late as 55 (Rouen et al., 2009; Ayub et al., 2006, and Gass et al., 2012).

Some ladies stress over menopause, this methods she will result in uncomfortable side effects. Be that as it may there are different strategies to treat indications and stay dynamic and solid. Typically, menopause is common. Really it happens on its own, and lady doesn't have help unless her side effects trouble her. In some cases, however, menopause is restoratively affected, so these methods it's brought about by surgery or prescription. All things considered, she ought to dependably work with her specialist to feel quiet and take great consideration of her wellbeing. Menopause is typically a typical, common life occasion that dependably happens between ages 40 and 55. Some ladies achieve menopause right on time (before age 40), as an aftereffect of surgical evacuation in the uterus and ovaries, chemotherapy or help, or common reasons. Every lady are encountering menopause in their interesting way. Some ladies may not precisely encounter any side effects influencing their expectation for everyday comforts. In anycase, for some ladies, the most incessant troublesome indications are hot flashes, sweating, and vaginal dryness. The best medication for these indications is hormone help (HT), albeit nonhormonal medicine treatment is helpfully realistic. HT ordinarily

recognizes two separate options (Rouen et al., 2009; Ayub et al., 2006; U.S. Department of Health and Human Services., 2005; Goodman et al., 2011; www.healthpromotion.ie., 2011 and Gass et al., 2012). If lady joins a uterus, she could conceivably be recommended consolidation estrogen in addition to progestin help (EPT). The progestin is incorporated with secure her uterus from disease that will happen when estrogen might be utilized alone. There are different HT potential outcomes open by solution from her social insurance supplier. Numerous dosages are accessible to help. Each of these choices has distinctive favorable circumstances and detriments that she can talk about with her specialist. Bioidentical hormones, that happens to be much the same as hormones made by the ovaries, might be purchased in well-tested, government-approved name items that is generally recommended via human services suppliers. The expression "bioidentical hormone help" is likewise regularly supportive to portray custom- intensified hormones which might be acquired at intensifying drug stores. They're not government affirmed and have by no means been tried for adequacy, wellbeing, measurements precision, or virtue (absence of contaminants). Albeit government-approved bioidentical hormones are a great choice for a ton of ladies, custom-compounded hormones normally are not suggested unless she gets an unfavorable susceptibility towards sanction items. In 2002, a significant study alluded to as Women's Health Initiative (WHI) reported an expanded danger of breast disease, coronary illness, stroke, and blood clumps while utilizing EPT. Inside the decade considering that the reporting of the individuals results, scrutinizes have demonstrated how the manifestation of HT (ET versus EPT), work is taken, and additionally the timing of beginning this medication (early versus late after menopause) produce distinctive profits and symptoms. Gambling potential unwanted impacts (for example acute myocardial infarction, stroke, blood clump, or breast malignancies) with HT in solid ladies ages 50 to 59 is low. As contradicted, utilizing HT for an amplified time or beginning HT when the young lady with numerous years past menopause is allotted to a higher hazard these negative(Rouen et al., 2009; Ayub et al., 2006; U.S. Department of Health and Human Services., 2005; Goodman et al., 2011; www.healthpromotion.ie., 2011 andGass et al., 2012):

HT keeps on being the best medication planned for menopausal indications, including hot flashes and night sweats that can interfere with slumber and debilitate quality lifestyle.

Some ladies may take it securely. For EPT, time is bound from the expanded danger of malignancy of the breast that may be seen surpassing short of what six various years of utilization. For ET, no indication of expanded danger of bosom growths was seen throughout normally 7 numerous years of medicine, a finding that permits more decision in the amount time she chooses ET. Most solid ladies underneath age 60 could have no surge in gambling potential cardiovascular ailment with HT. The dangers of stroke and thrombus inside the lungs are expanded yet, over these more youthful age sections, the potential dangers are a ton short of what 1 in every 1000 ladies a year taking HT. It conveyed all through the skin (by patch, cream, gel, or shower) and low-dose oral estrogen can have easier dangers of blood clumps and stroke than standard measurements of oral estrogen, yet the majority of the proof is simply not yet accessible. Research will keep on creating important data to support ladies utilizing choice about H

1.13.1 Pre-Menopausal

Menstrual issue without a doubt are a typical evidence for medicinal visits among ladies of conceptive and overwhelming menstrual draining influences pretty nearly 30% of ladies all through their regenerative lifetime. These grievances might fundamentally influence aggregate prosperity, cause a vacation day work, produce surgical mediation including hysterectomy, and at last have an extremely noteworthy effect on the consideration framework. The consequent rules provide for you a review of the present analysis and administration options for strange uterine draining among ladies of regenerative age. (Singh et al., 2013, and Mazess et al., 1991)

Institutionalized all inclusive wording is basic inside exchange of AUB to raise correspondence among professionals additionally to help aide research and training on this subject. Outline of current wording in medicinal and authentic writing uncovers confounding and conflicting definitions talking about menstrual dying. As a result of this, the FIGO Menstrual Disorders Working gathering (a worldwide master accord panel) has developed new rules for phrasing related this subject. The proposed classification for AUB intends to streamline depictions on this clinical presentation and dispense with phrasing for instance menorrhagia, metrorrhagia, and broken uterine dying.(Singh et al.,

2013; Mazess et al., 1991; Qureshi et al., 2010; Khokhar et al., 2010; Jesmin et al., 2013, and Jankowitz et al., 2013)

AUB could be characterized as any variety on the typical menstrual period, and incorporates changes in consistency and recurrence of menses, in span of stream, or even in amount of drain. Underneath the class of AUB, further definitions may be subdivided dictated by level of period, consistency, recurrence, term, chronicity, and timing related regenerative status. Draining not connected with menses could conceivably be further described in the meantime. Exemplary portrayals of AUB determine from the cyclicity alongside the level of menorrhea. Despite the fact that persistent's recognition with the draining genuinely isn't essentially quantifiable, is key for the administration of the issue. Eventually, over's experience and additionally the impact on her aggregate prosperity focus their training to which mediation could be needed. The singular's presentation of AUB is controlled by her subjective experience and impression from the measure of draining. Due to this, an all-encompassing methodology should be taken utilizing these definitions (Singh et al., 2013).

Heavy menstrual bleeding is one of common gripe for AUB. It's been understood to be “excessive menorrhea loss which disturbs over’s physical, social, emotional, and/or material standard of living that can take place alone or in combination with other symptoms (Singh et al., 2013).

1.13.2 Differences between Menopause and Post-Menopause

Ostensibly, basically the most stamped qualification between menopause and post menopause would be the diminishing in manifestations - as sample hot flashes are less incessant rather than as influential. A lady is typically considered post-menopausal succeeding ladies evacuation of the ovaries as it viably closes menstrual dying. A fair pointer with the begin of post menopause is the follicle animating hormone (FSH). At the point when ladies move into post menopause, FSH is going to be to a great degree high, showing her ovaries begin to seal down. This bringing down in estradiol is inside turn what causes the ascent in FSH. The follicle invigorating hormone endeavors vainly to

animate egg preparation. Essentially, it demonstrates the completion of periods, richness, and menopause. Numerous post-menopausal ladies start to visit a rest from menopause indications at this point. This after menopause shifts essentially and therefore does the end, or need thereof, from the manifestations. The test with suspecting menopause to secure could be the instability very nearly day by day outline in addition to the cruelty of the manifestations. Moreover, while post menopause may bring diminished menopause indications; uneven hormones put her helpless against specific conditions. Low estrogen e.g., abandons her at danger for osteoporosis, coronary illness and genitourinary decay. (Jankowitz et al., 2013)

1.14 Leptin with Menopause

The association between leptin , estradiol and FSH, without impedances of other pituitary and ovarian hormones, brought on by concealment of pituitary capacity after organization of GnRHa. (Haffner et al., 1997; Hadji et al., 2000, and Shams et al., 2006)

These patients were enlisted in a course of egg gift a consequence of the historical backdrop of low or no ovarian reaction, normally connected with the build old. With respect to, each one gathering demonstrated indistinguishable extent of large patients, along these lines prohibiting inclination to look in, out and all around with shod and non-shod. When we assessed the variety in leptin levels as delineated by BMI, the results showed that large patients had serum leptin levels fundamentally over the ones from non-corpulent, both before medication. These email address particulars are regarding the reported at one time. Albeit mean chronilogical time of both gatherings was diverse, numerous specialists have beforehand showed that there isn't any contrast in phases of serum leptin between junior and postmenopausal ladies. (Haffner et al., 1997; Hadji et al., 2000, and Shams et al., 2006)

Pretreatment and post-medicine assessments demonstrated that serum amounts of estradiol altogether expanded both in gatherings after medication. These impacts were normal, since patients exhibited hypoestrogenism before medication because of the use of Gnrh simple and after were recorded in controlled ovarian incitement keeping in mind the

end goal to the utilization of estradiol for endometrial arrangement. When you think about the serum degrees of estradiol caught after medication inside the two gatherings, we watched essentially larger amounts inside GnRh/FSH bunch, because of a response to the ovarian incitement. Inside GnRh/E2 bunch, patients gained settled measurements of estradiol valerate, hence the level of estradiol discovered in serum achieve an edge relating to the regulated dosage. Some exploration has indicated that estradiol expands leptin levels in ladies with typical menstrual cycles. With respect to the assessment of leptin, we likewise watched a significant build in its serum levels after medicine, in both gatherings. These discoveries show, at first, a rise of serum leptin levels optional on the change in estradiol, without impact of whatever available ovarian or pituitary hormone (GnRh ward), recommending a prompt association between these hormones and likewise a part of estradiol in fortifying the preparation or maybe the arrival of leptin. We speculate make angle a surge in ob gene (in charge of leptin blend by adipocytes) articulation impelled by estradiol would be the accountable for these results. An alternate conceivable system is truly a leptin gene promoter enactment through specific regulation by means of estrogen receptors. (Gower et al., 2000; Petzel et al., 2007; Yamada et al., 2003; Haffner et al., 1997; Hadji et al., 2000, and Shams et al., 2006)

Comparable results have as of now been acquired with females submitted to controlled ovarian incitement for IVF without the utilization of GnRh. On the other hand, uniquely in contrast to the work in which ladies had pituitary concealment, patients fused into these studies were put through the impact of ovarian and pituitary hormones, that may thwart or predisposition the conclusions. Despite the fact that serum degrees of leptin were comparative in the gatherings after medication, the leptin rate was fundamentally higher in the arrangement of patients treated with rFSH when contrasted with the gathering treated with estradiol. This result could be clarified by the higher degrees of estradiol influencing this gathering after medicine. Then again, we can't avoid the capability of an immediate movement of FSH inside the yield of leptin, through a conceivable ovarian preparation or an aberrant impedance inside the union with this hormone by adipocytes, through other ovarian substances. (Gower et al., 2000; Petzel et al., 2007; Yamada et al., 2003; Haffner et al., 1997)

RELATED RESEARCH

2.1 Overview

Leptin is a hormone delivered primarily by adipocytes (fat cells) that is included in the regulation of muscle to fat ratios. Leptin connects with zones of the cerebrum that control craving and conduct and indicators that the body has had enough to consume. A little number of individuals has hereditary transformations in the leptin quality, prompting a more prominent interest for sustenance.

2.2 Related Work

Gower et al. (2000) surveyed the study depending on three cases: 1) distinguished the components affecting coursing leptin in postmenopausal ladies; 2) figured out if hormone-replacement therapy (HRT) impacts serum leptin in the wake of modifying for bewildering variables; and, 3) recognized potential autonomous impacts of HRT or leptin on resting energy expenditure (REE).

Petzel, (2007)proposed both fringe and focal activities of leptin on bone digestion system. In vitro and in vivo confirmation upholds the speculation that leptin can act specifically or in a roundabout way on bone rebuilding by tweaking both osteoblast and osteoclast exercises. In any case, thinks about in people have not yet possessed the capacity to confirm these activities potentially on account of the moving harmony between stimulatory immediate activity and suppressive backhanded activity of leptin on bones by means of the hypothalamus. Where, the effects of estrogen decrease and deficiency amid common or artificially prompted menopause and organization of hormone substitution treatment has on leptin generation stays questionable. Different studies have demonstrated differences in leptin values in pre-and postmenopausal women.

Petzel et al. (2008) expressed that the mean serum leptin focuses did not differ measurably in any of the mulled over gatherings first and foremost and toward the end of the study of progesterone, testosterone, free testosterone and DHEAS concentrations in any of groups before and after treatment.

Sumner et al. (1998) investigated the relationship of the leptin with the gender, sex hormones, menopause, age, also diabetics, and ending with the fat mass. Moreover, the percent body fat using the Siri formula was calculated, as much as the fat mass (FM) according to the weight multiplied by the percent of the body fat also calculated, ending with the calculation of the fat-free mass (FFM) as the weight minus the FM. Concluded with the fasting plasma assayed for the leptin, estradiol, free testosterone, glucose, and insulin concentrations.

Michael et al., (1996) demonstrated that leptin vary more directly with absolute fat mass compared to fractional body fatness by itself; just as much as the relationship between leptin and fat mass is significantly affected by gender and also menopausal status. Although largely secreted by simply adipose cells, leptin, the polypeptide hormone of which affects body size, satiety as well as lipid metabolic rate, as well as receptor are likewise indicated in individual osteoblasts. Leptin leads to this core, hypothalamic modulation regarding navicular bone enhancement, plus locally within the skeleton by simply bettering difference regarding navicular bone marrow stroma directly into osteoblasts as well as inhibiting it's difference directly into osteoclasts as well as adipocytes.

Scariano et al., (2003) compared serum leptin values to bone mineral density (BMD) measurements filmed by dual X-ray absorptiometry and also to biochemical markers of bone procollagen propeptides (PINP) and bone-specific alkaline phosphates (bAP). In obese individuals these cancers can be more frequent plus more aggressive resulting in reduced survival. Considered one of one of the most prominent and well characterized cancers in this connection is breast cancers. Obesity plays an intricate role in cancer of

the breast and it is related to increased inflammation, angiogenesis and alterations in serum numbers of potential growth factors for instance insulin, adiponectin, leptin and estrogen. Reduced degrees of serum adiponectin happen to be reported in cancer of the breast patients as compared to healthy controls, specifically in postmenopausal as well as the level of adiponectin can be inversely related to insulin resistance. The role of serum leptin levels in breast cancers is apparently more advanced. Some reports have shown leptin to be increased in females with cancer of the breast but other reports have found leptin for being decreased or unchanged. This is because of a amount of confounding issues.

Grossmann et al., (2012) proposed that it could be function as the numbers of adiponectin and leptin along with the balance of adiponectin and leptin which are the critical factors in breast as well as other obesity related cancer tumorigenesis. Moreover, focused on around the current comprehension of the interplay between obesity and also the functions of leptin and adiponectin. It is going to then examine what's known about their potential roles in cancer particularly as concerns breast cancers you bet the number of adiponectin to leptin may be the cause in tumorigenesis. Thus leptin can be a predictor for BMD in the population that is at risk of employ a low BMD, and this also relationship is in addition to the effect of body weight on leptin levels.

Jen et al., (2005) demonstrated that Blood leptin level has not been associated with breast cancers risk. Leptin levels were significantly higher in black women than in white females and were also significantly higher in obese and overweight women in comparison to normal-weight women. Black women weighed countless stood a higher body mass index (BMI) than white women.

Douchi et al., (2002) investigated the variations in leptin production between pre- and postmenopausal women, and concluded that menopausal status don't even have any significant affect leptin production. Osteoprotegerin (OPG) and leptin are crucial cellular factors in the damaging bone remodeling. The serum OPG in middle-aged Chinese women was significantly above that in middle-aged Austrian and Icelandic women; when

it comes to old-aged women. The values of serum leptin in Chinese women were significantly under those involved with white, black, and Mexican American women.

Wu et al., (2008) concluded that OPG and leptin in Chinese adult women. The serum of OPG and leptin differ with ethnicity. Leptin is a crucial hormone associated with regulating diet plan, energy expenditure and reproduction in mammals, nevertheless its role in acanthomorph fishes remains scant.

Shan et al., (2013) examined polymorphism of leptin gene in wild and cultivated populations of Chinese perch by direct sequencing of 120 fish. No SNP was discovered in leptin gene. Leptin mRNA of Chinese perch was highly expressed in liver, and expressed at lower levels in brain, visceral fatty tissue, intestine, spleen and muscle. Moreover, the initial gene structure, the conservation of both cysteines that form the single disulfide bridge in leptin, and stable clustering in phylogenetic analyses substantiate the unambiguous orthology of mammalian and fish leptins, despite low aminoalkanoic acid identity.

Prokop et al., (2012) demonstrated that the avian leptin sequences have much less variation inside the binding site than does the leptin receptor. This analysis further suggests that avian leptins are artifactual. Moreover, proposed that leptin A has a higher separation energy than does B. differing binding energies are proof either divergent functions, different binding confirmations, or other protein partners of leptin B. Leptin is a crucial hormone for that damaging food consumption, energy expenditure and reproduction in mammals, but information regarding its role in teleosts remains scant.

Li et al., (2010) suggested that leptin has an acute relation to the regulating food intake, energy expenditure and lipid metabolism in grass carp, however the effect can result in rapidly counteracted through mechanisms that are currently unknown. Leptin is really a peptide hormone most common for its role in feedback regulation of adiposity in eutherian mammals. Normally a rise in adipose tissue mass results in a rise in circulating

leptin which increases energy expenditure and limits food consumption, playing with hibernating eutherian mammals this relationship may plunge to allow prehibernatory fattening. The echidna (*Tachyglossus aculeatus*) is usually a monotreme mammal which accumulates significant fat reserves before entering hibernation, and mates immediately towards the end of hibernation.

Sprent et al., (2012) hypothesised that echidnas would show a robust relationship between body mass and plasma leptin for almost all of the year which may change through the pre-hibernatory period. Moreover, inferred a weak negative relationship between adiposity and plasma leptin as has been seen as in reptiles and birds, instead of the strong positive relationship found in other mammals. Effective immune responses are coordinated by interactions among the nervous, endocrine, and natural defenses. Mounting immune, inflammatory, and sickness responses requires substantial energetic investments, and thus, an organism may ought to balance energy allocation about bat roosting processes using the energetic demands of other competing physiological systems. The metabolic hormone leptin is apparently mediating trade-offs between the disease fighting capability and other physiological systems through its actions on immune cells plus the brain.

Elizabeth et al., (2012) discussed recent data in support of leptin as a mediator of seasonal sickness responses and still provide a theoretical model that outlines how seasonal cues, leptin, and proinflammatory cytokines may interact to coordinate seasonal immune and sickness responses. As much as, reviewed the data in mammalian and non-mammalian vertebrates that suggests leptin is linked to regulating immune responses, inflammation, and sickness behaviors. Leptin is implicated inside regulation of seasonal immune responses, including sickness; however, the precise physiological mechanisms remain unclear. Leptin plays a huge role in the regulation of extra fat homeostasis, and potential associations of leptin receptor gene (LEPR) polymorphisms with obesity happen to be suggested. Obesity is regarded as to correspond with cancers of the breast.

Woo et al., (2005) indicated that it's difficult to clarify breast cancers on the basis of serum leptin concentrations or polymorphisms inside LEPR gene. leptin's action, beyond the management of appetite, to several sites of metabolic regulation.

Fajardo et al., (2004) studied its production in subcutaneous and visceral fat compartments pre and post menopause and indicated that in the compartments, leptin production is sensitive to different but overlapping stimuli, conveying information about energy availability to central and peripheral sites under different conditions of estrogen exposure. Because the cloning of leptin by Friedman's laboratory in 1994, over 3000 papers are published on leptin, turning it into essentially the most active research areas altogether of science. Leptin definitely seems to be a pleiotrophic hormone affecting numerous tissues in the body.

Brann et al., (2002) centered on the role of leptin in reproduction. Evidence is accumulating that leptin potentially has roles inside damaging GnRH and LH secretion, puberty, pregnancy, and lactation. Moreover, Signaling pathways utilized by leptin are beginning for being clearer, particularly JAK/STAT, MAPK, and SOCS3 are implicated as mediators/modulators of leptin effects at the cellular level. For the hypothalamic level, there is possibly evidence that CART (cocaine and amphetamine-related transcript) is involved to be a downstream mediator of leptin effects, especially with regards to overpower of GnRH secretion. Since its discovery in 1994, leptin, a protein hormone synthesized and secreted by adipose tissue, can regulate feed intake in lots of species including sheep and pigs. Although a nimiety expertise exists with regards to the physiological role of leptin in rodents and humans, the regulation and action of leptin in domestic animals is less certain. Emerging evidence in a number of species points too leptin might also affect the hypothalamo-pituitary-gonadal axis.

Spicer, (2002) presented showed that leptin receptors are functional, since leptin inhibits insulin-induced steroidogenesis of both granulosa and thecal cells of cattle in vitro. Leptin receptor mRNA can also be based in the testes of rodents. Similar to the ovary, these receptors are functional, no less than in rats, since leptin inhibits HCG-induced

testosterone secretion by Leydig cells *ex vivo*. While pregnant, placental output of leptin may be a major cause of the increase in maternal leptin in primates although not rodents. However, in both primates and rodents, leptin receptors exist in placental tissues and may regulate metabolism of the fetal-placental unit. As specific leptin immunoassays are developed for domestic animals, *in vivo* associations will then be made among leptin, body energy stores, dietary energy intake and reproductive function. Serum leptin concentration is owned by age, fat and bone mineral density (BMD), high are ethnic variations in physique and BMD values. The relationship between serum leptin concentration and BMD in Chinese women is presently unknown.

Zhong et al., (2005) examined their bond of serum leptin concentration as they grow old, bodyweight, BMI and BMD in mainland Chinese women. (Zhong, 2005) concluded that serum leptin concentration was not correlated with height. Serum leptin concentration was significantly higher in postmenopausal than premenopausal women ($p < 0.001$). Serum leptin concentration was correlated with Lat BMD ($R^2 = 0.012$, $p < 0.018$), FN BMD ($R^2 = 0.013$, $p < 0.004$) from the whole population.

Markowska et al., (2003) stated that the relations in other cancers of genital organs (ovary, cervix, vulva) along with endometriosis require some further studies. It is known that the circulating levels of leptin, the adipocyte hormone implicated in the control of energy balance, are correlated with fat body mass (FBM), even though the influences of other physiological the weather is not fully understood.

Carraro et al., (2006) figured that the positive relationship of leptin with insulin secretion and also the negative one with testosterone, indicate direct implications of leptin in insulin signaling, along with male sexual development. Finally, the truth that how much secreted insulin is dependent upon LBM plus the latter on testosterone and IGF-1, indicates the need for muscle tissue inside control over insulin secretion. Mammary fat is an important source of paracrine mitogens and anti-mitogens, including insulin-like growth factor, transforming growth factors, and cytokines (especially, $TNF\alpha$ and $IL-1\beta$). Nevertheless, additionally it is a crucial way to obtain the adipocytokine, leptin. Recently,

leptin was reported to stimulate the proliferation of various cell types (pancreatic β cells, prostate, colorectal, lung, etc.) to be a new growth factor. It was also shown to stimulate the proliferation of breast cancer cell lines.

Caldefie-Che'zet et al., (2005) conducted an immunohistochemical analysis of leptin expression in normal tissue and benign and malignant ductal breast cell, representing the different states on the invasion process as much as, determined to the first time that leptin is expressed both by ductal breast tumors through benign lesions as atypical hyperplasia. Moreover, postulated that leptin can be a prognostic or diagnostic factor for ductal cancer of the breast.

Chen et al., (2006) analyzed the correlations involving the serum levels of adiponectin and leptin plus the various clinicopathological parameters in 100 newly diagnosed, histologically confirmed breast cancer patients and 100 controls. Moreover, (Chen,2006) suggested that low serum adiponectin levels and high serum leptin levels are associated with a higher risk for breast cancer. Also, in addition to the effect of BMI, the raised serum ratio of L/A may indicate a good aggressive breast cancers.

2.3 Summary

In this chapter, an overview of the related research is introduced and different research on leptin and its effects.

MATERIALS AND METHODS

3.1 Overview

This study examined patients who attended the clinic of the gynecology department Near East Hospital in the Turkish Republic of Northern Cyprus between January 2014 and June 2014. The study included 85 subjects, and these subjects consisted of two groups as 50 menopausal patients and 35 healthy subjects. The control group consisted of healthy patients without any history of menopausal stage. All subjects provided written informed consent before the study, and the study was approved by our Local Research Ethic Committee. General health characteristics such as age, sex, smoking status, and alcohol consumption were investigated by self-administered questionnaire.

The hip (cm), weight (kg) and waist circumference (cm) of each subject were recorded and body mass index (BMI) was calculated (kg/m^2).

Blood samples were drawn from the ante-cubital vein, after overnight fasting and centrifuged at 4000 RPM for ten minutes and separated. The serum samples were stored at -80°C until they were analyzed for leptin.

3.2 General Laboratory Equipment

- Centrifuge
- Automated spectrophotometer
- Automated chemistry analyzer
- Immunoassay analyzer
- Hot plate with stirrer
- Vortex
- Sensitive Electronic balance

- Refrigerator
- Beakers 100 ml, 250 ml
- Dark bottle 250 ml
- Volumetric Flask 100 ml
- Micropipettes(50-200) μ l,(100-1000) μ l

3.3 Disposable Laboratory Equipment

- Absorbent paper
- Test tubes
- Distilled water
- Gloves
- Para film
- Plain tubes
- Syringes, 10 ml
- Antagonistic Liquid

3.4 Laboratory Analyses

The levels of serum glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined using a fully automated clinical chemistry analyzer (Abott Architect C8000). Leptin levels were measured by ELISA method.

3.5 Leptin Determination

Diagnosis-related group(DRG)Leptin (Sandwich) ELISA. The DRG Leptin ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of Leptin in serum and plasma.

The DRG Leptin ELISA Kit is a solid phase enzyme –linked immunosorbentassay (ELISA)based on the sandwich principle.

The Microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule.

An aliquot of patient sample containing endogenous Leptin is incubated in the coated well with a specific biotinylated monoclonal anti Leptin antibody. A sandwich complex is formed. After incubation the unbound material is washed off and a streptavidin peroxidase Enzyme Complex is added for detection of the bound Leptin.

Having added the substrate solution,the intensity of color developed is proportional to the concentration of Leptin in the patient sample.

3.6 Reagents

3.6.1 Reagents provided

- Microtiter wells, 12×8 (break apart) strips, 96 wells;
Wells coated with anti-Leptin antibody (monoclonal).
- Standard (Standard 0.5), 6 vials, (lyophilized), 0.5 ml; concentration; 0-2-5-25-50-100 ng/ml. Contain non-mercury preservative.
- Control (Low & High), 2 vials, (lyophilized),0.5 ml; for control values and ranges it must refer to vial label or QC-Datasheet. Contain non-mercury preservative.

- Assay buffer, 1 vial, 11 ml, ready to use, Contain non-mercury preservative.
- Antiserum, 1 vial, 11 ml, ready to use, monoclonal biotinylated anti-Leptin antibody; contain non-mercury preservative.
- Enzyme Complex, 1 vial, 11 ml, ready to use, Streptavidin conjugated to horseradish peroxidase; contain non-mercury preservative.
- Substrate Solution, 1 vial, 14 ml, ready to use, Tetramethylbenzidine(TMB).
- Stop Solution, 1 vial, 14 ml, ready to use, contains 0.5M H₂ SO₄, Avoid contact with stop solution. It may cause skin irritations and burns.
- Wash solution, 1 vial, 30 ml(40X concentrated).

3.6.2 Materials required but not provided

- A microtiter plate calibrated reader ($450 \pm 10\text{nm}$) (e.g. the DRG instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

3.6.3 Storage Conditions

When stored at 2°C – 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2°C – 8°C. Microtiter wells must be stored at 2°C – 8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

3.7 Specimen Collection and Preparation

Serum or plasma can be used in this assay. Do not use haemolytic, icteric or lipaemic specimens.

3.7.1 Specimen Collection

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma: Whole blood should be collected into centrifuge tubes containing anti-coagulant and centrifuged immediately after collection.

3.7.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 24 hours at 2°C – 8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

3.7.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with standard 0 and reassayed as described in Assay Procedure.

3.8 Quality Control

The control and the corresponding results of the Quality Control (QC) laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

3.9 Assay Procedure

3.9.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As general rule the enzymatic reaction is linearly proportional to time and temperature.

3.9.2 Test Procedure

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense 15 μL of each Standard, controls and samples with new disposable tips into appropriate wells.
3. Dispense 100 μL Assay Buffer into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for 120 minutes at room temperature (without covering the plate).
5. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (300 μL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
6. Add 100 μL Antiserum to each well.
7. Incubate for 30 minutes at room temperature.
8. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (300 μL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

9. Dispense 100 μ L Enzyme Complex into each well.
10. Incubate for 30 minutes at room temperature.
11. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (300 μ L per well). Strike the wells sharply on absorbent paper to remove residual droplets.
12. Add 100 μ L of Substrate Solution to each well.
13. Incubate for 15 minutes at room temperature.
14. Stop the Enzymatic reaction by adding 50 μ L of Stop Solution to each well.
15. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

RESULTS

The aim of the study was to compare the anthropometric parameters in both menopausal and control groups. Also, fasting triglycerides, cholesterol, HDL and LDL cholesterol values were also compared. The leptin levels were studied for both groups.

Glucose, triglyceride, cholesterol, HDL and LDL cholesterol were significantly higher in the menopausal group when compared to the control group ($P < 0.001$). Waist, BMI and hip measurements were also found significantly higher in the patient group than the control group ($P < 0.001$). As for leptin, it was also significantly higher in the patient group when compared to the control group ($P < 0.001$).

Descriptive analysis of metabolic characteristics of the populations are presented in Table 4.1.

Table 4.1: Comparison of anthropometric and metabolic values in menopausal and control group.

	Control (n = 35)	Menopausal (n = 50)	P
Age	40.12 \pm 3.46	58.16 \pm 2.73	0.13
BMI (kg/m ²)	21.13 \pm 1.86	25.27 \pm 2.31	< 0.001
Waist (cm)	89.15 \pm 7.05	120.12 \pm 8.75	< 0.001
Hip (cm)	92.18 \pm 9.25	130.13 \pm 9.32	< 0.001
Glucose (mg/dl)	89.15 \pm 6.25	105.14 \pm 7.28	< 0.001
Cholesterol (mg/dl)	190.22 \pm 22.85	240.13 \pm 32.25	< 0.001
HDL-Cholesterol (mg/dl)	54.23 \pm 4.25	32.12 \pm 3.25	< 0.001
LDL-Cholesterol (mg/dl)	117.52 \pm 22.56	150.15 \pm 55	< 0.001
Triglyceride (mg/dl)	89.83 \pm 42.74	158.38 \pm 22.5	< 0.001
Leptin (ng/ml)	5.56 \pm 0.86	10.03 \pm 1.08	< 0.001

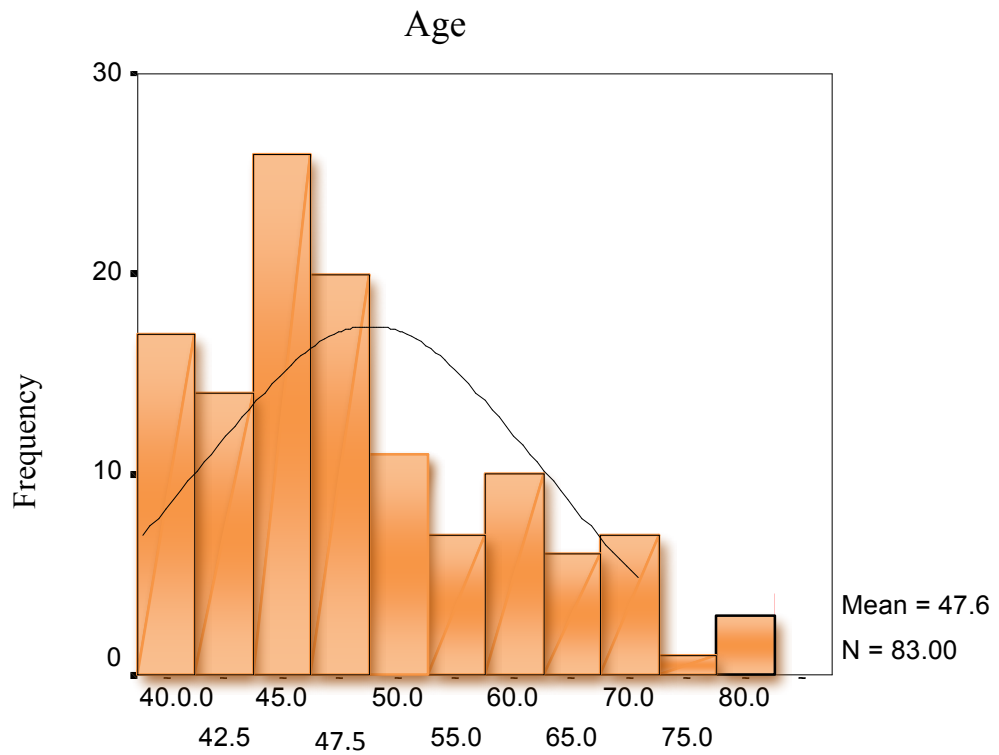


Figure 4.1: Histogram of age

The mean of the menopausal group was 58.16 ± 2.73 and that of the control group was 40.12 ± 3.46 .

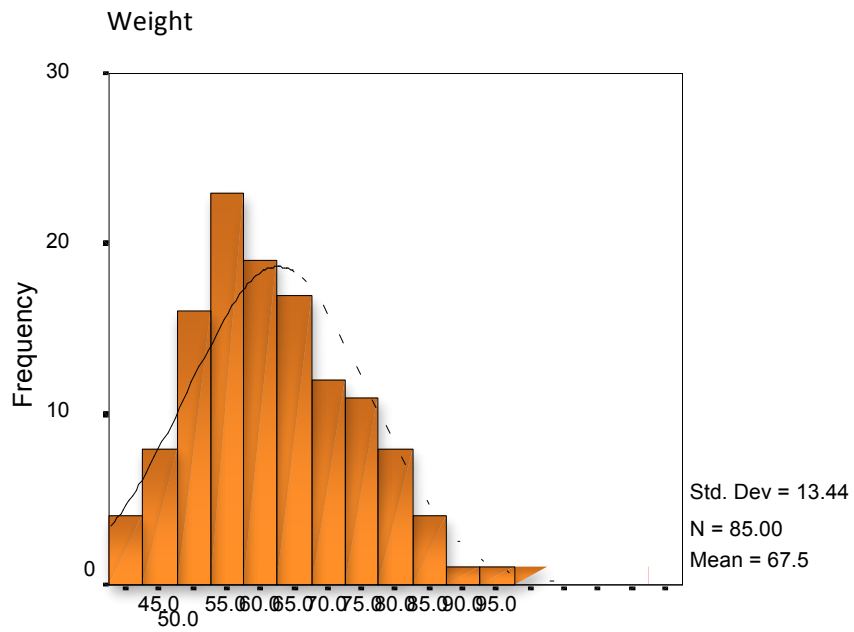


Figure 4.2: Histogram of weight

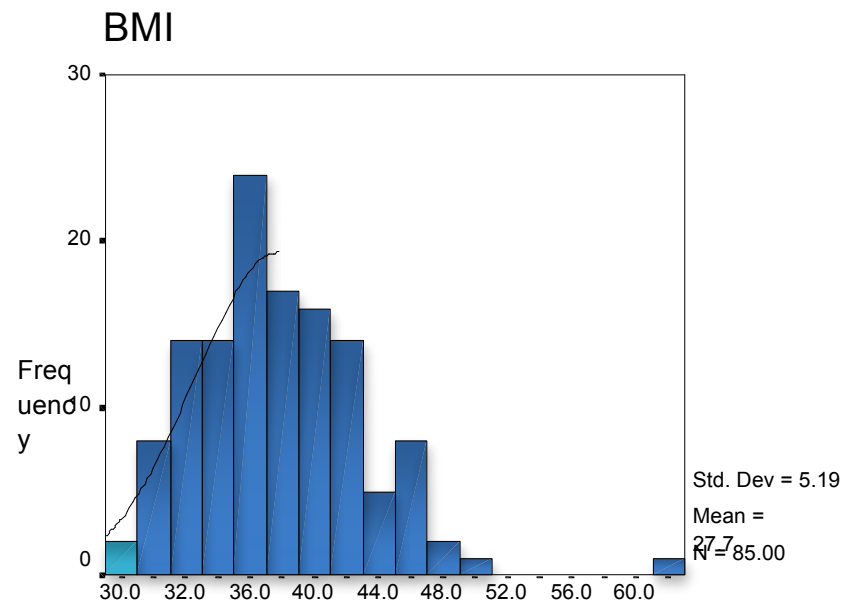


Figure 4.3: Distribution histogram of BMI

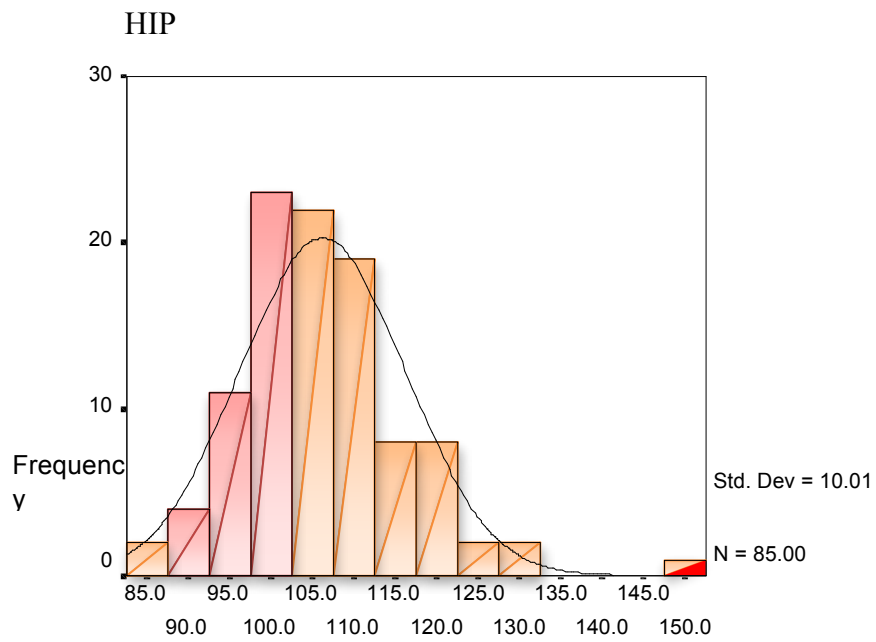


Figure 4.4: Hip study

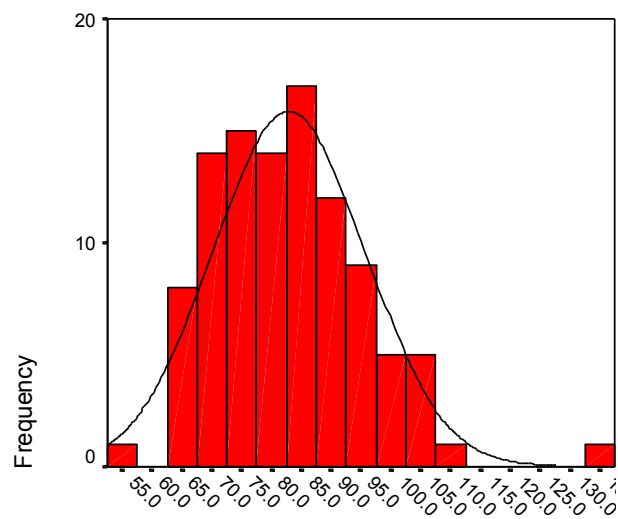


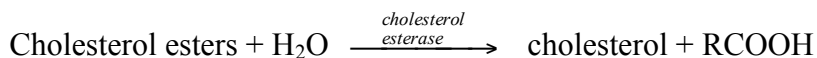
Figure 4.5: Waist diagram in cm

4.1 Cholesterol Analysis

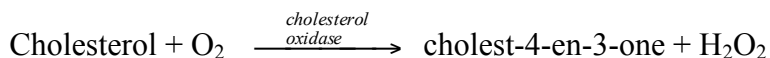
Principle of the Procedure

Enzymatic calorimetric test: Cholesterol was determined enzymatically using cholesterol esterase and cholesterol oxidase.

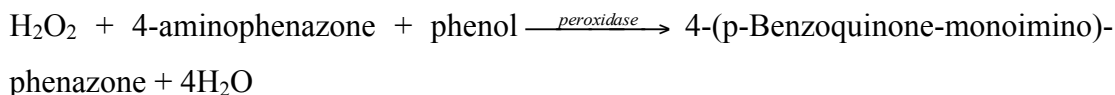
Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids.



Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.



Hydrogen peroxide created forms a red dyestuff by reacting with 4-aminophenazone and phenol under catalytic action of Peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.



The 4-(p-Benzoquinone-monoimino)-phenazone is a colored reagent and was measured spectrophotometrically.

Assay Procedure

100µl of sample and controls were placed in the required cups and arranged in the specified places in the instrument and the instrument was calibrated and the setup of the assay was done. The results were recorded.

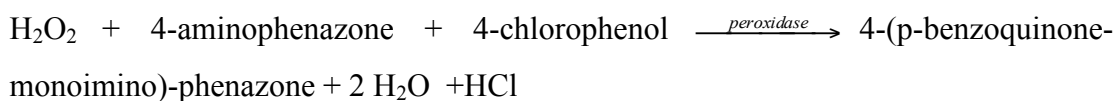
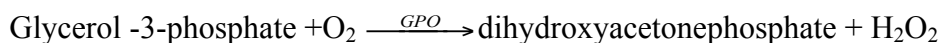
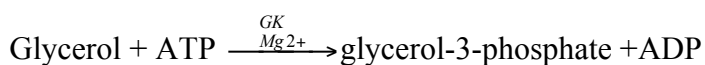
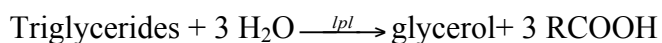
Quality control:

The within batch and between batch coefficient of variation were calculated for cholesterol assay and were 3.03 % and 0.97 % respectively.

4.2 Triglyceride Analysis

Principle of the Procedure

Triglycerides were estimated using an enzymatic colorimetric method, which contained lipase, glucokinase, glycerol-3-phosphate oxidase, and peroxidase. The following reactions occurred upon addition of different reagents:



The 4-(p-Benzoquinone-monoimino)-phenazone is a colored reagent and was measured spectrophotometrically.

Assay Procedure

100µl of samples and controls were placed in the required cups and arranged in the specified places in the instrument and the instrument was calibrated and the setup of the assay was done. The results were recorded.

Quality control:

The within batch and between batch coefficient of variation were calculated for triglycerides assay and were 4.26 % and 0.0% respectively.

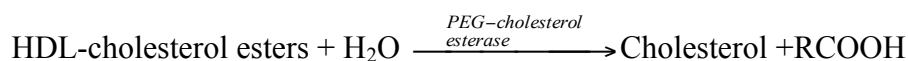
4.3 HDL-Cholesterol Analysis

Principle of the Procedure

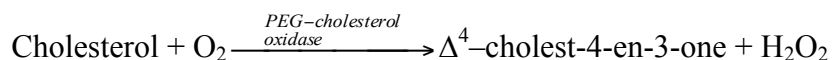
High-density lipoproteins were estimated using a homogeneous enzymatic colorimetric method. Upon mixing the sample with R1 (sulfated α -cyclodextrin/buffer) in the presence of slightly alkaline buffer and magnesium sulfate, sulfated α -cyclodextrin and dextrin sulfate selectively form water soluble complexes with LDL, VLDL, and chylomicrons which are resistant to PEG-modified enzymes.

Upon addition of R2 (PEG-modified enzymes/4-aminophenazone/buffer) and start of reaction: The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to amino groups (approx. 40%)

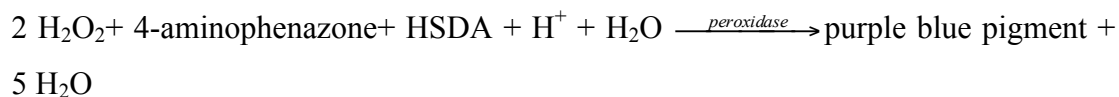
Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.



In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4 -cholestenone and hydrogen peroxide.



In the presence of Peroxidase, the hydrogen peroxide generated reacts with 4-aminophenazone and N- (2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) to form a purple blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.



Assay Procedure

100µl of samples and controls were placed in the required cups and arranged in the specified places in the instrument and the instrument was calibrated and the setup of the assay was done. The results were recorded.

Quality control:

The within batch and between batch coefficient of variation were calculated for HDL-Cholesterol assay and were 2.61 % and 1.21% respectively.

LDL-C estimation

Low-density lipoproteins were estimated using Friedwald formula, which required total cholesterol, triglycerides, and HDL cholesterol

LDL-cholesterol (mmol/l) = total cholesterol – (HDL-cholesterol + TG/2.2) (*Rifai et al., 2003*).

Leptin level in the patients and control groups was checked and the obtained results are tabulated as shown in Table 4.2 and Figure 4.9.

Table 4.2: Leptin value compared in patients and control groups

	Patients (n = 50)	Control (n = 35)	P Value
Leptin (ng/ml)	10.03 \pm 1.08	5.56 \pm 0.86	P < 0.001

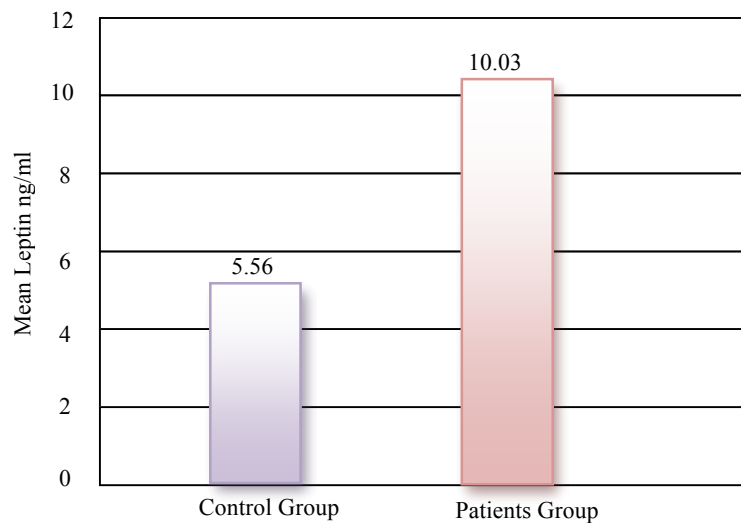


Figure 4.6: Leptin Levels

Leptin levels were found to be significantly higher in the menopausal group when compared to the control group.

Discussion

Menopausal status is related with weight gain, abnormal lipid and glucose metabolism. It is well known that, in menopause as a result of estrogen depletion weight gain is a problem. Also a consequence of the weight gain. The amount of the adipose tissue increases. The circulating leptin levels are directly proportional to the amount of adipose tissue. This is the first study ever done in Cyprus about menopause and its relationship with leptin.

Women at menopause often experience weight gain, particularly around abdomen due to declining estrogen levels, age-related loss of muscle tissue and lifestyle factors such as diet and lack of exercise. As for the studies carried out before BMI were all found to be significantly higher in the menopausal women which are in consistence with our results. As a result of fat deposits in the body after menopause, also the blood lipid profile changes. While total cholesterol, LDL-cholesterol and glucose increases, the HDL-cholesterol synthesis decreases in the menopause group as a result of the cholesterol metabolism which is distorted. Also the HDL- cholesterol decreases with the same reason ($P < 0.001$). (Gower et al. 2003)

Triglyceride levels were observed to increase in menopause in this study, which is in consistent with some previous studies. Although, there are some studies showing no changes with the triglyceride values, it is well shown in this study that triglyceride increases significantly in menopause ($P < 0.001$). (Derby et al. 2009)

Leptin plays an important role in body weight management, as it contributes to appetite control. In this prospective study, serum leptin levels in the menopausal group were found to be significantly higher than the control group ($P < 0.001$). Leptin levels are positively correlated to indices of body fat, namely total fat mass, percent body fat and BMI. There are several studies which are carried out in order to evaluate the levels of leptin in menopause. Although the results are contradictory. There are many studies which imply the increase in leptin in menopause and which is in consistence with our results. Menopause results in fat deposition, which in turn increases serum leptin levels, due to both insulin and leptin resistance. (Darel et al 2002, Prokop et al 2012)

Serum leptin levels are reported to be higher in women than in men. The gender difference has been explained partly by variable degree and distribution of the amount of body fat depots. Women tend to have a higher overall obesity which is more pronounced in subcutaneous fat than in visceral fat; together with menopause, women are expected to have high values of leptin.

CONCLUSION

The effects of menopause on leptin levels, glucose, and lipid profile and also the anthropometric parameters were investigated in this study and it can be concluded that leptin levels increase in menopause significantly and in order to understand the effects of menopause on women's health in accordance with leptin and also other adipokins in more details, further research needs to be done with a larger cohort.

References

All about Cholesterol, (2009), American Diabetes Association 1–800–DIABETES (342–2383), www.diabetes.org ©2009 by the American Diabetes Association, Inc. 11/09.

Bonen, G. Dohm, L., and Luc J.C. van Loon, (2006). Lipid metabolism, exercise and insulin action, 2006 *The Biochemical Society, Essays in Biochemistry* volume 42 2006.

Barbara C., and Nedergaard, (2004). Brown Adipose Tissue: Function and Physiological Significance, *Physiol Rev*, 84: 277–359, 2004; 10.1152/physrev.00015.2003, www.prv.org 2770031-9333/04 Copyright © 2004 the American Physiological Society.

Barbara L P, (2010). Optimal management of perimenopausal Depression, *International Journal of Women's Health, Department of Psychiatry*, University of California, San Diego, USA, and 2010:2 143–151.

Bret H., G., Jing H., Simon W., and David E. K., (2001). Skeletal Muscle Lipid Content and Insulin Resistance: Evidence for a Paradox in Endurance-Trained Athletes, *the Journal of Clinical Endocrinology & Metabolism* 86(12):5755–5761, Copyright © 2001 by the Endocrine Society.

Da-Chung C., Yueh-Fang C., Yao-Tsug Y., Hso-Chi C., Fu-Chen K., Ou-Yang F., Hue-Yong C., Ming-Feng H., Shyng-Shou F. Y., (2006). Serum adiponectin and leptin levels in Taiwanese breast cancer patients, *Cancer Letters* 237 (2006) 109–114, 0304-3835/\$ - see front matter q 2005 *Elsevier Ireland Ltd.* All rights reserved. doi:10.1016/j.canlet.2005.05.047.

Darrell W. B., Marlene F. W., Krishnan M. D., Virendra B. M., Clint D. B., (2002), Leptin and reproduction, *Steroids* 67 (2002) 95–104, 0039-128X/02/\$ – see front matter © 2002 *Elsevier Science Inc.* All rights reserved. PII: S0039-128X (01)00138-6.

Derby CA, Crawford SL, Pasternak RC, Sowers M, Sternfeld B, Matthews KA. (2009). Lipid changes during the menopause transition in relation to age and weight: the Study of Women's Health across the Nation. *American Journal of Epidemiology*. Oxford Journals. doi: 10.1093/aje/kwp043

Dr P. P., Nishida C., Porter, (2003). Obesity And Overweight, World Health Organization, Global Strategy On Diet, Physical Activity And Health, Who 2003.

Elena V. K., M.D., Ph.D.; Margaret D. C., M.S.P.H.; Kate M. S., M.S.; and Rosemarie H., M.D., M.P.H., (2013). Trends in High LDL Cholesterol, Cholesterol-lowering Medication Use, and Dietary Saturated-fat Intake: United States, 1976–2010, NCHS Data Brief No. 117, March 2013.

Elizabeth D. C., Gregory E. D., Susannah S. F., (2012). Leptin, a neuroendocrine mediator of immune responses, inflammation, and sickness behaviors, Contents lists available at SciVerseScienceDirect, *Hormones and Behavior*, journal homepage: *Hormones and Behavior* 62 (2012) 272–279.

F. Caldefie-Che'zet, M. D., M. de L., G. K., Mishellani F., C. Fusillier, M. Guerry, F. Penault-Llorca, J. Guillot, M.-P. Vasson, (2005). Leptin: A proliferative factor for breast cancer? Study on human ductal carcinoma, *Biochemical and Biophysical Research Communications* 334 (2005) 737–741, 0006-291X/\$ - see front matter _ 2005 Elsevier Inc. All rights reserved. doi:10.1016/j.bbrc.2005.06.077.

Gower, B. A., Nagy, T. R., Goran, M. I., Smith, A., and Kent, E. (2000), Leptin in Postmenopausal Women: Influence of Hormone Therapy, Insulin, and Fat Distribution, 0021-972X/00, Vol. 85, No. 5, *The Journal of Clinical Endocrinology & Metabolism* Printed in U.S.A., Copyright © 2000 by The Endocrine Society.8 jcem.endojournals.org.

Guan-Gui Li, Xu-Fang Liang, QiulingXie, Guangzhao Li, Ying Yu, Kaaseng Lai, (2010), Gene structure, recombinant expression and functional characterization of grass carp leptin, Contents lists available at ScienceDirect. General and Comparative Endocrinology, journal homepage: General and Comparative Endocrinology 166 (2010) 117–127.

Hamid Javaid Qureshi, Ghulam Hussain, Zafar AltafJafary, Muhammad Usman Bashir, NaghmanaLatif, ZeeshanRiaz, (2010), Calcium Status In Premenopausal And Postmenopausal Women, J Ayub Med Coll Abbottabad 2010;22(2),

H. J. Mersminn, J. R. Goodman, and L. J. Brown, (2014), Development of swine adipose tissue: morphology and chemical composition Journal of Lipid Research Volume 16, 1975 by guest, on April 22, 2014.

Hee-Yeon Woo, Hyosoon Park, Chang-Seok Ki, Yong Lai Park, Won Gil Bae, (2005), Relationships among serum leptin, leptin receptor gene polymorphisms, and breast cancer in Korea, 0304-3835/\$ - see front matter q 2005 Elsevier Ireland Ltd. All rights reserved. doi:10.1016/j.canlet.2005.05.041.

Hiroaki Okazaki, Jun-ichiOsuga,Yoshiaki Tamura, Naoya Yahagi, Sachiko Tomita, FutoshiShionoiri, Yoko Iizuka,Ken Ohashi, Kenji Harada, Satoshi Kimura, TakanariGotoda, Hitoshi Shimano, Nobuhiro Yamada, and Shun Ishibashi, (2002), Lipolysis in the Absence of Hormone-Sensitive Lipase Evidence for a Common Mechanism Regulating Distinct Lipases, DIABETES, VOL. 51, DECEMBER 2002.

HuangLu, CaiLi, (2000), **Leptin: a multifunctional hormone**, The University of Texas South western Medical Center 5323H. Hines Boulevard Dallas, TX75390-8854,USA, Cell Research(2000), 10,81-92.

Jenny Sprent, Susan M. J., Stewart C N., (2012). Does leptin signal adiposity in the egg-laying mammal, *Tachyglossus aculeatus*?, Contents lists available at Sci Verse Science Direct, General and Comparative Endocrinology, journal homepage: General and Comparative Endocrinology 178 (2012) 372–379.

Jeong C. R., Seob S., Sang H. K., Sung K. Kang, B.Cheol K., Hang Y. L., Youn J. K., Jung Y. Jo, E.Ji Y., Hyung J. C., and Euna K., (2010). Safety of Intravenous Infusion of Human Adipose Tissue-Derived Mesenchymal Stem Cells in Animals and Humans, Original Research Reports, Stem Cells and Development, Volume 20, Number 8, 011_ Mary Ann Liebert, Inc. DOI: 10.1089/scd.2010.0466.

J P Després, S Moorjani, M Ferland, A Tremblay, P J Lupien, A Nadeau, S Pinault, G Thériault, and C Bouchard, (2014). Adipose tissue distribution and plasma lipoprotein levels in obese women. Importance of intra-abdominal fat, ARTERIOSCLEROSIS VOL 9, No 2, MARCH/APRIL 1989, 2, 2014.

John K. Scariano, Philip J. Garry, George D. Montoya, Ali K. Chandani, Janice M. Wilson, Richard N. Baumgartner, (2003), serum leptin levels, bone mineral density and osteoblast alkaline phosphatase activity in elderly men and women, Mechanisms of Ageing and Development 124 (2003) 281_ 286,

Jonas Burén, (2012), Glucose and lipid metabolism in insulin resistance – an experimental study in fat cells, Umeå University Medical Dissertations, New Series No 817 * ISSN 0346-6612 * ISBN 91-7305-359-7, 2012.

J.W. Prokop, R.J. Duff, H.C. Ball, D.L. Copeland, R.L. Londraville, (2012), Leptin and leptin receptor: Analysis of a structure to function relationship in interaction and evolution from humans to fish, Contents lists available at Sci Verse Science Direct, Peptides journal home page : Peptides 38 (2012) 326–336.

Kawaljit Kaur Khokhar, Gurcharan Kaur, and Sharda Sidhu, (2010), Prevalence of Obesity in Working Premenopausal and Postmenopausal Women of Jalandhar District, Punjab, *J Hum Ecol*, 29(1): 57-62 (2010).

K.-L. Catherine Jen, Anne Buison, Linda Darga, and Dorothy Nelson, (2005), The relationship between blood leptin level and bone density is specific to ethnicity and menopausal status, Volume 146, Number 1, © 2005 Mosby, Inc. All rights reserved. doi:10.1016/j.lab.2005.02.014.

Laura C. Schulz and Eric P. Widmaier, (2007).LEPTIN RECEPTORS, chapter 2, Department of Biology, Boston University, Boston, MA,.

Leon J. Spicer, (2001), Leptin: a possible metabolic signal affecting reproduction, *Domestic Animal Endocrinology* 21 (2001) 251–270, 0739-7240/01/\$ – see front matter © 2002 Elsevier Science Inc. All rights reserved. PII: S0739-7240(01)00120-5.

Markowska, Drews, Malendowicz, and Szenajch, (2003). The Role of Leptinin the Development of Tumoursof Female Genital Organs, *Praca w ramach grantu KBN pt.: Leptynaijejreceptory miesniakachmacicy.* Nr 3PO5A01622 nr.umowy 0371/P05/2002/22., *Rep. Pract. Oncol.Radiother.*8 (3) 2003.

M. A. Pothoven, D. C. Beitz and J. H. Thornton, (2013), Restricted-Fed Beef Cattle during Growth and Ad Libitum, The online version of this article, along with updated information and services, is located on the World Wide Web at:

Magda O Seixas, Larissa C Rocha, Mauricio B Carvalho, Joelma F Menezes, Valma ML Nascimento, Ricardo D Couto, Ájax M Atta, Mitermayer G Reis, Isa M Lyra, Marilda S Goncalves, (2010), Levels of high-density lipoprotein cholesterol (HDL-C) among children with steady-state sickle cell disease, Seixas et al. *Lipids in Health and Disease* 2010, 9:91

Margery L.S. Gass, MD, NCMP (Co-Chair); JoAnn E. Manson, MD, DrPH, NCMP (Co-Chair); Felicia Cosman, MD; Francine Grodstein, ScD; V. Craig Jordan, OBE, PhD, DSc, FMedSci; Richard H. Karas, MD, PhD; Andrew M. Kaunitz, MD; Pauline M. Maki, PhD; Peter J. Schmidt, MD; Jan L. Shifren, MD, NCMP; Cynthia A. Stuenkel, MD, NCMP; and Wulf H. Utian, MD, PhD, DSc(Med), NCMP., (2012), POSITION STATEMENT, The 2012 Hormone Therapy Position Statement of The North American Menopause Society, The Board of Trustees approved the position statement on January 15, 2012, *Menopause: The Journal of The North American Menopause Society* Vol. 19, No. 3, pp. 257/271, DOI: 10.1097/gme.0b013e31824b970a, 2012 by The North American Menopause Society.

Martin Petzel, (2007), Action of Leptin on Bone and Its Relationship To Menopause, *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2007, 151(2):195–199.

Martha E. Fajardo, Juan M. Malacara, Herminia G. Mart'inez-Rodr'iguez, Hugo A. Barrera-Saldaña, (2004), Hormone and metabolic factors associated with leptin mRNA expression in pre- and postmenopausal women, *Steroids* 69 (2004) 425–430, 0039-128X/\$ – see front matter © 2004 Elsevier Inc. All rights reserved. doi:10.1016/j.steroids.2004.03.013.

Masayo YAMADA, Toshiya MATSUZAKI, Takeshi IWASA, Fumi SHIMIZU, Naoko TANAKA, Rie OGATA, Machiko KIYOKAWA, Toshiyuki YASUI, Minoru IRAHARA and Toshihiro AONO, (2003), Serum Leptin Levels in Women throughout Life; Relationship to Body Mass Index and Serum Estradiol Levels, *pn J Reprod Endocrinol* @2003 by Japan Society of Reproductive Endocrinology, *Japanese Journal of Reproductive Endocrinology* (2003) 8 : 55-60.

Menopause, A Guide, July 2008, The Women's Health Council, W.www.whc.ie/menopause, W.www.healthpromotion.ie, W.www.thebms.org.uk, W.www.menopausematters.co.uk, W.www.aware.ie. Published by: The Women's Health Council and Health Service Executive Publication date: July 2008, Review date: July 2011, Order code: HPM00069.

Michael E. Grossmann, Margot P. Cleary, (2012), The balance between leptin and adiponectin in the control of carcinogenesis-Focus on mammary tumorigenesis, Contents lists available at SciVerseScienceDirect,Biochimie, journal homepage: Biochimie 94 (2012) 2164e2171.

Michael Ho, Susan Foxall, Michael Higginbottom, David M. Donofrio, Jinfang Liao, Peter J. Richardson, Yannick P. Maneuf, (2006), Leptin-mediated inhibition of the insulin-stimulated increase in fatty acid uptake in differentiated 3T3-L1 adipocytes, Metabolism Clinical and Experimental 55 (2006) 8 – 12.

Michael Rosenbaum, Margery Nicolson, Jules Hirsch, Steven B. Heymsfield, Dymrna Gallagher, Florence Chu, And Rudolph L. Leibel, (1996), Effects of Gender, Body Composition, and Menopause on Plasma Concentrations of Leptin, 0021.972x/96, Journal of Clinical Endocrinology and Metabolism, Copyright © 1996 by The Endocrine Society.

Michael T. McDermott MD, (2008), Cholesterol and Triglycerides, Division of Endocrinology, Metabolism and Diabetes University of Colorado Health Sciences Center Denver, Colorado 80262, 2008.

Naveed, Rajput & Mahmood, (2010). Effect of Variations in Body Mass Index on Serum Leptin Levels in Pre and Post-Menopausal Women, *Global Journal of Health Science*, Vol. 2, No. 1; April 2010.

Neil F. Goodman, MD, FACE; Rhoda H. Cobin, MD, MACE; Samara Beth Ginzburg, MD; Ira A. Katz, MD, FACE; Dwain E. Woode, MD, (2011), AACE Menopause Guidelines Revision Task Force, AACE Menopause Guidelines, Endocr, 2011, 17(Suppl 6).

Ni Zhong, Xian-Ping Wu, Zhang-Rong Xu, Ai-Hong Wang, Xiang-Hang Luo, Xing-Zhi Cao, Hui Xie, Peng-Fei Shan, Er-Yuan Liao, (2004), Relationship of serum leptin with age, body weight, body mass index, and bone mineral density in healthy mainland Chinese women, ClinicaChimicaActa 351 (2005) 161–168, 0009-8981/\$ - see front matter D 2004 Elsevier B.V. All rights reserved. doi:10.1016/j.cccn.2004.09.003.

NishatAyub, Sohail Rafi Khan, Fauzia Syed, (2006), Leptin Levels in Pre and Post-Menopausal Pakistani Women, Vol. 56, No. 1, January 2006.

N T Lam, A T Cheung, M J Riedel, P E Light, C I Cheeseman, and T J Kieffer, (2004), Leptin reduces glucose transport and cellular ATP levels in INS-1 β -cells, *ournal of Molecular Endocrinology* (2004) 32, 415–424, 0952–5041/04/032–415 © 2004 Society for Endocrinology *Printed in Great Britain*.

Patricia A. Zuk, Min Zhu, Peter Ashjian, Daniel A. De Ugarte, Jerry I. Huang, Hiroshi Mizuno, Zeni C. Alfonso, John K. Fraser, Prosper Benhaim, and Marc H. Hedrick, (2002), Human Adipose Tissue Is a Source of Multipotent Stem Cells, © 2002 by The American Society for Cell Biology , *Molecular Biology of the Cell*, Vol. 13, 4279–4295, December 2002.

Patricia Anne Rouen, (2009), Study of Women Veterans in Menopause, A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Michigan, 2009.

Paul Trayhum, Jacqueline S. Duncan, Nigel Hoggard and D. Vernon Rayner, (1998), Regulation of leptin production: a dominant role for the sympathetic nervous system?, *Proceedings of the Nutrition Society* (1998), 57,413419.

Peter J. Havel, (2000), Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance, *Proceedings of the Nutrition Society* (2000), 59, 359–371.

P Hadji, O Hars, K Bock, G Sturm, T Bauer, G Emons, and K-D Schulz, (2000), The influence of menopause and body mass index on serum leptin concentrations, *European Journal of Endocrinology* (2000) 143 55±60, ISSN 0804-4643.

PZ Zimmet, VR Collins, MP de Courten, AM Hodge, GR Collier, GK Dowse, KGMM Alberti, FHemraj, H Gareeboo, P Chitson, and D Fareed, (1998), Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean nation of Mauritius, *International Journal of Obesity* (1998) 22, 171±177.

R. Carraro, A. Ruiz-Torres, (2006), Relationship of serum leptin concentration with age, gender, and biomedical parameters in healthy, a non-obese subjects, *Archives of Gerontology and Geriatrics* 43 (2006) 301–312, 0167-4943/\$ – see front matter # 2005 Elsevier Ireland Ltd. All rights reserved. doi:10.1016/j.archger.2005.11.004.

Rachel C. Jankowitz, Kandace P. McGuire, Nancy E. Davidson, (2013), Optimal systemic therapy for premenopausal women with hormone receptor-positive breast cancer, journal homepage: *The Breast* 22 (2013) S165eS170.

Richard B Mazess and Howard S Barden, Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills, *Am J C/in Nuir* 1991;53:132-42. Printed in USA.© 1991 American Society for Clinical Nutrition.

Ríos-Lugo, María J.; Cano, Pilar; Jiménez-Ortega, Vanesa; Fernández-Mateos, María P.; Scacchi, Pablo A.; Cardinali, Daniel P.; Esquifino, Ana I., (2010), Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high-fat fed rats, Preprint del document opublicado en Journal of Pineal Research, 2010, 49, 342-348, *Publicado en: Journal of Pineal Research 49:342–348; 2010.*

S. F. Clarke and J. R. Foster, (2006), A history of blood glucose meters and their role in self-monitoring of diabetes mellitus, British Journal of Biomedic Al Science 2012 69.

Shams M, Homayouni K, Hamidi A, Sadegholvad A, Omrani GR, Leptin and Bone Mineral Density in Healthy Postmenopausal Iranian Women: A Populationbased Study, E-mail: hormone@sums.ac.ir , Int J EndocrinolMetab 2006; 4: 70 -77.

Shan He, Xu-Fang Liang, Ling Li, Wei Huang, Dan Shen, Ya-Xiong Tao, (2013), Gene structure and expression of leptin in Chinese perch, Contents lists available at Science Direct, General and Comparative Endocrinology, journal homepage: General and Comparative Endocrinology 194 (2013) 183–188.

Simon W. Beaven, Aleksey Matveyenko, Kevin Wroblewski, Lily Chao, Damien Wilpitz, Tu Wen Hsu, acob Lentz, Brian Drew, Andrea L. Hevener, and Peter Tontonoz, (2013), Reciprocal Regulation of Hepatic and Adipose Lipogenesis by Liver X Receptors in Obesity and Insulin Resistance, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA Correspondence: Cell Metabolism 18, 106–117, July 2, 2013^a2013 Elsevier Inc.

Steven M. Haffner, LeenaMykkanen, and Michael P. Stem, (1997), Leptin Concentrations in Women in the San Antonio Heart Study: Effect of Menopausal Status and Postmenopausal Hormone Replacement Therapy, American Journal of Ep Wemtokagy, Copyright @ 1997 by The Johns Hopkins University School of Hygiene and Public Health, All rights reserved,

SubrinaJesmin, AM Shahidul Islam, ShamimaAkter, MdMajedul Islam, SayeedaNusrat Sultana, Naoto Yamaguchi, Osamu Okazaki, Masao Moroi, Michiaki Hiroe, Sosuke Kimura,Kawano Saturo, and Taro Mizutani, Tetsu Watanabe, (2013), Metabolic syndrome among pre- and post-menopausal rural women in Bangladesh: result from a population-based study, Jesmin et al. BMC Research Notes 2013, 6:157.

Sukhbir.Singh,.MD,.Ottawa.ON,Carolyn.Best,.MD,.Toronto.ON,Sheila.Dunn,.MD,.Toronto.ON,Nicholas.Leyland,.MD,.Toronto.ON, Wendy.Lynn.Wolfman,.MD,.Toronto.ON, (2013), Abnormal Uterine Bleeding in Pre-Menopausal Women, , J.Obstet.Gynaecol.Can.2013;35(5.eSuppl):S1–S28, No. 292, May 2013 (Replaces No. 106, Aug 2001).

Terrence P. McGarty, (2010), Obesity And Type 2 Diabetes: Cause and Effects, Copyright © 2010, The Telmarc Group, All Rights Reserved, The Journal of Gabriellus : The World of The Seventh Century (2006) The Squirrel Tales (2010).

Tsutomu Douchi , Ichiro Iwamoto, Nobuyuki Yoshimitsu, ShoichiroKosha, Yukihiro Nagata, (2002), Leptin production in pre- and postmenopausal women, Maturitas 42 (2002) 219–223, 0378-5122/02/\$ - see front matter © 2002 Elsevier Science Ireland Ltd. All rights reserved. PII: S0378-5122(02)00078-6.

U.S. Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute, (2005), NIH Publication No. 05-5200, Originally Printed October 2002, Revised June 2005.

W H Howell, D J McNamara, M A Tosca, B T Smith, and J A Gaines, (1997), Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis, Copyright © 1997 by The American Society for Clinical Nutrition, Inc.

William P. Castelli, MD, Robert J. Garrison, MS; Peter W. F. Wilson, MD; (2009), Incidence of Coronary Heart Disease and Lipoprotein Cholesterol Levels, The Framingham Study, by guest on August 25, 2009.

Xi-Yu Wu, Xian-Ping Wu, Hui Xie, Hong Zhang, Xiang-Hang Luo, Shi-Ping Liu, Yi-Qun Peng, Ru-Chun Dai, Er-Yuan Liao, (2008), Relationship between age-related reference values of serum osteoprotegerin and leptin in native Chinese women and compared with those in women of other races, *Clinica Chimica Acta* 389 (2008) 72–78, Available online at, 0009-8981/\$ - see front matter © 2007 Elsevier B.V. All rights reserved. doi:10.1016/j.cca.2007.11.024.

T.R.N.C
NEAR EAST UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

ROLE OF LEPTIN IN MENOPAUSAL WOMEN

PSHTIWAN ABDULLAH YOUSIF

BIOCHEMISTRY PROGRAM

MASTER THESIS

NICOSIA2014

ROLE OF LEPTIN IN MENOPAUSAL WOMEN

**A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF APPLIED SCIENCES
OF
NEAR EAST UNIVERSITY**

by

Pshtiwan Abdullah Yousif

**In Partial Fulfillment of the Requirements for
the Degree of Master of Science
in
Biochemistry**

NICOSIA 2014

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : PSHTIWAN ABDULLAH YOUSIF

Signature :

Date:

To my parents and my wife...

T.R.N.C
NEAR EAST UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

ROLE OF LEPTIN IN MENOPAUSAL WOMEN

PSHTIWAN ABDULLAH YOUSIF

BIOCHEMISTRY PROGRAM
MASTER THESIS

ADVISOR
Prof. Dr. Güldal MEHMETÇİK

NICOSIA.2014