T.R.N.C

NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

ROLE OF ADIPONECTIN IN MENOPAUSAL WOMEN

MUHAMMAD IBRAR KHAN AFRIDI

BIOCHEMISTRY PROGRAM

MASTER THESIS

NICOSIA.2014

T.R.N.C

NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

ROLE OF ADIPONECTIN IN MENOPAUSAL WOMEN

MUHAMMAD IBRAR KHAN AFRIDI

BIOCHEMISTRY PROGRAM

MASTER THESIS

ADVISOR

Prof. Dr. Güldal MEHMETÇİK

NICOSIA.2014

AKNOWLEDGEMENT

More than something else, I offer my humble thanks to ALLAH Almighty for bestowing upon me and giving me the potential for the completion of this research work. My special praise to Prophet Muhammad (Peace be upon him) who is forever a source of guidance for the humanity as a whole.

My unreserved appreciation goes to my supervisor ***Prof. Dr. Güldal MEHMETÇİK*** for her guidance and encouraging behavior, you brought best in me. Thank you very much**.**Without her kind cooperation and guidance I would have not achieved this goalas her academic advice and kind guidance was available to me during all stages of the study.

I owe lots of thanks to Prof.Dr.Nihal Salmayenli,and my class fellows Rameez Hassan, Faraidun Barznji and PshtiwanAbdullh Shkake.

I strongly acknowledged and pay my deepest gratitude to my friends from Pakistan at KKTC specially Zahoor Ahmad, Imad Khan, Muhammad Tayyab, Abdul Aziz, Mazher Ali Shah, Abdul Latif, Shah Faisal, Mushtaq Ahmad, Anwar Saeed, Adnan Khan, Jamal Shah, Sana -Ul- allah and Shah wali.for their sincere cooperation and encouragement.

I wish to pay homage to my Fiancée and other family members.

In last but not the least I owed lots of thanks to my loving parents whom they always help, motivate and encourage me in each and every step of my life throughout my education career.

Muhammad Ibrar Khan AFRIDI

ABSTRACT

Muhammad. I. K.Afridi., Role of Adiponectin in Menopausal Women. Near East University North Cyprus.Institute of Health Science, Biochemistry program, Master Thesis, Nicosia, 2014.

Adipogenesis refers to the differentiation of pre-adipocytes into mature fat cells, i.e. the development of adipose tissue, which varies according to sex and age. Adipocytes differentiate from stellate or fusiform precursor cells of mesenchymal origin.Adiponectin has been postulated to act an important role in the modulation of glucose and lipid metabolism in insulin-sensitive tissue in both humans and animals. The transition from pre to post menopause is associated with the emergency of many features of metabolic state. The intra abdominal body fat increases, low density lipoprotein and triglyceride levels increase while high density lipoprotein decreases. As the results to date are conflicting, in our study we aimed to study the changes in adiponectin and anthropometric parameters after menopause.. A total of 70 female in menopause and 90 control subjects were included in this study. For this purpose the ELISA methods was used in the study to evaluate the values of adiponectin The results showed that adiponectin levels decreased while BMI and blood pressure increased with menopause and in order to investigate the effect of menopause on these parameters, further work must be carried out in the near future.

Key Words: Menopause, Adiponectin and Adipose tissue.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADP**:**	Adenosine di phosphate
ATP**:**	Adenosine tri phosphate
TNF**:**	Tumor necrosis factor
WAT:	White adipose tissue
BAT:	Brown adipose tissue
VLDL:	Very low level density lipoprotien
TAGs:	Triacyle glycerols
GLUT4:	Glucose transport type 4
GBP28:	Gelatin binding protien 28
HMW:	High molecular weight
LWM:	Low molecular weight
MMW:	Middle molecular weight
ER:	Endoplasmic reticulum
SREBP1c:	Sterol regulatory element binding protien 1c
PPARg:	Peroxisome proliferation-activated receptors
TZD:	Thiazolidinediones
ELISA:	Enzyme-linked immunosorbent assay
HFD:	High-fat diet
FSH:	Folicale stimulating harmones
E1:	Estrone
E2:	Estadiol
E3:	Estriol
LH:	Luteinizing hormone
DHEA:	Dehydroandrostenedion sulfate
CVD:	Cardiovascular disease
LDL:	Low density lipoprotein
HDL:	High density lipoprotein
BMI:	Body mass index
VLDL:	Very low density lipoprotein

VO2:	Maximal oxygen consumption
CT:	Computed tomography
TG:	Triglyceride
MATLAB:	Matrix Laboratory
Mbps:	Megabyte per second
CETP:	Cholestrol ester transfer protein
FFA:	Free fatty acid
PCOS:	Polycystic ovary syndrome
VAT:	Visceral adipose tissue
hs-CRP:	High sensitive C-reactive protien
HOMA-IR:	Homeostatic model assessment insulin resistence
OW:	Over weight
NW:	Normal weight
BMD:	Bone mineral density
SHBG:	Sex- harmone binding glubuline
MS :	Metabolic syndrome
TBBMC:	Total body bone mineral content
NGT:	Normal glucose tolerance
IGT:	Impaired glucose tolerance
OGTT:	Oral glucose tolerance test
OX:	Oxidized
HRP:	Horseradish peroxidase
PAY:	Physical
TMB:	Tetramethyle benzidine
GDM:	Gestational diabets mellitus
TRNC:	Turkish Republic of Northern Cyprus

1. Introduction

Adipocytes are the cells that mainly compose adipose tissue, specific in storing energy as fat. There are two sorts of adipocytes, brown and white, which vary in numerous important properties. White adipose tissue is important for maintaining energy metabolism of the organism by storing excess energy as lipid. Adipose tissue is made up from a mixture of distinctive cells. The most conspicuous part is experienced adipocytes which store and discharge lipids in light of circling hormones. White adipocyte cells from distinctive areas can have different molecular and physiological properties, for example, expanded instinctive adipose tissue is connected with an expanded risk of insulin resistance and cardiovascular disease, Furthermore adipocyte size has been interfaced to an expanded risk of metabolic problems, for example, Type 2 diabetes or cardiovascular issue. Recent research has demonstrated that adipose tissue is not just an inactive storage station for lipids yet is also an important endocrine organ that plays a key part in the integration of endocrine, metabolic, and inflammatory signals for the control of energy homeostasis. The adipocyte has been indicated to secrete a variety of bioactive proteins into the circulation. These secretory proteins, which have been all in all named adipocytokines , incorporate leptin , tumor necrosis factor (TNF)-a , plasminogen-activator inhibitor type 1 (PAI-1) , adipsin , resistin and adiponectin(manju et al., 2003).

1. Adipose tissue

Originally considered as simply a storage organ for triacylglycerol, interest in the biology of adipose tissue has increased substantially. Over the last decades there has been considerable accumulation of experimental data about the biology and biochemistry of adipose tissue. This tissue is no longer considered to be an inert tissue that just stores fat (Ottaviani E et al,.2011). Adipose tissue is a metabolically dynamic organ that is the primary site of storage for excess energy but it serves as an endocrine organ capable of synthesizing a number of biologically active compounds that regulate metabolic homeostasis. This dynamic tissue is composed not only of adipocytes, but also of other cell types called the stroma-vascular fraction, comprising blood cells, endothelial cells, pericytes and adipose precursor cells among others (Bernlohr DA et al,.2002 and Saely C et al,.2012). Several studies have evidenced that adipose tissue is not uniform. Depending on the location in the body, they differ in their capacity to secrete adipocytokines, as well as cellular composition with varied phenotype, as well as the quantity

and proportion of adipocytes forming it, blood vessel stromal cells and immune system cells (Trzeciak-Ryczek A et al, .2011). It is now generally recognized that adipose tissue is an important organ of a complex network that participates in the regulation of a variety of quite diverse biological functions (Figure 1) (Costa JV et al,.2006 and Laclaustra M et al,.2007).

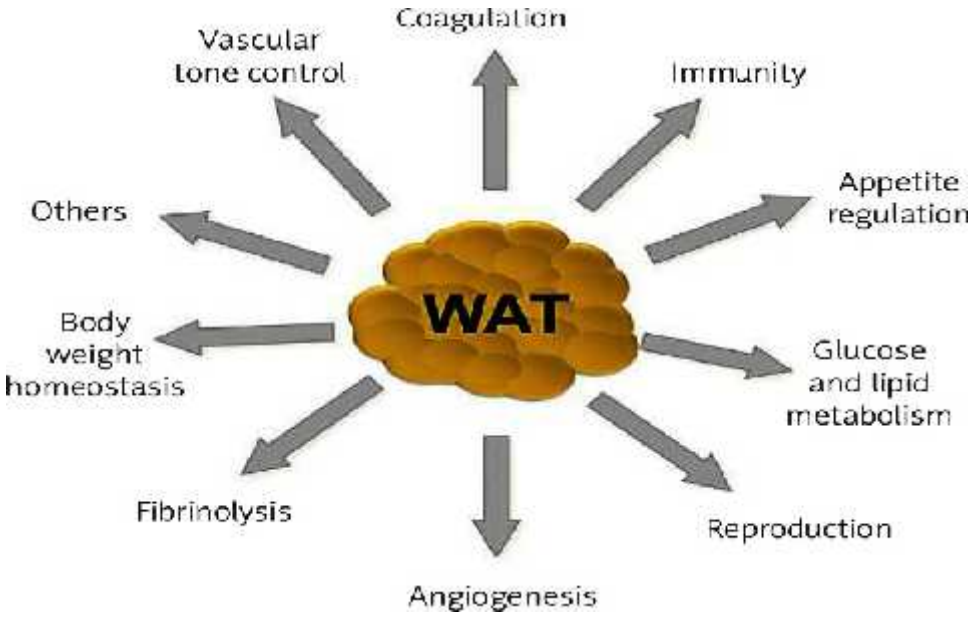


Figure.1.The most significant physiological functions of white adipose tissue such as coagulation,appetite regulation,glucose and lipid metabolism, angiogenesis, fibrinolysis, body weight homeostasi and vascular tone control. *Arch Med Sci. Apr 20, 2013; 9(2): 191200.*
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3648822/figure/F0001/

Adipogenesis refers to the differentiation of pre-adipocytes into mature fat cells, i.e. the development of adipose tissue, which varies according to sex and age. Adipocytes differentiate from stellate or fusiform precursor cells of mesenchymal origin. The morphological and functional changes that take place in the course of adipogenesis correspond to a shift in transcription factor expression and activity leading from a primitive, multipotent state to a final phenotype characterized by alterations in cell shape and lipid accumulation (Fonseca-Alaniz MH et al,.2007 and Saely C et al,.2012).

Pre-adipocytes within adipose tissue can differentiate into mature adipocytes throughout life, thus enabling hyperplastic expansion of adipose tissue when increased storage requirements are needed. In addition, the mature adipocytes can expand in size to accommodate increased storage needs and in situations of overnutrition become hypertrophic. As a result, adipocyte number and morphology transform in response to energy balance via the biochemical processes involved in lipid uptake, esterification, lipolysis and differentiation of pre-adipocytes (Grasy SL et el,.2007).

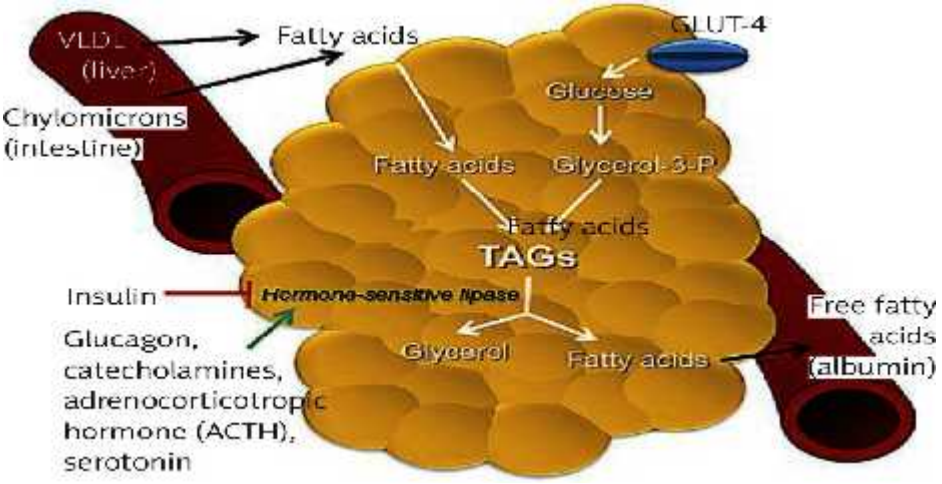
In mammals, there are two types of adipose tissue: white and brown. The adipocytes in these two types exhibit different morphology and function. Brown adipose tissue specialized in heat production (thermogenesis) is almost absent in adult humans, but is found at birth. Brown adipocytes, with an average diameter, are smaller than adipocytes of white adipose tissue. They have a number of cytoplasmic lipid droplets of different sizes, cytoplasm relatively abundant, a spherical core and slightly eccentric and numerous mitochondria that release heat by oxidation of fatty acids. Brown adipose tissue also stores energy in lipid form, but more regularly produces heat by oxidizing fatty acids within the adipocyte, rather than supplying free fatty acids for use by other cell types (Bernlohr DA et al,.2002, Fonseca-Alaniz MH et al,.2007and Saely C et al,.2012). Brown fat derives its color from extensive vascularization and the presence of many densely packed mitochondria. It is traversed by many more blood vessels than white fat. These blood vessels assist in delivering fuel for storage and oxidation, and in dispersing heat generated by the numerous mitochondria to other parts of the body (Fonseca-Alaniz MH et al,.2006 and Kiess W et al,.2008).

Although its participation in thermogenesis is irrelevant, white adipose tissue's functional capacity is much broader and more comprehensive. It has extensive distribution in the body, involving, or infiltrating, almost the entire region subcutaneously by organs and hollow viscera of the abdominal cavity or mediastinum and several muscle groups, for which it offers mechanical protection, softening the impact of shocks and allowing appropriate sliding of muscle bundles, one on the other, without compromising their functional integrity (Bernlohr DA et al,.2002 and Fonseca-Alaniz MH et al,.2007). Because it is an excellent thermal insulator and has a wide distribution, including the dermis and subcutaneous tissue, it plays an important role maintaining body temperature (Saely C et al,.2012). By this ability to accumulate and provide energy when necessary, it assumes the status of the most important buffering system for lipid energy balance, particularly fatty acids, which are an exceptionally efficient fuel storage species.

The highly reduced hydrocarbon tail can be readily oxidized to produce large quantities of ATP (adenosine triphosphate)(Kiess W et al,.2008).

1.2.Lipogenesis and lipolysis

Fat accumulation is determined by the balance between fat synthesis (lipogenesis) and fat breakdown (lipolysis/fatty acid oxidation). Lipogenesis is a process that occurs preferentially in adipose tissue (Figure 2), but it also happens in liver, and it is the synthesis of fatty acids, which are used as energy reserves. This process is responsive to changes in the diet (Kersten S 2001). It is stimulated by a high carbohydrate diet leading to elevated postprandial plasma triglyceride levels, whereas lipogenesis is inhibited by polyunsaturated fatty acids and by fasting. Fasting is related to a decrease in plasma glucose and an increase in plasma-free fatty acids. These effects are partly mediated by hormones, which inhibit (leptin) or stimulate (angiotensin, acylation stimulating protein) lipogenesis. Glucose itself is a substrate for lipogenesis. It increases the process by stimulating the release of insulin and inhibiting the release of glucagon from the pancreas (Kersten S et al.,2001).



Arch Med Sci. Apr 20, 2013; 9(2): 191200.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3648822/figure/F0001/

Figure 2.Primary metabolic role of adipose tissue. In the feeding state, insulin-dependent glucose transport 4 (GLUT 4) allows the uptake of glucose from the bloodstream to adipocytes. Glycolysis occurs, producing glycerol-3-phosphate (glycerol-3-P), a substrate required for lipogenesis. Fatty acids from liver carried by very low-density lipoproteins (VLDL) and

chylomicrons from the intestine are esterified with glycerol-3-P to form lipid droplets of triacylglycerols (TAGs). In the fasting state and in stress conditions, hormonesensitive lipase is activated for lipolysis. Some steps are required to produce glycerol, which travels to the liver, and fatty acids. These free fatty acids will tra vel in the bloodstream to the liver, muscle and to other organs to be oxidized. In the bloodstream fatty acids are immediately bound to albumin.

Lipolysis occurs in adipose tissue and is the breakdown of fat, in other words, from energy reserves (triglycerides) for energy production by which triacylglycerol molecules are hydrolyzed to free fatty acids and glycerol (Figure 2). During times of metabolic stress (i.e. during fasting or prolonged arduous exercise when the body's energy needs exceed the circulating nutrient levels), the adipocyte's triacylglycerol droplet is degraded to provide free fatty acids to be used as an energy source by other tissues. Numerous stimuli are able to elicit the lipolytic response in adipocytes. However, ultimately the same pair of enzymes, hormone-sensitive lipase and monoacylglycerol lipase, is responsible for catalyzing the hydrolysis of the triacylglycerol ester bonds. Complete hydrolysis of triacylglycerol involves the breakage of 3 ester bonds to release free fatty acids and a glycerol moiety. The same enzyme, hormone-sensitive lipase, is responsible for facilitating hydrolysis of the esters at positions 1 and 3 of the triacylglycerol. A second enzyme, 2-monoacylglycerol lipase, catalyzes hydrolysis of the remaining ester to yield a third free fatty acid and glycerol. Hormone-sensitive lipase is inhibited by insulin and is favored by the presence of glucagon and epinephrine (Kiess W et al,.2008, Laclaustra M et al.,2007 and Kersten S 2001). Glycerol is effluxed out of adipocytes via an aquaporin type of transport molecule and must be shuttled back to the liver for use in oxidation or gluconeogenesis. However, under maximal lipolytic conditions, substantial recycling of fatty acids occurs such that on average about two fatty acid molecules are released per glycerol molecule. Outside the adipocyte, fatty acids are immediately bound to albumin and carried in the bloodstream to the liver, muscle and other tissues for oxidation (Laclaustra M et al.,2007).

β-Oxidation is a catabolic process in which the free fatty acids resulting from lipolysis are used by the body as a source of energy. The fatty acid molecules are converted into acetyl coenzyme A molecules (Bernlohr DA et al,.2002).

1.3. Adiponectin.

Adiponectin was found between 1995 and 1996 and given different names which are apM1(adipose most abundant gene transcript 1)(Medaa et al.,1996),Acrp30(adipocyte complement-related protein of 30KDa)(scherer et al.,1995),GBP28(gelatin binding protein of 28 KDa)(Nakano et al.,1996) and adipQ(Hu et al.,1996).

Adiponectin is a protein hormone which act important role in metabolic process. These are the glucose regulation and fatty acid catabolism and produced from adipose tissue into the bloodstream and its very abundant in plasma relative to many hormone (Takashi and Toshimasa,2005). Adiponectin has beneficial effects on obesity-related medical complications. In contrast to other adipokines, circulating diponectin levels are reduced in obesity,type 2 diabetes and associated diseases(Li et al.,2009).

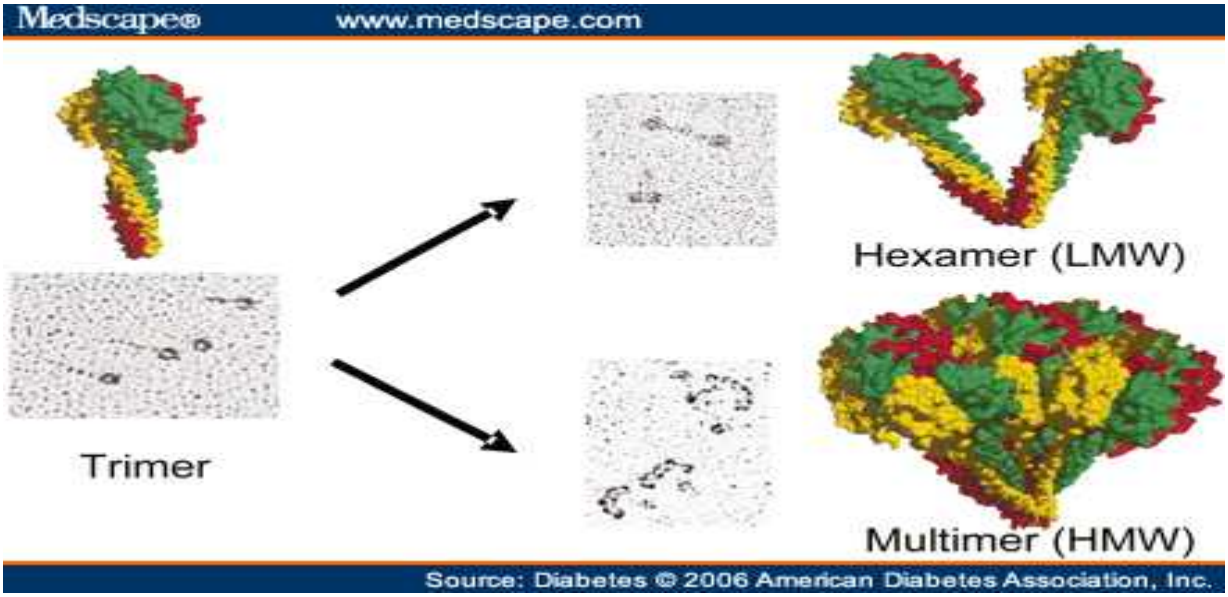
Adiponectin has been postulated to act an important role in the modulation of glucose and lipd metabolism in insulin-sensitive tissue in both humans and animals(Yamauchi et al., 2001).Decreased adiponectin levels have been demonstrated in genetic and diet- induced murine models of obesity.as well as in diet-induced forms of human obesity(Arita et al.,1999).Low adiponectin levels have also been strongly implicated in the development of insulin resistance in mouse models of both obesity and lipoatrophy(Yamauchi et al,. 2001).

1.4. Structure of Adiponectin

Adiponectin is a protein of 247 amino acids composed of four domains, an amino-terminal signal sequence,a variable region, a collagenous domain(Cad), and a carboxy-terminal globular domain(gAD)(Scherer et al.,1995).The basis of both its primary amino acid sequence and its subunit domain structure. Adiponectin is most similar to C1q, which is a member of the complement related family of protein(Berg et al,. 2002).

The basic building block of adiponectin is a tightly associated trimer, which is formed by association between three monomers at the globular domains. Monomeric (30-KDa) adiponectin has not been observed in the circulation and appears to be confined to the adipocytes. Four to six trimers associate through their collagenous domains to form higher-order structure or oligomers, which circulate in plasma (Scherer et al.,1995 and Berg et al.,2002 and Arita et al.,1999).

Adiponectin circulates in several different size complexs in serum (Fig.1). Its basic unit is a homotrimer.These homotrimers can assemble into higher-order structures, such as a hexamer,and several of these hexamers can assemble into a high-molecular weight (HMW) complex. All three forms can be found in serum (www.medscape.com).



(Fig.2) *Adiponectin multimerization. Shown are the three major forms of adiponectin found in circulation. Electronmicrographs of purified complexes are shown. Schematic representations of the different complexes are shown as well, with each adiponectin subunit in the basic trimeric building blocks represented in a different color.*

1.5. Receptor of Adiponectin

Two receptor for adiponectin are AdipoR1 and AdipoR2 which have been characterized that mediate effects of adiponectin in various tissues.The receptors contain 7-transmembrane domains but are structurally and functionally distinct fro G-protien-coupled receptors, displaying intra-cellular and extracellular N-and C- termini and signaling via alternate, non-classic GPCR path ways (Hayley et al.,2010 and Sahar et al.,2013).

AdipoR1 is expressed omnipresence, most abundantly in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. AdipoR1 functions as a high-affinity receptor for globular adiponectin and low –affinity one for full length adiponectin (Hyun et al., 2010).

1.6.**BiosynthesisandSecretionPathwaysofAdiponectin Multimers**

Adiponectin present in a wide range of multimer complexesin plasmaand combines viaits collagen domain to create 3 major oligomeric forms: a low-molecular-weight (LMW) trimer, a middle-molecular-weight(MMW)hexamer,and a high-molec- ular-weight (HMW) 12- to 18-mer (Pajvani et al., 2003; Waki etal.,2003).Importantly,HMW adiponectin has been shown to be able to activate AMP kinase most potently (Kobayashi etal.,2004;Hada etal.,2007).

A truncated form ofadiponectin that includes the globular domain cleaved proteolytically fromfull-length adiponectin has been reported to present in plasma, although in very small amounts (Fruebis et al.,2001). The Adiponectin gene expressed exclusively in adipocytes has been reported to be regulated by tran- scriptional factors including C/EBPs(Saitoet al.,1999), sterol regulatory element binding protein 1c (SREBP1c) (Seo et al.,2004),

and PPARg (Maeda et al.,2001)(Figure2).Farmer and his colleague sreported that during adipocyte differentiation, SirT1 levels are decreased and PPARg levels are increased, both of which increase endoplasmicreticulum (ER) oxidoreductase Ero1-L,there by stimulating secretion of HMW adiponectin (Qianget al., 2007). Scherer and his colleagues showed that there is an abundant pool of properly folded adiponectin in the secretory pathway through thiol-mediated retention and that adiponectin is covalently bound to the ER chaperone ERp44. They also showed that another ER chaperone, Ero1- La, plays a critical role in the release of adiponectin from ERp44 and that these chaperones play a major role in the assembly of HMW adiponectin.They also reported that one mechanism for increasing circulating levels of specific adiponectin complexes by PPARgagonists may be selective up regulation of rate-limiting chaperones such as ERp44 and Ero1-La(Wang etal.,2007)(Figure2).

Many observations support the hypothesis that HMW adiponectin is the mos tactive form of the protein and has a more relevan trole ininsulin sensitivity and in protecting against diabetes. First,rare mutations—G84R and G90S—in the collagen domain are very closely associated with type 2diabetes (Wakietal.,2003). Subjects with either of these two mutations have extremely low levels of HMW adiponectin,although total plasma adiponectin.

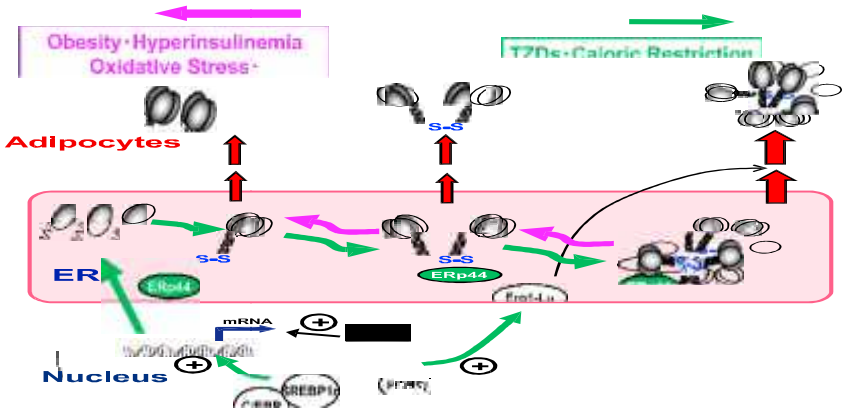


Figure3.*RegulatoryMechanismsofAdiponectinMultimer Formation*

TheAdiponectingeneexpressed normallyinadipocyteshasbeenreported toberegulated bytranscriptional factors,includingC/EBPs, sterol regulatory elementbindingprotein1c(SREBP1c),andperoxisomeproliferator-activated receptorg(PPARg).SirT1has recently beenreported todeacetylateLys268 and Lys293 ofPPARgand tocauseselectivePPARgmodulation,leading to upregulationofadiponectin.There isan abundantpool ofproperly folded adiponectininthe secretorypathwaythroughthiol-mediatedretention,and thatadiponectin iscovalentlybound to the ErchaperoneERp44.AnotherER chaperone, Ero1-La, plays acritical roleinthe releaseofadiponectin from ERp44,and thatthese chaperonesplayamajorrolein the assemblyofHMW adiponectin.Hyperinsulinemia,oxidativestressandinflammation observedin obesityhave beenreportedtoreduceHMWadiponectin,(ToshimasaYamauchi et al,.2013). Levelswerenotsignificantlychanged. Moreover,the two mutant adiponectinsrecombinantlyexpressed inNIH3T3fibroblasts were not able to form the HMWform of adiponectin(Waki etal.,2003).Second,increasesintheratioofplasmaHMWadiponectin levelstototaladiponectinlevelshave mutual relation with improvement in insulin sensitivity during treatment with an insulin-sensitizingdrug,TZD,inbothmiceand human diabetes, whereasincreasesintotalserumadiponectinlevelsdonotshow good correlations withimprovement ininsulinsensitivity during treatmentwith TZD(Pajvani et al., 2004). Third, the level of plasmaHMWadiponectinwas reportedtobe associated with parametersrelatedto glucosehomeostasisina cohortstudy (Lara-Castroetal.,2006).Itisnoteworthythattheratioofplasma HMWadiponectintototal adiponectincorrelated more signifi-cantlywithglucoseandinsulin

levelsthandidthetotaladiponec-tinlevel(Lara-Castroetal.,2006),suggesting thatalterations in plasmaHMWadiponectinlevel may be more relevant to the predictionofinsulinresistancethanaretotalplasmaadiponectin alterations. Consistent with this, levels of total adiponectin, HMWadiponectin,and the HMW-to-total adiponectinratio all inverselycorrelatedwithkeyfeaturesofcentralobesityandpositively correlatedwith the insulin stimulatedglucosedisposal rate. However,HMWadiponectinlevels, not total adiponectin levels,areprimarilyresponsiblefortheserelationships,suggestingthat measurementofthe HMWadiponectinlevel may be superiorto measurementof total adiponectin(Fisher et al.,2005).

They used an ELISAsystemforselective measurement of HMWadiponectin,they also found HMWadiponectinand the HMW to total adiponectin ratio have significantly better power forthepredictionofinsulinresistance and themetabolic syndromeinhumans(Haraetal.,2006).Thus,HMWadiponectin levelmaybethesuperiorbiomarkerforinsulinresistance,metabolic syndrome,and type 2diabetes. However,Blüher et al. failedtoobservethesuperiorityofHMW overtotaladiponectin inassessing metabolicvariablesat baselineorinresponseto physicaltraining (Blüher et al., 2007), suggestingthat further studies arerequiredforclinicaleffectivenessand usefulnessof measuringHMWadiponectin.

1.7. Discovery of the Insulin-Sensitizing Action of Adiponectin

Using DNA chips, they screened for secreted molecules in WAT, the expressions of which were increased in small adipocytes from insulin sensitive mice such as heterozygous peroxisome proliferator-activated receptor g (PPARg)-deficient mice, and thy found that increased expression of adiponectin correlates with increased insulin sensitivity in mouse models of altered insulin sensitivity.They next assessed whether adiponectin was able to improve insulin resistance in KKAy mice (KK mice overex- pressing the agouti protein), as a model of the metabolic syndrome and type 2 diabetes linked to obesity. Plasma adipo- nectin levels were decreased in KKAy mice fed a high-fat diet (HFD). Replenishment of adiponectin significantly ameliorated HFD-induced insulin resistance and hypertriglyceridemia, which led us to propose that adiponectin is an insulin-sensitizing adipokine (Yamauchi et al., 2001). These data also strongly suggested that the HFD-induced, obesity-linked decrease in

adiponectin level is causally involved in obesity-linked insulin resistance and the metabolic syndrome. Scherer and colleagues showed that an acute increase in the level of circulating adiponectin triggers a transient decrease in basal glucose level by inhibiting both the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production in both wild-type and type2 diabetic mice, and they proposed that adiponectin sensitizes the body to insulin, which is associated with inhibition of endog- enous glucose production (Berg et al., 2001; Combs et al., 2001). Lodish and colleagues reported that a proteolytic cleavage product of adiponectin, which structurally resembles globular adiponectin, increases fatty acid oxidation in muscle, decreases plasma glucose, and causes weight loss in mice (Fruebis et al., 2001).

Subsequently, the long term effects of adiponectin on insulin resistance in vivo were investigated through the use of adiponectin transgenic mice (Yamauchi et al., 2003; Combs et al., 2004) or adiponectin-deficient mice (Kubota et al., 2002; Maeda et al., 2002; Ma et al., 2002; Nawrocki et al., 2006). Adiponectin transgenic mice showed amelioration of insulin resistance and diabetes. Adiponectin deficient mice showed mild insulin resistance with glucose intolerance. Adiponectin deficient mice also exhibited other features of metabolic syndrome, such as dyslipidemia and hypertension (Kubota et al., 2002).

Scherer and his colleagues showed that adiponectin trans- genic mice displayed increased expression of PPARg target genes and became morbidly obese with improvement in insulin sensitivity (Kim et al., 2007).

2. Menopause.

2.1. Definition of menopause

According to Soules MR.et al.,(2001) menopause is specifically defined by the last menstrual period (documented by absence of menses for 12 months) but symptoms usually occur earlier, during a period termed primenopause. So therefore the menopausal transition divided into stages, early primenopause is defined by change in length of the menstrual cycle. Late primenopause occur after one or skipped menstrual or greater than 60 days of armeronrrhea, and early menopause is the five years after menopause followed by lifelong late menopause.

According Burger HG et al,.2002 menopause is best defined as the absence of menses for 12 consecutive months. Menstrual history is the most reliable indicator of the postmenopausal state,

as specific hormonal measures, such as estradiol (E2) and FSH levels both vary widely in the perimenopause during an individual menstrual cycle . The perimenopause has been defined as a period of menstrual irregularity and hormonal variability, beginning when menstrual cycle length changes from an established pattern into longer, shorter, or more variable cycles, with an average duration of 4 year, ending 1 yearr after the final menstrual period. This means that women can expect to have menstrual irregularities for approximately 4 yr before their final menses. Although it is commonly believed that E2 levels fall gradually throughout the perimenopause, concentrations are preserved until relatively late in the perimenopausal period, as E2 does not decline significantly until women experience at least 3 months of amenorrhea (Burger HG et al,.2002).

In medical term menopause means when the period stop for one year,menopause is natural part of life of female.It show the end of fertility. At puberty every female have monthly hormonal cycle, that resulted in their period which is called menstrual cycle. During the early part of each cycle the level of hormone estrogen rise and stimulating the growth of an egg in their ovaries. At ovulation an egg is released from one of the two ovaries then another hormone progesterone, stimulate the lining of the uterus to thicken. If the egg is not fertilized by sperm then hormonal levels drop and the lining is shed as period. As female get older than their ovulate less often and the fertility goes down with age. When the female approch menopause then the release of estrogen and progesterone become irregular, on this stage their ovaries less active and much less osterogen.Eventually the ovary stop functioning and release no more eggs. The release of estrogen and progesterone also stop. So these changing hormone levels are responsible for many of the symptoms of menopause.(The women Health Council, menopause A Guide, july 2008).

2.2. Premenopause

In this stage follicular artresia is associated with decrease in inhibin secretion, rising serum follicle stimulating hormone (FSH) levels and decrease4 fecundity. The major and abrupt reduction in ovarian estrogen production does not occur until about 6 months before menopause, in contrast there is a slow but steady age-related decline in estrogen levels (androstenedione and testosterone throughout the pre-menopausal period. In addition to a decreased ability to conceive, the premenopausal period characterized by irregular menstrual bleeding and vasomotor symptoms (hot flushes).(Lund KJ. et al,.2008.)

2.3. Menopause and late menopause

The most significant hormonal change associated with menopause is the marked reduction in levels of estradiol (E2) and estrone (E1). The decrease in E2 levels is more pronounced, since E1 is produce from peripheral aromatization of the more-slowly declining androgens.Associated changes include increased level of FSH and luteinizing hormone (LH) as a result of decreased E2 and inhibin levels, pituitary hormone are not affected significantly although protection levels may decrease slightly. Both the ovaries and the adrenal glands continue to produce androgens throughout the menopausal and postmenopause periods, although level decrease slowly with increasing age.The postmenopauseal ovaries produce androstenedione and testosterone(but not estradiol) while the adrenal glands produce and rostenedione, dehydroandrostenedion(DHEA) and dehydroandrostenedion sulfate(DHEA-S).(Lubo RA. et al,.2007).

3. Menopause Effect on Body Organs

3.1. Cardiovascular disease (CVD) risk after menopause

CVD is the primary cause of death in women of westernized countries, with more than one in two women dying from CVD. However, atherosclerotic disease occurrence is different in men and women, as sudden begins approximately 10 year later in women than men, and myocardial infarction is uncommon until women reach their sixth decade (American Heart Association 2001). Premenopausal women appear to be protected from CVD compared with men of similar age. But women below the age of 50 year rarely develop CVD, by age 70 year the incidence of CVD is equal in men and women, suggesting that estrogen deficiency causes a rapid acceleration in CVD risk. Controversy exists about whether menopause increases the risk of CVD independent of normal aging. Some studies have expressed increased risk of CVD after menopause, and others have not (Gohlke et al,.2000). For example, Framingham investigators found a 4-fold increase in CVD in the 10 yearr following natural menopause. Premature, surgically induced menopause has been shown to increase the risk for CVD (Gohlke et al,.2000).

Studies assessing the relationship of menopause with measures of atherosclerosis have yielded interesting results.(Sutton-Tyrrell et al. 1998.) showed that 45% of postmenopausal women (n =

294) had clinically significant carotid intima-media thickness (≥0.75 mm) compared with 16% of age-matched premenopausal women. Carotid intima-media thickness has been shown to be a strong predictor of CVD risk (Chambless LE et al,.1997). Aortic calcification, a measure of atherosclerosis, was higher in postmenopausal women, and the extent of calcification increased with the number of postmenopausal years (Witteman JC et al,.1989). Similarly, coronary artery calcium deposits in women, measured by computed tomography (CT), was half that in men until the age of 60 year when the difference decreased (Janowitz WR et al,.1993).

The relevant importance of factors that influence cardiovascular risk in postmenopausal women are unknown. Alterations in lipid metabolism with estrogen deficiency are thought to be a substantial component of CVD risk in postmenopausal women (Kannel WB ET et al 1994), but there are also direct effects of estrogen deficiency on body fat distribution (central obesity), insulin action, the arterial wall, and fibrinolysis that may influence cardiovascular risk. These factors contribute to an increased prevalence of the metabolic syndrome in postmenopausal women compared with premenopausal women (Park YW et al,.2003).

3.2. The metabolic syndrome after menopause

The metabolic syndrome has received more focus as the updated Adult Treatment Panel III guidelines emphasize treatment of the metabolic syndrome in addition to lowering of low density lipoprotein (LDL) levels (National Cholestrol Education Prograrm 2001) The metabolic syndrome may not be a single disease entity, but, rather, a constellation of closely related risk factors that together convey substantially increased cardiovascular risk after accounting for traditional CVD risk factors (Lakka HM et al,. 2002). The features of the metabolic syndrome include the accumulation of visceral (abdominal) adiposity, insulin resistance, hypertension, and dyslipidemia (hypertriglyceridemia, reduced high density lipoprotein (HDL), and small dense LDL particles (Despres JP et al,.1993). The metabolic syndrome is estimated to affect approximately 20–30% of the middle-aged population (Park YW et al,.2003), and prevalence appears to be increasing in the U.S. population with increasing obesity and sedentary lifestyle (Meigs JB et al,. 2002). Postmenopausal status is associated with a 60% increased risk of the metabolic syndrome, even after adjusting for confounding variables, such as age, body mass index (BMI), household income, and physical inactivity (Park YW et al,.2003). The risk of CVD attributed to the metabolic syndrome appears to be especially high in women, and it is estimated

that half of all cardiovascular events in women are related to the metabolic syndrome (Wilson PW et al,.1999).

Although syndrome X was initially coined by (Reaven GM et al,.1988), the features of the metabolic syndrome were first described by Vague (Vague J et al,.1956) and have subsequently been called the insulin resistance syndrome, the central obesity syndrome, and the deadly quartet. The etiology of the metabolic syndrome is unknown, but is thought to be a combination of factor. (Selby JV et al 1991.) studied 1028 male twins and found greater concordance of dyslipidemic hypertension in monozygotic than dizygotic twins. Within the discordant monozygotic twin pairs, the twin with dyslipidemic hypertension weighed significantly more as an adult, implying an interaction between genetic and environmental influences on the manifestation of the metabolic syndrome (Bouchard C et al,. 1995). Many believe that the underlying pathophysiology of the metabolic syndrome is related to increased visceral obesity and insulin resistance (Despres JP et al,.1993).

3.3. Effects of menopause on body composition

Two patterns of body fat distribution have been investigated, the accumulation of fat centrally, as intraabdominal fat (android or apple shape) and the accumulation of fat in the gluteo-femoral region . The accumulation of fat in a central distribution (intraabdominal) has emerged as a cardiovascular risk factor independent of overall obesity (Kannel WB et al,.1991). Android fat deposition is associated with a higher risk of diabetes, hypertriglyceridemia, small dense LDL particles, hypertension, and CVD (Despres JP et al,.1993). Estrogen promotes the accumulation of gluteo-femoral fat (Krotkiewski M et al,.1983), and the loss of estrogen with menopause is associated with an increase in central fat (Poehlman ET et al,.1995).

Although it is commonly suggest that menopause is associated with weight gain, most studies do not reveal increases in BMI independent of normal aging (Poehlman ET et al,.1997 and Crawford SL et al,.2000). Although it is estimated that middle aged women gain approximately 0.55 kg (~1 lb)/year, there does not appear to be an independent effect of menopause on body weight (Geu SS et al,. 1999, Kuller Let al,. 1997). However, even in the absence of weight gain, body fat distribution changes across the menopause. Cross-sectional (Zamboni M et al,.1992) and longitudinal studies (Poehlman ET et al,.1995, Bjorkelund C et al,.1996) have shown that the menopausal transition is associated with a preferential increase in abdominal adiposity,

independent of the effect of age and total body adiposity. .(Poehlman ET et al,.1995) prospectively compared women who became postmenopausal to age matched controls who remained premenopausal and found that the transition to menopause was associated with an increase in the waist to hip ratio and total body fat. Abdominal fat, measured by CT scan, has also been shown to increase with menopause in both cross-sectional (Toth MJ et al,. 2000) and prospective studies (Carr MC et al,. 2003). Visceral fat accumulation is thought by many to be the major determinant of the metabolic syndrome.

Women with high amounts of visceral fat have an excess of cardiovascular mortality and associated metabolic abnormalities (Lapidus L et al,.1984). When (Pascort A et al,.2001) matched women for abdominal fat (by CT scan) and menopausal status, the differences initially found in very low density lipoprotein (VLDL), LDL, HDL, large buoyant HDL2 particles, LDL particle size, fasting glucose, C peptide, and blood pressure were eliminated, implying that the differences in visceral fat and menopausal status accounted for the metabolic differences. Regional differences in adipose tissue lipoprotein lipase activity in postmenopause may account for the menopausal changes in fat accumulation, but results to date are conflicting (Ferrara CM et al,. 2002, Mauriege P et al,. 2000). Adiponectin, a novel adipocyte derived peptide, may play a role in the metabolic syndrome, as concentrations are inversely related to obesity and insulin resistance. However, the only study evaluating adiponectin in menopause revealed no difference in pre- and postmenopausal women (Nishizawa H et al,.2002).

Menopause is also associated with reduced lean body mass (muscle) and this appears to be related to decreased physical activity (Poehlman ET et al,. 2002).(Lynch NA et al.) recently showed lower maximal oxygen consumption (VO2 max) in sedentary postmenopausal (VO2 max) women compared with sedentary age-matched premenopausal women and found an inverse relationship between visceral adiposity and maximal oxygen consumption. The reductions in exercise capacity and activity may contribute to the reduced lean body mass and increased central adiposity with menopause.

3.4. Changes in LDL with menopause

Postmenopausal women have higher total cholesterol, LDL cholesterol, triglycerides (TG), and lipoprotein(a) [Lp(a)] levels and lower HDL cholesterol levels than premenopausal women (Jensen J et al,.1990, Campos H et al,. 1988,Li Z et al,.1996). Although elevated LDL is not a

component of the metabolic syndrome, LDL levels increase by 10–20% (Poehlman ET et al,.1997, Matthews KA et al,.1989) with menopause, and the greatest change in LDL concentration appears to occur early in the transition from premenopause to postmenopause (Matthews KA et al,.2001). Apo B, the primary apolipoprotein of LDL particles, and other apo B-containing particles are also higher in postmenopausal compared with premenopausal women (Li Z et al,.1996).

LDL particle composition also changes with menopause. The prevalence of small, dense LDL is low in premenopausal women (10–13%), but increases to 30–49% in postmenopausal women (Campos H et al,. 1988 Austin M et al,1988, Carr MC et al,.2000) (Table 4). LDL are comprised of a spectrum of particles that vary in size, density, chemical composition, and atherogenic potential. A preponderance of small, dense LDL is associated with an increased risk of myocardial infarction (Auistin M et al,. 1988) as well as the severity of CVD (Campos H et al,.1992). The risk of CVD is 3-fold higher in women with small, dense LDL than in those with large, buoyant LDL (Auistin M et al,. 1988). (Mackey et al. 2002) recently reprted by electron beam CT that postmenopausal women with high coronary calcium scores had smaller LDL particle size, higher LDL levels, and fewer large HDL2 particles than postmenopausal women with low coronary calcium scores.

3.5. Changes in TG with menopause

Saveral longitudinal studies have shown that TG levels increase with the transition through the menopause (Jenson J et al,. 1990), and the increase in TG also appears early in the postmenopausal period (Mathews KA et al,.2001). (Poehlman et al. 1997) repored that the prospective transition to postmenopause was associated with a 16% increase in TG. Although men generally have higher TG levels than women, TG increases in middle-age (between 40–69 year) in women, but not in men (Razay G et al,.1992), and TG appears to be a better predictor of CVD risk in women than in men (Hokanson JE et al,. 1996). (Lindquist 1982) reported a prospective increase in TG levels in women who became postmenopausal during a 6-year period, whereas there was no change in TG in the similarly aged women who remained either premenopausal or postmenopausal. Increasing TG with menopause may be related to the fact that TG levels are highly correlated with increasing abdominal fat content and insulin resistance.

3.6.Changes in HDL with menopause

Many studies showed that total HDL levels fall slightly with menopause (Poehlman ET et al,.1997, Jensen J et al,.1990,Matthews KA et al,. 1989, DO KA et al,.2000), whereas others reveal no changes (Kannel WB et al,.1976). Menopausal changes in HDL metabolism are more complex than the measurement of total HDL reveals, because the more antiatherogenic HDL2 levels decrease (by 25%), whereas HDL3 levels increase (Kuller L et al,.1997, Carr MC et al,.2003,Li Z et al,.1969, Stevenson JC et al,.1993). HDL2 particles are the large, buoyant, and more cardioprotective subspecies of total HDL. The strong inverse relationship between HDL cholesterol and abdominal adiposity appears to be largely dependent on variations in HDL2 levels (Lamarche B et al,.1997).

3.7. Changesin lipid metabolism with menopause

Proteins of lipid metabolism underlying the menopausal change in lipids have been evaluated in few studies. The increased prevalence of small, dense LDL with menopause may be explained by higher HL activity in postmenopausal women (Carr MC et al,.2003, Berg GA et al,.2001). Endogenous estrogen levels are inversely associated with HL activity (Tikkanen MJ et al,.1986). HL hydrolyzes the TG and phospholipid in LDL and HDL and is one factor that determines the size and density of LDL and HDL particles (Santamarina et al ,.1998). The higher the HL activity, the more TG and phospholipid hydrolyzed, resulting in smaller, denser more atherogenic lipoprotein particles. Lipoprotein lipase hydrolyzes TG in triglyceride-rich lipoproteins, generating FFA that can serve as an energy source or can be stored in adipocytes. We have recently shown a small, but significant, rise in lipoprotein lipase activity with the transition through menopause (unpublished observation). Cholesteryl ester transfer protein (CETP) catalyzes the exchange of cholesterol ester in HDL and LDL particles for TG in VLDL, and high CETP concentrations are associated with reduced HDL levels. Menopausal status does not appear to affect CETP activity (Lewis et al,.1999).

3.8. Insulin resistance changes with menopause

Two of the most important pathophysiological components of the metabolic syndrome are increased visceral fat accumulation and insulin resistance. Abdominal obesity is closely associated with increased insulin resistance, compensatory hyperinsulinemia, and increased risk

of type 2 diabetes, independent of an individual's total body fat content (Pouliot MC et al,.1992). The pathophysiology underlying the insulin-resistant state is complex. Insulin resistance, with inadequate compensatory hyperinsulinemia, diminishes the normal suppression of FFA arising from adipose tissue by insulin. The increased levels of FFA may impair peripheral glucose uptake, increase hepatic gluconeogenesis, and reduce hepatic clearance of insulin (Despres JP et al,.1993).

The literature to date is not clear as to whether menopause is associated with increased insulin resistance. What little data there are remain contradictory. Several groups have shown increased fasting insulin (Poehlman ET et al,.1995, Razay G et al,.1992) and increased fasting glucose levels (Lynch NA et al, Dallongeville J et al,.1995) in postmenopausal compared with premenopausal women, which would imply worsened insulin resistance with the menopause. However, insulin sensitivity is known to worsen with advancing age and increasing central obesity, making it difficult to tease out the effect of menopause from these processes. Studies using accurate measures of insulin resistance, such as the euglycemic-hyperinsulinemic clamp or the frequently sampled iv glucose tolerance test, are scarce (Lindhein SR et al,.1994, Walton C et al,.1993, Toth MJ et al,.2000, DeNino et al,. 2001).

Lindheim et al.,(1994) showed reduced insulin sensitivity (i.e. higher insulin resistance) in postmenopausal women compared with BMI-matched premenopausal women. However, others have shown no differences in insulin sensitivity in postmenopausal compared with premenopausal women (Walton C et al,. 1993, Toth MJ et al,.2000). (DeNino et al. 2001) compared measures of insulin resistance and visceral adipose tissue in age-grouped women ranging from 20–78 year. They found that reduced insulin sensitivity did not appear until women were older than 60 year and had accumulated levels of visceral fat that approximated the levels seen in men, suggesting a possible threshold effect of abdominal fat on insulin resistance (DeNino et al,. 2001).

Guthrie et al.,(2001) reported prospective data on 265 healthy perimenopausal women with normal fasting glucose. The group of women (16%) who developed impaired fasting glucose (≥6.1 mmol/liter) over the 5-yr period had higher baseline BMI, fasting glucose and insulin, waist circumference, and TG; lower HDL levels; as well as greater increases in BMI and insulin over the study period compared with women who maintained normal fasting glucose. There was no difference in menopausal status between the two groups,

4. REVIEW OF LITERARURE

Adiponectin is the most abundant protien secreted by the white adipose tissue.The circulation of adiponetin is in high level in the blood-stream and its serum and its concentration is inversely correlated with body fat mass. The distribution of adiponectin receptors (AdipoR1,AdipoR2 and T-cadherin) in peripheral tissues and organs allows adiponectin to exert pleiotropic effects on whole body metabolism.Besides its well-known antidiabetic, antiatherogenic, and anti inlammatory properties, accumulating evidence suggests a direct role for adiponectin in reprodutive tissues. Adiponectin shows evidence that also act on the release of gonadotropins. They repoted that underweight women have delayed puberty and higher risk of premature delivery and on the other hand overweight women have early puberty and are prone to develop polycystic ovary syndrom(PCOS),gestational diabetes mellitus(GDM) and preeclampsia(Palin MF et al,.(2012).(Sadashiv et al,. 2013) examined the adiponectin mRNA in adipose tissue and its associatiion with metabolic risk factors in postmenopausal obesewomen. They showed that adiponetin mRNA levels were significantly lower in obese than non obese. Further they reported that glucose level were significant higher, while HDL was lower in obese than non-obese. They also reported that VAT adiponectin mRNA also significantand inverse association with TG, while direct association with HDL and both association were independent of BMI and waist circumference.

Bonneau GA et al,(.2014) worked on adiponectin and waist cirumference as predictors of insulin- resistance in women.they found adiponectin was associated with the insulin resistance but not hs-CRP. They also found that adiponectin and waist circumference were predictors of IR only in women. They also reported that the levels of adiponectin in postmenopausal women were high as compared with premenopausal women. So according to their work adiponectin and waist circumference are important predictors of insulin resistance even in healthy non diabetic women.

Choudhury AB et al,(.2014) were observed that osteocalcin was significantly positively correlated with adiponectin in both pre menopausal and postmenopausal diabetic women and negative association between osteocalcin and HOMA-IR was observed only premenopausal diabetic women, but the associarion was partially reduced after additional adjustment for adiponectin. They observed that adiponectin slightly attenuated the inverse association between

osteocalcin and presence of type -2 diabetes in both premenopausal and post menopausal women.

In the study of Omentin -1, visfatin and adiponnectin levels in relation to bone mineral density in Iranian post-menopausal women they observed that in multivariable adjusted linear regression, serum omentin -1 levels were inversaly correlated with BMD, in multiple regression analysis serum visfatin and adiponectin levels were not significantly correlated with BMD at different skeletal sites after controlling for age, body mas index and bone related markers. According to their observetion the highest quartile of adiponectin compared to the lowest qurtile after adjusting for potential confounders, revealed an inverse association with BMD in the lumbar spine.(Tohidi M et al,.2012).

Wanig and his co workers studied plasma adiponectin has a protective role in the development of obesity related metabolic syndrom and viscular disorders. They showed that plasma adiponectin was inversely associated with the risk of hypertension among both white and black women. Their association appeared to be nonlinear in white womem does compared to black women. Their lifesyle factors, measure of obesity and obesity related clinical factors attenuated these association. The multivariable relative risk (95% CI) of hypertension across increasing quartiles of plasma adiponectin were 1.00, 0.98 (0.66-1.46), 0.63 (0.41-0.97), and 0.92 (0.60-1.42) in white women (P(trend): 0.38) and 1.00, 0.96 (0.64-1.46), 0.83 (0.53-1.29), and 0.58 (0.36-0.94) in black women (P(trend): 0.02). They found an inverse correlation between plasma adiponectin and risk of hypertension in white and black post-menopausal women. They also observed that the reduced hypertension was limited to only intermediate conceentration of adiponectin in white women which was graded across quartiles of adiponectin in black women.(Wang L et al,.2012).

Tenta R et al,.(2012) took 81 post menopausal women to examaine the association between cirulating levels of adiponectin and indices of bone mass and bone metabolism in middle-aged post-menopausal women. They reported that there was no association between total or HMW adiponectin and BMD (L2-L4) or TBBMC. On contrary the levels of adiponectin were positively associated with OPG levels and negatively with IGF-I, in multiple regression models after adjustment for potential confounders. They showed that HMW adiponectin have negative correlation with IGF-I in multiple regression models but not with OPG, TBBMC or BMD(L2-L4). Although they failed to show statistically significant correlation between circulating

adiponection levels and indices of bone mass in women during the postmenopausal period, they reported that the significant correlation with OPG and IGF-I levels suggesting an anabolic role of adiponectin which may contribute in the understanding of the inerplay between adipose tissue derived hormones and bone metabolism.

According to Lecke SB et al ,.(2011) adipose tissues are responsible for variety of adiopkines , composed leptin and adiponectin which are involved in endocrine processes regulating glucose and fatty metabolism, energy expenditure, inflammatory response, immunity ,cardiovascular function and reproduction. Their observation was describes the fluctuation in circulating leptin and adiponectin as well as their patterns of secretion in women from birth to menopause. They showed that during pregnancy leptin and adiponectin seem to play an autocrine /paracrine fashion in the placenta and adipose tissues, playing a the maternal fetal interface and contributing to glucose metabolism and fetal development. While in newborns adiponectin levels are 2 to 3 times higher than adults. They showed that plasma adiponectin levels at age five are inversely associated with the percentage of body fat. In adults they suggested that obese individual exhibit both leptin resistance and decreased serum adiponectin levels.

The women with Hashimoto;s thyroiditis were characterized by significantly eleveted serum concentration of IL-6 whereas thy found the concentration of leptin and adiponectin were same. Hashimoto;s thyroiditis patients had signifcantly higher serum levelvs of TSH as compaared with controls. According to their observetion the linear regression analysis of the Hashimoto;s thyroiditis group all of the studied women expressed that serum leptin levels have postively association with BMI, waist to hip ratio(WHR), TSH and IL-6 while on othere hand adiponectin have negtively relation. They found no association between serum adiponectin and TSH, ft(4),or TPOAbs. So they conclud that Hashimoto;s thyrodittis is chacacterized by an increased production of IL-6 but does not have direct influnce on leptin or adiponectin levels.(Sieminska L et al,.2010).

Jurimae J et,.al (2009) reported that adipontectin is responsible to regulate systemic insulin sensitivity as a part of a broader control mechanism in energy balance. They also observed that it is not clear whether adiponectin exerts its positive effects on insulin sentivity equally in a wide range of obesity. They investigated the association of plasma adiponectin concentartion with insulin resistance (IR) in cross-sectional sample of middle aged premenopausal women with wide range of obesity. In addition, they also studied the relationship between adiponectin, body

composition, and blood biochemical and cardiorespiratory fitness variables. Body composition and fat distribution were measured via dual-energy x-ray absorptiometry in normal-weight (NW) (n = 41, body mass index [BMI] < 25 kg/m(2)) and overweight (OW) (n = 57, BMI > or = 25 kg/m(2)) women. Fasting blood samples were collected ; adiponectin, leptin, insulin, glucose, and insulin-like growth factor-I were measured; and IR index were calculated. The IR index from fasting plasma insulin and plasma glucose levels were estimated using the homeostasis model assessment (HOMA), as follows: fasting plasma insulin (in microliter units per milliliter) x fasting plasma glucose (in millimoles per liter)/22.5. Adiponectin was significantly higher (P = .0001) in NW (14.7 +/- 4.7 microg/mL) compared with OW (9.9 +/- 3.1 microg/mL) women. Significant differences (P < .003) in body mass, BMI, percentage of fat mass, fat mass, trunk fat, trunk fat-leg fat ratio, leptin, insulin, and HOMA were also observed between NW and OW groups. Leptin was independently related to plasma adiponectin (beta = -.259, P = .001) in the overall study group. Plasma adiponectin was only related to trunk fat-leg fat ratio (beta = -.242, P = .002) among NW subjects, whereas plasma adiponectin was related to fat-free mass (beta = .182, P = .0001) and HOMA (beta = -.576, P = .002) among OW women. They also observed inverse relationship between adiponectin and leptin concentrations suggests that leptin may be involved in the regulation of adiponectin in middle-aged premenopausal women. Their data also demonstrate that adiponectin may play an important role in sustaining insulin sensitivity only in OW middle-aged premenopausal women.(Park HT et al,. 2009) worked on the relationship between serum adipocytokine levels and metabolic syndrome in menopausal women. They observed that adipocytokine are bioactibve substances which are derived from adipose tissues, especially visceral fat and act a important role in the development of metabolic syndrome. They reported that there were no significant differences in adiponectin or resitin levels in women with metabolic syndrome when they were compared with controle group. They also showed that TNF-alpha levels were signicantly higher in women with metabolic syndrome and multivariate logistic regression analysis indicated that TNF-slpha was signifcantly associated with mtabolic syndrome. They suggest that TNF-alpha in menopausal women is a binifical biomerker for diagnosing mtabolic syndrome.

The serum adiponectin concentration showed a significant negative association with serum estradiol concentration in post-menopausal women but not premenopausal women and perimenopausal women. The serum adiponectin concentration also reported a significant

negative association with serum monocyte chemotactic protein -1 level concentration in post menopausal women. They conclude that adiponectin concentration may be associated with protective role against insulin resistance and atherosclerosis during the post-menopausal stage. (Miyatani Yet al, .2008.).

Goodarzi MT et al,.(2007) studied to compare the levels of adiponectin hs-C-protien (CRP). HBA1c, and blood lipids among diabetic and healthy post-menopausal women and they also determind the relationship between circulating adiponectin and development of type 2 diabetes. .The levels Adiponectin were significantly decreased in the diabetic patients as compared to normal control subjects.Adiponectin levels were also negatively correlated with hs-CRP, LDL cholestrol, HbA1c, TG, and total cholesterol (TC). They also found A positive correlation between adiponectin and HDL cholestrol. Their data showed that diabetic women have lower adiponectin levels compared to healthy women. HbA1c as an indicator of glycemic control has a negative correlation with serum adiponectin. Adiponectin may play an important role in the pathogenesis of diabetes, and may be an independent predictor of the development of diabetes in women.

According to Chanprasertyothin S et al,.(2006). The lumbar spines, factors associated with BMD were age ($p < 0.01$) and lean body mass ($p < 0.001$). No independent association with fat mass was demonstrated likewise, at the femoral neck, only lean body mass was related to BMD ($p < 0. 01$). In terms of the relation of serum adiponectin to BMD, no association of serum adiponectin to BMD at the lumbar spines or femoral neck was found.

Sieminska L et al,.(2006) studied that the visceral fat accumulation was found in post-menopausal women because of hypoestrogenism, decreased secretion of sex hormone binding globuline and with rise of free testetrone. They reported that adipocytokines produced by adipose tissue; adiponectin , leptin and resistin might play a role. According to their research adiponectin is a hormone which act as a insulin sensitivity and lipid oxidation and possesses anti-inflammatory properties. Increased levels of androgens post- menopause and low sex-hormone binding globuline (SHBG) are related with decreased levels of adiponectin. They reported that adipocytokines may have a link connecting postmenopausal hormonal changes and also suggest that the excess of visceral fat and is a risk factor of cardiovascular disease.

Sieminska L et al., (.2006) reported that low level of adiponectin was related to an increased number of MS variable in post-menopausal and pre-menopausal women. In post-menopausal

women with MS had lower adiponectin levels as compared with pre-menopausal women with MS. They showed that the inverse relationship of adiponectin to leptin in post-menopausal women. They had suggested that leptin is associated with several MS components while the adipcyokines appears to play a role only in post-menopausal women.

In the study of blood leptin and adiponectin as possible mediators of the relation between fat mass and BMD in peri-menopausal women. They shown that serum leptin levels were negatively correlated with BMD (beta = -0.005, p = 0.027) and TBBMC (beta = -14.32, p = 0.013). The above correlation observed only when serum insulin levels were included, as an independent variable, in the regression analysis model. Adiponectin was not significantly correlated with BMD(L2-L4) nor with TBBMC, either in the presence or absence of insulin. They reported that circulating adiponectin does not show any effect on bone mass. While circulating leptin showed a negative correlation with bone mass which is dependent on serum insulin levels.(Kontogianni MD et al,.2004).

Tanja Adam et al,.(2010) reported that in pre-menopausal women negative mood and nocturnal urinary epinephrine were significantly associated to adiponectin independent of BMI. While they observed in post-menopausal women, negative mood was not related with adiponectin cross sectionally, while the negative mood was significant predictor for lower levels of adiponectin one year later, independent of intial adiponectin concentration and changes in body mass index. They also observed the depressive order was associated with the lower levels of adiponectin.

In this study theleptinlevelsandthe Leptin/adiponectin ratiosweresignificantlyhigher inecidentcasesofendometrial cancer (8.2±0.5, 2.05±1.08 ng/ml) thanin the controls subjects(4.5±0.5,0.98±0.18,Pb0.0001), whereasthe adiponectinlevelsweresignificantlylowerinthe incidentcases(6.2±0.4μg/ml)thaninthe controlsubjects(9.0±0.4μg/ml,Pb0.0001).Forthe incidentcases,the serumlevelsofthe adipokineswere significantlycorrelatedwiththe patientbodymassindex(BMI)(Pb0.001forleptin,Pb0.05foradiponectin), andthe leptin levelsand the lepti/adiponectinratiosweresignificantlyassociated withthe homeostasismodelassessment ratio(HOMA-R)andthe fastinginsulinlevels(Pb.001).Higherleptin/adiponectin ratioswerefoundto be significantly correlatedwithanincreasedrisk ofendometrialcancer[OR(95%CI)forthe top

vsthe bottomtertileofthe leptin/adiponectinratiowas 6.0(3.2–11.9),P-valueb0.0001].Moreover,the ORsoft the leptin/adiponectinratioswerehigherthanthoseof leptinor adiponectinalone.The associationofthe leptin/adiponectin ratioswithendometrialcancerrisk remainedafter adjustingfor the obesity indices,hypertension andpresenceofdiabetesmellitus. Their result showed that leptin/adiponectin ratio was independently correlated with an increased risk for endometrial cancer development.(Naohiro Ashizawa et al,.2010).

Samir Ben Ali et al,(.2011) worked on the relationship of plasma leptin and adiponectin concentration with menopausal status in Tunisian women. They suggested that pre-menopausal women had significantly higher leptin and L/A ratio and lower adiponectin levels as compared with post-menopausal women. The effect of menopause on the mean values of BMI, insulin or HOMA-IR, HDL cholestrol and TG had not found. They used multiple linear regression model, menopausal status was identified, as significant independent predictor for leptin and adiponectin levels. In menopausal status of obese women showed higher leptin and L/A ratio and lower levels of adiponectin by comparison with normal weight subjects.

Sumika Mastsui et al,.(2013) studied that the serum adiponectin level in late menopausal women was high as compared with early post-menopausal women.The level of adiponectin did not show a significant association with glomerular filtration rate(eGFR) in late post-menopausal women. While the levels of adiponectin in late post-menopausal women showed a significant negative association with triglyceride(TG) and positive correlation with high density lipo-protien cholesterol (HDL-C) after adjustments for age and BMI. Their result showed that in the lat post-menopausal women with normal renal function, high adiponectin levels is related with favorable lipid profiles and high adiponectin levels may just not involved in eGFR but also other factors in late post-menopausal women.

Park YW et al,.(2003) studied that diponectin has been postulated to act an important role in the modulation of glucose and lipd metabolism in insulin-sensitive tissue in both humans and animals(Yamauchi et al., 2001).Decreased adiponectin levels have been demonstrated in

genetic and diet- induced murine models of obesity.as well as in diet-induced forms of human obesity(Arita et al.,1999).Low adiponectin levels have also been strongly implicated in the development of insulin resistance in mouse models of both obesity and lipoatrophy(Yamauchi et al,. 2001).

Kannel WB ET et al,.(1994) studied that the relevant importance of factors that influence cardiovascular risk in postmenopausal women are unknown. Alterations in lipid metabolism with estrogen deficiency are thought to be a substantial component of CVD risk in postmenopausal women , but there are also direct effects of estrogen deficiency on body fat distribution (central obesity), insulin action, the arterial wall, and fibrinolysis that may influence cardiovascular risk. These factors contribute to an increased prevalence of the metabolic syndrome in postmenopausal women compared with premenopausal women .

E. Kassi et al,.(2010) studied that NGT, hsCRP was positively correlated with fasting leptin and HOMA, while in subjects with IGT negatively with QUICKI. In both groups they were reported that hsCRP was positively associated with fasting insulin, body mass index (BMI) and waist circumference. They observed that the fasting adipopnectin was positively correlated with high density lipo-protien (HDL) in both groups and negatively with triglyceride in subjects with NGT as well as with serum glucose levels at time 120 min of OGTT in subjects with IGT. The association between oxidized LDL and adipokines was not found, while between oxidized LDL and HOMA was a positive association in subjects with IGT.

5.MATERIALS AND METHODS

This study examined the patients who attended the Gynecology Department in Near East Hospital in Turkish Republic Of Northern Cyprus between January 2014 andJune 2014. The population consisted of 160 subjects divided into two group,70 menopausal patients and 90 healthy control subjects. The control group of healthy patients without any history of menopausal stage. All subjects provided written informed conset were the study, and the study was approved by our Local Research Ethic Committee. General health characteristics such as age, sex, smoking status, and alcohol consuption were investigated by self administed questionaire.

The height (m), weight (kg) and waist circumference (cm) of each subject were recorded and body mass index (BMI) was calculated (kg/m2).

Blood samples were drawn from the antecubital vein, after overnight fasting and centrifuged at 4000 rpm for ten (10) minutes and separated. The serum samples were stored at -80°**C** until they were analyzed for adiponectin.

5.1 General Laboratory Equipment

- Centrifuge
- Automated spectrophotometer
- Automated chemistry analyzer
- Immunoassay analyzer
- Hot plate withrrer
- Vortex
- Sensitive Electronic balance
- Refrigerator
- Beakers 100 ml, 250 ml
- Dark bottle 250 ml
- Volumetric Flask 100 ml
- Micropipettes(50-200)µl,(100-1000)µl

5.3 Laboratory Analyses

The levels of serum glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), ALT, AST, uric acid and low-density lipoprotein cholesterol (LDL-C) were determined using a fully automated clinical chemistry analyzer(Roche Diagnosticscubes 6000).

5.4.Adiponectin analysis.

In the BioVendor Human Adiponectin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human adiponectin antibody. After 60 minutes incubation and washing, polyclonal anti-human adiponectin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured adiponectin. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of adiponectin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5.5.Technical Hints

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

5.7. PREPARATION OF SAMPLES

The kit measures adiponectin in serum, plasma (EDTA, citrate, heparin, cerebrospinal fluid (CSF). Samples should be assayed immediately after collection or should be stored at -20°C. Mixthoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles,which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

5.8. Serum and plasma samples

Dilute serum and plasma 300x with the Dilution Buffer prior to the assay in two steps:

5.8.1. Dilution A (10x):

Add 10 µl of samples to 90 µl of Dilution Buffer. **Mix well** (not to foam).

5.8.2. Dilution B (30x):

Add 10 µl of Dilution A into 290 µl of Dilution Buffer to prepare final dilution (300x). **Mix well** (not foam). One step-dilution can be performed (add 5 µl of samples to 1495 µl of Dilution Buffer). Beware of imprecision in pipetting and mix the samples very thoroughly!

5.9. Assay Procedure

5.9.1 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 tha 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.

5.9.2.Test Procedure

Adiponectin levels are significantly lower (2-3 orders of magnitude) in breast milk, urine and CSF than in serum and plasma. Therefore, different protocols have to be used.

5.9.2.1. Protocol (a)

For serum and plasma samples:

Sample dilution is 300x

Standard range is 5-100 ng/ml (the Standards of 150 ng/ml and/or 2 ng/ml can be added optionally) Incubation with Substrate Solution is 10 minutes

1. Pipet 100µl of Standards (5-100 ng/ml for serum and plasma samples, 1-50 ng/ml for milk, urine and CSF samples), diluted Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 µl of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for 10 minutes (serum and plasma samples) or 25-30 minutes (milk, urine and CSF samples) at room temperature (20-30°C). The incubation time may beextended [up to 20 minutes for serum and plasma samples or up to 50 minutes for milk, urine and CSF samples] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the color development by adding 100 µl of Stop Solution.
10. Determine the absorbance of each well using a micro plate reader set to 450 nm,

preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650nm). Subtractreadings at 630 nm (550 - 650 nm) from the readings at 450 nm.Theabsorbance should be read within 5 minutes following step 9.

6. RESULTS AND DISCUSSION

6.1. Overview

Using the analysis results towards the end on the last chapter, it may be deduced that many of the procedures in the hospital that takes reasonable length of time ought to be reduced, and the taken results according to the AdiponectinELISA are explained here.

6.2. Parameters

A clinical characteristics of the study groups, as well as laboratory variables are shown in Table 5.1. No significant differences were observed between the patients and the control groups in all of these parameters.

Table 6.1:Study Table

Variables	**Control Subjects N = 90**	**Patient Subjects N =70**	**P Value At ∞ = 0.10**
BMI (Kg/m²)	23.32±1.93	28.30±4.25	P = 0.00021
Waist (cm)	85.23±6.53	110.23±10.20	P < 0.0001
Hip (cm)	99.22±5.33	122.15±11.20	P < 0.0001
Adiponectin (µg/ml)	5.56 ± 0.865	6.45 ± 1.01	P < 0.001
Cholesterol (mg/dl)	179.23±20.33	240.23±22.30	P < 0.0001
LDL-Cholesterol (mg/dl)	118.10±22.55	145.22±13.20	P < 0.0001
HDL-Cholesterol (mg/dl)	50.22±11.08	43.22±12.10	P < 0.0001

Triglyceride (mg/dl)	89.22±22.15	182.10±12.10	P < 0.0001
Glucose (mg/dl)	89.25±5.39	105.23±13.20	P < 0.0001

(P value < 0.10 considered significant)

6.3. Results

The result was conducted by using the statistical Analysis in calculating the P values for patient and control subjects of all the different variables including BMI, Waist, Hip, glucose, HDL, LDL cholesterol, Triglyceride. As the number of patient subjects is 70 and the number of control subjects is 90. As the number of both the test groups is more the 30 so for this purpose the Z test was used and then P value was calculated for each the respective variables for both patient and control subjects at 90 percent with confidence interval by using the two tailed test.

It was found that all the varibales BMI, Waist, Hip, glucose, cholesterol, LDL cholesterol, Triglyceride with both the parameters were statistically significant at an alpha 90 percent which means that these variables affects the Adiponectin in the menopausal woman.

Table 6.2: Adiponectin level in the study group

	Patient Subjects (n = 70)	**Control Subjects (n = 90)**	**P value**
Adiponectin (µg/ml)	6.45 ± 1.01	5.56 ± 0.865	P < 0.0001

Table 6.3: Control Level and laboratory variables in MENUPAUSE patients

		Correlation coefficient	**P Value**
1	Age	0.273	0.055
2	BMI	-0.201	0.161

3	Adiponectin	-0.047	0.745

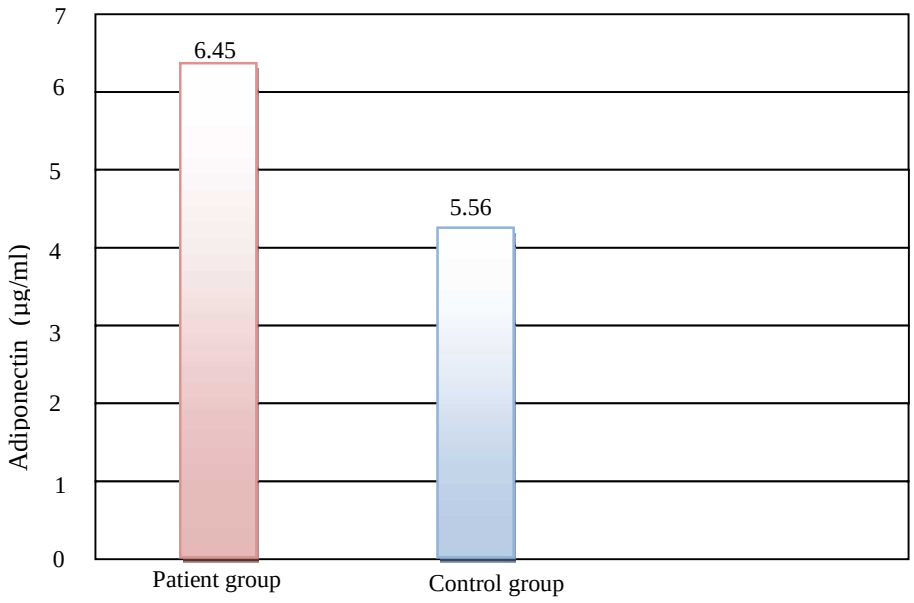


Figure4: Adiponectin in Patients and Control Groups

The obtained results are presented in Table 6.3 and Figure 4 showed that adiponectin level in patients with MENUPAUSE was 5.56 ± 0.865 µg/ml. The P value of the Adiponectin is less then 0.0001 which means that Adiponectin is statistically significant and affects the menopausal women.

6.4. Discussion

Adiponectin is a protein hormone which has important role in metabolic process. These are the glucose regulation and fatty acid catabolism and they areproduced fromadipose tissue into the bloodstream and its very abundant in plasma relative to many hormone (Takashi and Toshimasa,2005). Adiponectin has beneficial effects on obesity-related medical complications. In contrast to other adipokines, circulating adiponectin levels are reduced in obesity, type 2 diabetes and associated diseases (Li et al.,2009).

The aim of this study was to measure adiponectin levels in North Cyprus females and to see the correlation betweenAge, BMI and adiponectin. A total of 160 North Cyprus females participated in the study, 90 control and 70 patients. Personal information was recorded in specially designated form, anthropometrics measurements were taken, blood pressure was measured and fasting blood samples were collected.Adiponectin level showed a significant positive correlation with age, weight ($P<0.0001$), BMI ($P<0.0001$).

In current study revealed the significant association between plasma adiponectin levels and menopausal status. Indeed, adiponectin levels increased from premenopausal stage to postmenopausal stage. Irrespective of the menopausal status, adiponectin levels were lower in obese subjects with normal weight.

It is common clinical observation that a large number of women gain body weight after menopause. Several clinical studies have demonstrated this effect. (Tchernof A et al.,1998, Kanaley JA et al.,2001). Central obesity increase after menopause, indicating that the loss of estrogen may not only increase body fat and weight, but also change body fat distribution (Lee CG et al., 2009, Lovejoy JC et al., 2008). Accumulation of abdominal fat is associated with an increase in risk for the development of insulin resistance, hypolepidimia,hypertension and cardiovascular disease (Lemieux S et al.,1996).

These progressions bring about loss of the tight input circle in charge of menstrual cycle consistency and changes in the menstrual draining example get to be clear. Eventually, there is

end of menses and estrogen creation. Characteristic menopause has happened when there has been 12 months of amenorrhea after a last menstrual period (Patricia, 2009).

7. Conclusion:

The aim of this study was to measure adiponectin levels in North Cyprus females and to see the correlation between Age, BMI. A total of 160 North Cyprus females participated in the study,90 control subjects and 70 subjects with the menopause. Personal information was recorded in specially designated form, anthropometrics measurements were taken, blood pressure was measured and fasting blood samples were collected.

In conclusion, the adiponectin levels were found to be increased with menopause also increasing the BMI and waist/hip ratio, therefore it can be concluded that menopause affects the metabolism in females. Adiponectin p value is less than 0.0001 which means that Adiponectin is statistically significant in menopause and in order to see this effect in more detail, further cohort study is needed.

REFRENCES

American Heart Association 2001 Heart and stroke statistical update: *American Heart Association. http:*//216.185.102.50/statistics/

Arita,Y.,Kihara,S.,Ouchi,N.,Takahashi,M.,Maeda,K.,Miyagawa,J.,Hotta, K., Shimomura, I., Nakamura,T., &Miyaoka, K., et al. (1999). Paradoxical decrease of an adipose-specific protein,adiponectin,in obesity.*Biochem. Biophys. Res. Commun*.257,79–83.

Austin M, Breslow J, Hennekens C, Buring J, Willett W, Krauss R 1988 Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 260:1917–1921 [CrossRef][Medline]

Austin M, King M-C, Vranizan K, Newman B, Krauss R 1988 Inheritance of low density lipoprotein subclass patterns: results of complex segregation analysis. Am J Hum Genet 43:838–846 [Medline]

Berg AH.Combs TP.,& Scherer PE,2002. Acrp30/adiponectin; an adipocytokine regulating glucose and lipid metabolism.*Trends Endocrinol Metabomlism.*13; 84-89.

Berg GA, Siseles N, Gonzalez AI, Ortiz OC, Tempone A, Wikinski RW 2001 Higher values of hepatic lipase activity in postmenopause: relationship with atherogenic intermediate density and low density lipoproteins. Menopause 8:51–57 [CrossRef] [Medline]

Berg,A.H.,Combs,T.P.,Du,X.L.,Brownlee, M.,& Scherer,P.E.(2001).The adipocyte-secreted protein Acrp30 enhances hepatic insulin action.*Nat.Med.*7,947–953.

Bjorkelund C, Lissner L, Andersson S, Lapidus L, Bengtsson C 1996 Reproductive history in relation to relative weight and fat distribution. Int J ObesRelatMetabDisord 20:213–219 [Medline]

Bouchard C 1995 Genetics and the metabolic syndrome. Int J ObesRelatMetabDisord 19(Suppl 1):S52–S59

Bruschi F, Meschia M, Soma M, Perotti D, Paoletti R, Crosignani PG 1996 Lipoprotein(a) and other lipids after oophorectomy and estrogen replacement therapy. ObstetGynecol 88:950–954[CrossRef] [Medline]

Burger HG, Dudley EC, Robertson DM,,& Dennerstein L 2002. Hormonal changes in the menopause transition. *Recent ProgHorm Res* 57:257–275 [CrossRef] [Medline]

Campos H, Genest JJ, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, Wilson PW, Schaefer EJ 1992 Low density lipoprotein particle size and coronary artery disease. ArteriosclerThromb 12:187–195 [CrossRef] [Medline]

Campos H, McNamara JR, Wilson PW, Ordovas JM, Schaefer EJ 1988 Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women.JClinEndocrinolMetab 67:30–35 [Abstract] [Medline]

Carr MC, Brunzell JD Increased hepatic lipase activity and intraabdominal fat across the transition from pre- to postmenopause. Program of the 85th Annual Meeting of The Endocrine Society, Philadelphia, PA, 2003 (Abstract P2-280)

Carr MC, Kim KH, Zambon A, Mitchell ES, Woods NF, Casazza CP, Purnell JQ, Hokanson JE, Brunzell JD, Schwartz RS 2000 Changes in LDL density across the menopausal transition. J Invest Med 48:245–250 [Medline]

Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX 1997Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. Am J Epidemiol146:483–494 [CrossRef] [Medline]

Combs,T.P.,Pajvani,U.B.,Berg,A.H.,Lin,Y.,Jelicks,L.A.,Laplante,M.,Na-wrocki,A.R., Rajala,M.W.,Parlow,A.F., &Cheeseboro,L.,etal.(2004).A trans- genic mouse with a deletion in the collagenous domain ofadiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocri- nology* 145,367–383.

Crawford SL, Casey VA, Avis NE, McKinlay SM 2000 A longitudinal study of weight and the menopause transition: results from the Massachusetts Women's Health Study. Menopause 7:96–104 [CrossRef] [Medline]

Dallongeville J, Marecaux N, Isorez D, Zylbergberg G, Fruchart JC, Amouyel P 1995Multiple coronary heart disease risk factors are associated with menopause and influenced by substitutive hormonal therapy in a cohort of French women. Atherosclerosis 118:123–133 [CrossRef][Medline]

DeNino WF, Tchernof A, Dionne IJ, Toth MJ, Ades PA, Sites CK, Poehlman ET 2001Contribution of abdominal adiposity to age-related differences in insulin sensitivity and plasma lipids in healthy nonobese women. Diabetes Care 24:925–932 [CrossRef] [Medline]

Despres JP 1993 Abdominal obesity as important component of insulin-resistance syndrome.Nutrition 9:452–459 [Medline]

Do KA, Green A, Guthrie JR, Dudley EC, Burger HG, Dennerstein L 2000 Longitudinal study of risk factors for coronary heart disease across the menopausal transition. Am J Epidemiol151:584–593 [CrossRef] [Medline]

Ferrara CM, Lynch NA, Nicklas BJ, Ryan AS, Berman DM 2002 Differences in adipose tissue metabolism between postmenopausal and perimenopausal women. J ClinEndocrinol Metab87:4166–4170 [Abstract] [Medline]

Gideen R.Hajer, Timan W. van Haeften.,& Frank L.J. Visseren.(2008).Adipose tissue dysfuntion in obesity.diabetes, and vasular diseases.5 September 2008.

Gohlke-Barwolf C 2000 Coronary artery disease: is menopause a risk factor? Basic Res Cardiol 95(Suppl 1):I77–I83

Guo SS, Zeller C, Chumlea WC, Siervogel RM 1999 Aging, body composition, and lifestyle: the Fels Longitudinal Study. Am J ClinNutr 70:405–411 [Medline]

Guthrie JR, Ball M, Dudley EC, Garamszegi CV, Wahlqvist ML, Dennerstein L, Burger HG2001 Impaired fasting glycaemia in middle-aged women: a prospective study. Int J ObesRelatMetabDisord 25:646–651 [CrossRef] [Medline]

Hayley KC Julie W, Sarah K, Fiona S, Ayanthi AR., &Jonathan PW.2010. Erp46 bindsto AdipoR1, but not AdipoR2, and modulates adiponectin signaling.*Biochemical and Biophysical Research Communications*. 392(2); 234-239.

Hokanson JE, Austin MA 1996 Plasma triglyceride is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 3:213–219 [CrossRef] [Medline]

Hu,E.,Liang,P.,and Spiegelman, B.M.(1996).AdipoQ is a novel adipose- specific gene dysregulated ino besity.*J.Biol.Chem.* 271,10697–10703.

Hyun JP, Young MK, Cho HK.,& Myeong HJ.2010. ATF3 negatively regulates adiponectinreceptor 1 expression.*Biochemical and Biophysical Research Commuincations*.400;72-7.

Janowitz WR, Agatston AS, Kaplan G, ViamonteJr M 1993 Differences in prevalence and extent of coronary artery calcium detected by ultrafast computed tomography in asymptomatic men and women. Am J Cardiol 72:247–254 [CrossRef] [Medline]

Jenner JL, Ordovas JM, Lamon-Fava S, Schaefer MM, Wilson PW, Castelli WP, Schaefer EJ 1993 Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. The Framingham Offspring Study. Circulation 87:1135–1141 [CrossRef] [Medline]

Jensen J, Nilas L, Christiansen C 1990 Influence of menopause on serum lipids and lipoproteins. Maturitas 12:321–331 [CrossRef] [Medline]

Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Steppan CM. Abdominal fat distribution in pre- and postmenopausal women: the impact of physical activity, age, and menopausal status. Metabolism 2001;50:976–82

Kannel WB, Cupples LA, Ramaswami R, Stokes JD, Kreger BE, Higgins M 1991 Regional obesity and risk of cardiovascular disease; the Framingham Study. J ClinEpidemiol 44:183–190[CrossRef] [Medline]

Kannel WB, Hjortland MC, McNamara PM, Gordon T 1976 Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med 85:447–452 [CrossRef] [Medline]

Kannel WB, Wilson PW 1995 Risk factors that attenuate the female coronary disease advantage. Arch Intern Med 155:57–61 [CrossRef] [Medline]

Khandouzi ,M.,Morteza.K,Syed Mehdi Defashti and Dr.ManabDeka.(2013) *International Journal of Scientific and Engineering Reseach*,volume 4(11),ISSN 2229-5518.

Kim,J.-Y.,vandeWall,E.,Laplante,M.,Azzara,A.,Trujillo,M.E.,Hofmann,S.M.,Schraw,T.,Durand, J.L.,Li,H.,&Li,G.,etal.(2007).Obesity-associated improvements in metabolic profile through expansion of adipose tissue.*J. Clin.Invest.* 117,2621–2637.

Krotkiewski M, Bjorntorp P, Sjostrom L, Smith U 1983 Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. J Clin Invest 72:1150–1162[CrossRef] [Medline]

Kubota,N.,Terauchi,Y.,Yamauchi,T.,Kubota,T.,Moroi,M.,Matsui,J.,Eto,K.,Yamashita,T.,Kamon, J.,&Satoh,H.,etal.(2002).Disruption of adiponectin causes insulin resistance and neointimal formation. *J.Biol.Chem.*277,25863–25866.

Kuller L, Meilahn E, Lassila H, Matthews K, Wing R 1997 Cardiovascular risk factors during first five years postmenopause in nonhormone replacement users. In: Forte T, ed. Hormonal, metabolic, and cellular influences on cardiovascular disease in women. Armonk: Futura; 273–287

Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT 2002 The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288:2709–2716 [CrossRef] [Medline]

Lamarche B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP 1997Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. ArteriosclerThrombVascBiol 17:1098–1105[CrossRef] [Medline]

Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L 1984 Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J 289:1257–1261 [CrossRef][Medline]

Lee CG, Carr MC, Murdoch SJ, Mitchell E, Woods NF, Wener MH, et al. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. J Clin Endocrinol Metab 2009;94:1104–10.

Lemieux S, Prud'Homme D, Bouchard C, Tremblay A, Despres JP. A single threshold value of waist girth identifies normal weight and overweight subjects with excess visceral adipose tissue. Am J Clin Nutr 1996;64:685–93. Inoue M, Yano M, Yamakado M, Maehata E, Suzuki S. Relationship between the

Lewis-Barned NJ, Sutherland WH, Walker RJ, Walker HL, De Jong SA, Edwards EA, Markham VH 1999 Plasma cholesterol esterification and transfer, the menopause, and hormone replacement therapy in women. J ClinEndocrinolMetab 84:3534–3538 [Abstract] [Medline]

Li S, shin HJ, Ding EL., &van DRM.2009. Adiponectin levels and risk of type 2 diabetices; a synthematic review and meta-analysis. JAMA.302; 179-88.

Li Z, McNamara JR, Fruchart JC, Luc G, Bard JM, Ordovas JM, Wilson PW, Schaefer EJ1996 Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes.J Lipid Res 37:1886–1896 [Medline]

Lindheim SR, Buchanan TA, Duffy DM, Vijod MA, Kojima T, Stanczyk FZ, Lobo RA 1994Comparison of estimates of insulin sensitivity in pre- and postmenopausal women using the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. J SocGynecol Invest1:150–154 [Medline]

Lindquist O 1982 Intraindividual changes of blood pressure, serum lipids, and body weight in relation to menstrual status: results from a prospective population study of women in Goteborg, Sweden. Prev Med 11:162–172 [CrossRef] [Medline]

Lovejoy JC, Champagne CM, DeJorge L, Xie H, Smith RS.Increased visceral fat and decreased energy expenditure during the menopausal transition. Int J Obes 2008;32:949–58.

Lubo RA menopause; endocrinology, consequences of estrogen deficiency effect of hormone replacement therapy, treatment regimens. Katz VI, Lentz GM, Lobo RA, Gershenson DM, editors comprehensive gynocolgy. *5th Edition.Philadelphia; Mosby Elservier;* 2007.p.1039-7.

Lynch NA, Ryan AS, Berman DM, Sorkin JD, Nicklas BJ Comparison of VO2max and disease risk factors between perimenopausal and postmenopausal women. Menopause 9:456–462

Mackey RH, Kuller LH, Sutton-Tyrrell K, Evans RW, Holubkov R, Matthews KA 2002Lipoprotein subclasses and coronary artery calcium in postmenopausal women from the healthy women study. Am J Cardiol 90:71i–76i [CrossRef]

Maeda,K.,Okubo, K.,Shimomura, I.,Funahashi,T.,Matsuzawa,Y.,&Mat- subara, K.(1996).cDNA cloning and expression of a novel adipose specific collagen-likefactor, apM1 (AdiPose Most abundant Gene transcript1).*Bio- chem.Biophys. Res. Commun.*221,286–289.

Maeda,N.,Shimomura,I.,Kishida,K.,Nishizawa,H.,Matsuda,M.,Nagare-tani,H.,Furuyama,N.,Kondo, H.,Takahashi,M., &Arita,Y.,etal.(2002).Diet-induced insulin resistance in mice lacking adiponectin/ACRP30.*Nat. Med*.8,731–737.

Manju C**,** Susan AP, Theodore C and Robert RH**.** 2003 .Adiponectin: More Than Just Another Fat Cell Hormone.*Diabetes Care*. 26(8) : 2442-50

Matthews KA, Kuller LH, Sutton-Tyrrell K, Chang YF 2001 Changes in cardiovascular risk factors during the perimenopause and postmenopause and carotid artery atherosclerosis in healthy women. Stroke 32:1104–1111 [CrossRef] [Medline]

Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR 1989 Menopause and risk factors for coronary heart disease. N Engl J Med 321:641–646 [CrossRef] [Medline]

Mauriege P, Imbeault P, Prud'Homme D, Tremblay A, Nadeau A, Despres JP 2000Subcutaneous adipose tissue metabolism at menopause: importance of body fatness and regional fat distribution. J ClinEndocrinolMetab 85:2446–2454 [Abstract] [Medline]

Meigs JB 2002 Epidemiology of the metabolic syndrome, 2002. Am J Manag Care 8(Suppl 11):S283–S296

Nakano,Y.,Tobe, T.,Choi-Miura, N.H.,Mazda,T.,& Tomita, M.(1996). Isolation and characterization of GBP28, anovelgelatin-bindingprotein puri- fied from human plasma.*J.Biochem*.120,803–812.

National Cholesterol Education Program 2001 Executive Summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 285:2486–2497[CrossRef] [Medline]

Nawrocki, A.R.,Rajala, M.W.,Tomas,E.,Pajvani, U.B.,Saha,A.K.,Trumba-uer, M.E., Pang,Z., Chen, A.S., Ruderman,N.B.,& Chen, H., et al. (2006). Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activatedreceptor gamma agonists.*J.Biol.Chem.* 281,2654–2660.

Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H, Nagaretani H, Matsuda M, Kondo H, Furuyama N, Kihara S, Nakamura T, Tochino Y, Funahashi T, Matsuzawa Y 2002Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes51:2734–2741 [CrossRef] [Medline]

Otto TC.,&Lan MD.(2005). Adipose tissue developmenty from stem cell to adipocyte.*crit Rew BiochemMolBiol*,40.229-242.

Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB 2003 The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. Arch Intern Med 163:427–436[CrossRef] [Medline]

Pascot A, Despres JP, Lemieux I, Almeras N, Bergeron J, Nadeau A, Prud'homme D, Tremblay A, Lemieux S 2001 Deterioration of the metabolic risk profile in women. Respective contributions of impaired glucose tolerance and visceral fat accumulation. Diabetes Care 24:902–908[CrossRef] [Medline]

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.

Poehlman ET 2002 Menopause, energy expenditure, and body composition. ActaObstetGynecolScand 81:603–611 [CrossRef] [Medline]

Poehlman ET, Toth MJ, Ades PA, Rosen CJ 1997 Menopause-associated changes in plasma lipids, insulin-like growth factor I and blood pressure: a longitudinal study. Eur J Clin Invest 27:322–326 [CrossRef] [Medline]

Poehlman ET, Toth MJ, Gardner AW 1995 Changes in energy balance and body composition at menopause: a controlled longitudinal study. Ann Intern Med 123:673–675 [CrossRef] [Medline]

Pouliot MC, Despres JP, Nadeau A, Moorjani S, Prud'Homme D, Lupien PJ, Tremblay A, Bouchard C 1992 Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. Diabetes 41:826–834 [CrossRef] [Medline]

Razay G, Heaton KW, Bolton CH 1992 Coronary heart disease risk factors in relation to the menopause. Q J Med 85:889–896 [Medline]

Reaven GM 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes37:1595–1607 [CrossRef] [Medline]

Sahar K, Felicity JR, Hayley KC, Nicole LS, Julie W, Yu HK, Choaping N, Robert GP., & Jonathan PW. 2013. Characterisation N-terminal region of AdipoR2 prevents its expression at the cell-surface. *Biochem Biophys Res Commun.*432(1);28-33.

Santamarina-Fojo S, Haudenschild C, Amar M 1998 The role of hepatic lipase in lipoprotein metabolism and atherosclerosis. CurrOpinLipidol 9:211–219 [CrossRef] [Medline]

Scherer,P.E.,Williams,S.,Fogliano,M.,Baldini,G.,&Lodish,H.F.(1995).A novel serum protein similar to C1q,produced exclusively in adipocytes.*J.Biol. Chem.* 270,26746–26749.

Selby JV, Newman B, Quiroga J, Christian JC, Austin MA, Fabsitz RR 1991 Concordance for dyslipidemic hypertension in male twins. JAMA 265:2079–2084 [CrossRef] [Medline]

Shlipak MG, Simon JA, Vittinghoff E, Lin F, Barrett-Connor E, Knopp RH, Levy RI, Hulley SB 2000 Estrogen and progestin, lipoprotein(a), and the risk of recurrent coronary heart disease events after menopause. JAMA 283:1845–1852 [CrossRef] [Medline]

Soules MR, Sherman S. Parrott E, Rebar R, Santoro N., &Utian W.et al. Execulsive Summary stages of Reproductive aging workshop (STRAW). Climateric 2001;4(4);267-72.

Stevenson JC, Crook D, Godsland IF 1993 Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis 98:83–90 [CrossRef] [Medline]

Sutton-Tyrrell K, Lassila, HC, Meilahn E, Bunker C, Matthews KA, Kuller LH 1998 Carotid atherosclerosis in premenopausal and postmenopausal women and its association with risk factors measured after menopause. Stroke 29:1116–1121 [CrossRef] [Medline]

Takashi K and Toshimasa Y.2005.Adiponectin and Adiponectin Receptors.*Endoer Rev*.26 (3);439-51.

Tchernof A, Poehlman ET. Effects of the menopause transition on body fatness and body fat distribution. Obes Res 1998;6:246–54.

The women Health Council, menopause A Guide, july 2008.Lund KJ. Menopause and Menopausal transition. *Med Clin North Am* 2008;92; 1253-71.

Tikkanen MJ, Kuusi T, Nikkila EA, Stenman UH 1986 Variation of postheparin plasma hepatic lipase by menstrual cycle. Metabolism 35:99–104 [CrossRef] [Medline]

Toth MJ, Sites CK, Eltabbakh GH, Poehlman ET 2000 Effect of menopausal status on insulin-stimulated glucose disposal: comparison of middle-aged premenopausal and early postmenopausal women. Diabetes Care 23:801–806 [CrossRef] [Medline]

Toth MJ, Tchernof A, Sites CK, Poehlman ET 2000 Effect of menopausal status on body composition and abdominal fat distribution. Int J ObesRelatMetabDisord 24:226–231 [CrossRef][Medline]

Vague J 1956 The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. Am J ClinNutr 4:20[Medline]

Walton C, Godsland IF, Proudler AJ, Wynn V, Stevenson JC 1993 The effects of the menopause on insulin sensitivity, secretion and elimination in non-obese, healthy women. Eur J Clin Invest 23:466–473 [CrossRef] [Medline]

Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB 1999 Clustering of metabolic factors and coronary heart disease. Arch Intern Med 159:1104–1109 [CrossRef] [Medline]

Witteman JC, Grobbee DE, Kok FJ, Hofman A, Valkenburg HA 1989 Increased risk of atherosclerosis in women after the menopause. Br Med J 298:642–644 [CrossRef] [Medline]

Yamauchi,T.,Kamon,J.,Waki,H.,Imai,Y.,Shimozawa,N.,Hioki,K.,Uchida,S.,Ito,Y.,Takakuwa,K., &Matsui,J.,etal.(2003a).Globular adiponectin pro- tectedob/obmice from diabetes and ApoE-deficientmice from atheroscle- rosis. *J.Biol.Chem.* 278,2461–2468.

Yamauchi,T.,Kamon,J.,Waki,H.,Terauchi,Y.,Kubota, N.,Hara,K.,Mori,Y., Ide,T.,Murakami, K., &Tsuboyama-Kasaoka,N.,etal.(2001).Thefat-derived hormone adiponectin reverses insulin resistance associated with both lipoa- trophy and obesity. *Nat.Med.*7,941–946.

Zamboni M, Armellini F, Milani MP, De Marchi M, Todesco T, Robbi R, Bergamo-Andreis IA, Bosello O 1992 Body fat distribution in pre- and post-menopausal women: metabolic and anthropometric variables and their inter-relationships. Int J ObesRelatMetabDisord 16:495–504[Medline].