ABSTRACT

Title of Document: HYPERLEPTINEMIA, METABOLIC SYNDROME, AND MORTALITY IN OLDER ADULTS.

Suruchi Mishra, PhD, 2010

Directed By: Dr. Nadine R. Sahyoun, Ph.D., R.D. and Dr. Mira Mehta, Ph.D.
Department of Nutrition and Food Science

Background: Abdominal adiposity and fat mass increase with aging, and as does insulin resistance which is frequently associated with hyperleptinemia and leptin resistance. Serum leptin may predict risk of metabolic syndrome and mortality among older adults.

Objectives: The objectives of the present study were to evaluate the relationship of serum leptin with risk of metabolic syndrome and mortality and to examine these associations in relation to the measures of body adiposity and proinflammatory cytokines. The influence of leptin receptor (I/D) gene polymorphism on diabetes as a contributing cause of mortality was also examined. Gender specific serum leptin cut off values as a biomarker for the risk of metabolic syndrome were determined.

Design: The Health, Aging and Body Composition (HABC) study is a prospective cohort of 3,075 older adults aged 70 to 79 years. Body composition, demographic information, biochemical variables including, markers of systemic inflammation, and genetic variation were assessed in detail.

Results: Women in quintile 2, 3, 4 and 5 of serum leptin were at significantly lower risk for metabolic syndrome as compared to those in quintile 1 after controlling for confounders. Serum leptin was independently associated with risk of metabolic syndrome after sequentially adjusting for demographic variables ($p<0.0001$), fat depots ($p=.0024$), and proinflammatory cytokines ($p=.0098$) in women. Among men, the association between serum leptin and risk of metabolic syndrome was explained by body adiposity. Women in quartile 2 and 3 of serum leptin were at lower risk than women in quintile 1 for all-cause mortality and mortality from cardiovascular disease independent of body fat and proinflammatory cytokines. Additionally, elevated level of serum leptin was associated with increased risk for diabetes as a contributing cause of mortality for both genders after sequentially adjusting for potential confounders, body fat and proinflammatory cytokines. Significant interaction was found between leptin receptor genotype and total percent fat ($p=0.008$) in association with diabetes as a contributing cause of mortality among women. The cut off serum leptin level that suggests the possible risk of metabolic syndrome was determined to be 6.45 ng/ml with 60% sensitivity and 63% specificity among men and 18.25 ng/ml with 55% sensitivity and 62% specificity among women.

Conclusion: Elevated levels of serum leptin may be associated with increased risk of metabolic syndrome and risk of diabetes as a contributing cause of mortality among older women. However, intermediary levels of serum leptin may lower the risk of all-cause mortality and mortality from CVD, suggesting a paradoxical association of serum leptin with cardiovascular risk factors and mortality from CVD among older women.

HYPERLEPTINEMIA, METABOLIC SYNDROME, AND MORTALITY IN OLDER ADULTS.

By

Suruchi Mishra

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2010

Advisory Committee:
Professor Nadine R. Sahyoun, Chair
Professor Mira Mehta, Co-Chair
Professor Mark Kantor
Professor Thomas W. Castonguay
Professor Stephen M. Roth

Acknowledgements

I would like to express the deepest gratitude to Dr. Nadine Sahyoun for her insightful advice, unflinching encouragement and giving me extraordinary experiences throughout my research. Her perpetual energy and enthusiasm in research has motivated all her advisees, including me. Without her guidance and persistent help this dissertation would not have been possible.

I gratefully acknowledge Dr. Mira Mehta for her valuable guidance, constant support and trust in me from the beginning. I would also like to thank my other committee members: Dr. Thomas W. Castonguay, Dr. Mark Kantor and Dr. Stephen M. Roth for their critical scientific advice and willingness to share their bright thoughts.

In addition, I would also like to thank all of the faculty members, and staff in the Department of Nutrition and Food Science for their support. I extend my appreciation to my lab group, including Dr. Amy Anderson for sharing her knowledge and experiences.

Finally, I also give my heartfelt thanks to my husband and daughter and my parents in India for their unflagging love, support and encouragement.

Special thanks go to the Department of Nutrition and Food Science at the University of Maryland for providing me financial support during the doctoral program.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Based on the National Cholesterol Education Program, Adult Treatment Panel III definition, slightly more than one-third of the adults in United States have been characterized as having metabolic syndrome (1). The prevalence of metabolic syndrome increased from 20.3% and 15.6% among males and females, respectively, under 40 years of age to 51.5% and 54.4% for males and females, respectively, 60 years of age and older (1) **(Figures 1.1).** This is an important issue because the population of older adults is expanding and as this trend which is projected to continue **(Figures 1.2)** (3 and 5), will concurrently increase the prevalence of age-associated diseases (2 and 4).

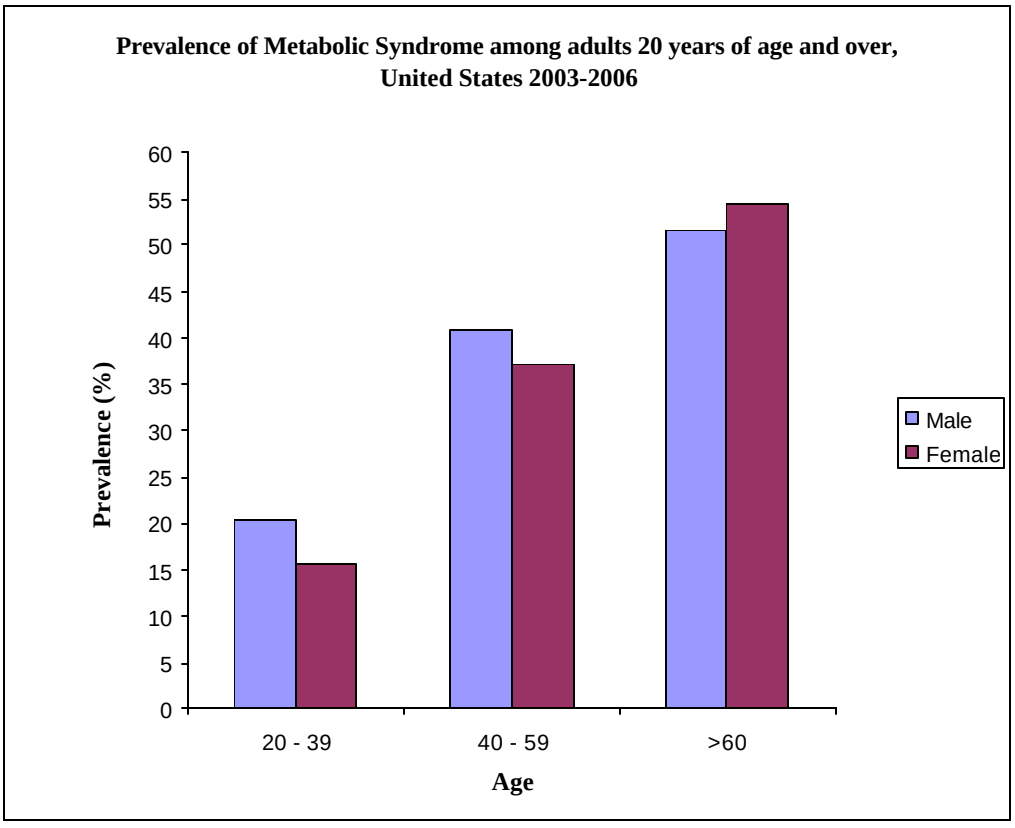

Figure 1.1 Prevalence of Metabolic Syndrome Among Adults 20 Years of Age and Over, 2003-2006 (1)

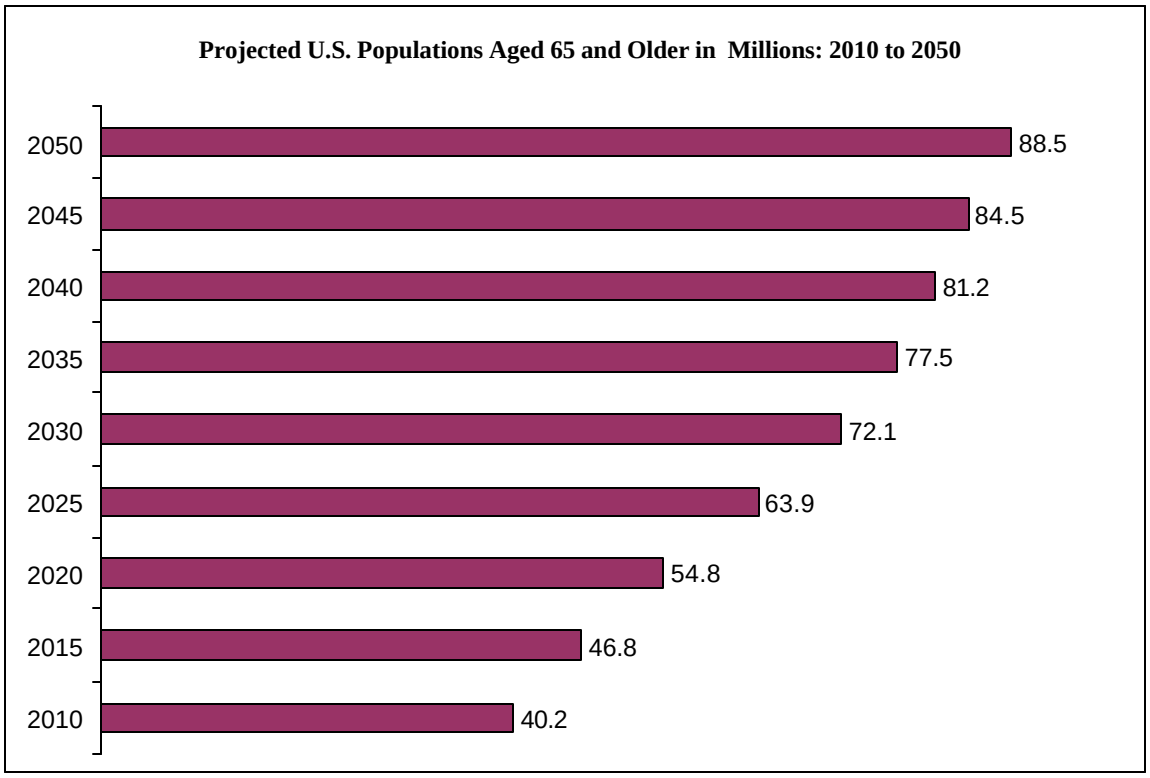

Figure 1.2 Projected U.S. Populations Aged 65 and Older: 2010 to 2050 (5)

In the past decade, identification of the hormone leptin in adipose tissue established the latter as a metabolically active organ that plays a crucial role in food intake and energy expenditure (6). Recently, elevated levels of serum leptin in obese state have been shown to result in the development of leptin resistance (7 and 8). This has drawn considerable attention to the role of hyperleptinemia on the risk of metabolic diseases and survival. There is a concomitant increase in serum leptin levels and in the expression of suppressor of cytokine signaling 3 (SOCS3) (11 and 12) along with the amplification in fat mass and insulin resistance (9 and 10) among older adults, suggesting that aging is associated with leptin resistance, a mediator of metabolic disorders (9 and10).

Serum leptin reflects the amount of energy stored in body. Elevated levels of serum leptin leads to accumulation of lipid in non-adipose tissue such as liver, pancreas, and muscles and is

linked to impaired insulin action (14) and insulin resistance (15). Leptin resistance in pancreatic ß - cells contributes to dysregulation of adipoinsular axis leading to hyperinsulinemia and type 2 diabetes (16). Leptin is not only implicated in the lipotoxicity of peripheral tissues but, has also been shown to play a critical role in the inflammatory response because of structural and functional similarities with the IL-6 family of cytokines (7). A parallel increase in proinflammatory cytokines and serum levels of leptin has been associated with aging, dementia, atherosclerosis, metabolic syndrome (7 and 13) and mortality.

Several previous epidemiological studies have linked serum leptin levels to indicators of health and showed that elevated levels of serum leptin increase the risk of metabolic diseases cross-sectionally (17, 18, and 19). It would be interesting to see if serum leptin is prospectively associated with the risk of metabolic syndrome among older adults. Pro-inflammatory cytokines are related to serum leptin levels and have been implicated in the pathogenesis of several chronic diseases and mortality, but the contribution of pro-inflammatory molecules to the association of serum leptin with metabolic syndrome and mortality has not been explored in previous studies. Although several studies have shown positive association between hyperleptinemia and risk of cardiovascular disease (17, 18, and 19), some have also reported protective effect of leptin in heart failure (61 and 62). Studying the association of serum leptin with metabolic syndrome and mortality in context of body adiposity and inflammatory factors will provide a complete picture of the role of serum leptin on the risk of metabolic syndrome and mortality.

The objectives of the present study are:

1. To determine the prospective association of serum leptin with metabolic syndrome and to examine whether these associations are influenced by:

- Measures of body adiposity such as, BMI, total percent fat, visceral fat, and subcutaneous fat
- Markers of inflammation such as, C-reactive protein, plasminogen activator inhibitor -1, tumor necrosis factor – a, and interleukin - 6

2. To examine the association between serum leptin with all-cause mortality, mortality from cardiovascular disease and from diabetes as a contributing cause of mortality and by leptin receptor (I/D) genotype. These associations were examined by:

- Total percent fat
- Markers of inflammation such as, C-reactive protein, plasminogen activator inhibitor -1, tumor necrosis factor – a, and interleukin - 6

3. To determine the gender specific cut off point of serum leptin as a biomarker of metabolic syndrome among older adults.

Chapter 2: Literature Review

A. Leptin and Metabolic Health

Leptin has been associated with inflammation, obesity, hypertension and insulin sensitivity, all of which are believed to increase risk of several chronic diseases and mortality (29, 33, 36, 51 and 60). Because of its association with several components of metabolic diseases, circulating levels of serum leptin may be a potential predictor of metabolic syndrome and play an important role in survival.

What is Leptin?

Leptin is a 16-kDa adipocyte-derived protein hormone. It is comprised of four a-helices and two short ß-strands that contain an intra-chain disulfide bond responsible for its biological activity (20). Its structure suggests that it belongs to the cytokine family (7). White adipose is the principle source of circulating leptin. Lesser amounts are also synthesized in and secreted from the hypothalamus, pituitary, skeletal muscle, and bone, arterial endothelium, intestines, fetus, testes and placenta (20).

Leptin signaling occurs when leptin attaches to its receptor. The leptin receptor (Ob-R) is a member of the cytokine receptor family and is encoded by the db gene (20). While there are several forms of Ob-R (20 and 21), ObRb is main mediator of leptin signaling in several tissues (20, 12 and 11). The receptors for leptin are present in many areas of the brain, including the hypothalamus, cerebellum, cortex, hippocampus, thalamus, and brain capillary endothelium (21). These are also expressed in peripheral tissues, including the lung, kidney, liver, pancreas, adrenals, ovaries, hematopoietic stem cells, and skeletal muscles (20 and 21).

Functions of Leptin

In hypothalamus, leptin interacts with the receptors on neuropeptide Y/ Agouti related peptide (NPY/AgRP) neurons to suppress these orexigenic peptides (7, 22, 23, and 24) and trigger anorexigenic (POMC/CART) peptides (7, 23, and 24). This results in sympathetic stimulation and food intake inhibition (26). However, during the low levels of circulating leptin the orexigenic peptides are stimulated, anorexigenic peptides are inhibited (26) leading to reduced sympathetic activity, reduced energy expenditure and stimulated feeding behaviour (26). In liver and muscle, leptin activates 5' - Adenosine Mono Phosphate – activated Protein Kinase (AMPK). Activated AMPK decreases glucose regulated transcription, protein synthesis, cholesterol synthesis, triglyceride synthesis and increases ß –oxidation, glucose transport and glycolysis (74). Thus, the net action of leptin is to inhibit appetite, stimulate thermogenesis, enhance fatty acid oxidation, decrease glucose, and reduce body weight and fat (22).

Mechanism of Action of Leptin

Binding of leptin to Ob-Rb receptor provides intracellular signaling by activating Janus kinase–signal transducers and activators of transcription (JAK–STAT) pathway via STAT 3 proteins in the hypothalamus. Leptin receptor signaling through the JAK2-STAT3 pathway is under the negative feedback control of suppressor of cytokine signaling 3 (SOCS3) proteins (22, 12, and 11). Binding of Leptin to its receptor induces SOCS3 in NPY and pro-opiomelanocortin (POMC) neurons and over expression of SOCS3 reduces JAK-STAT signaling and inhibits leptin signaling (22 and 12) (Fig 2.1). Expression of SOCS-3 increases

(12 and 11) along with the increase in fat mass and abdominal adiposity during aging, as well as an increase in insulin resistance, suggesting that it could be a mediator of leptin resistance in aging individuals (37 and 57).

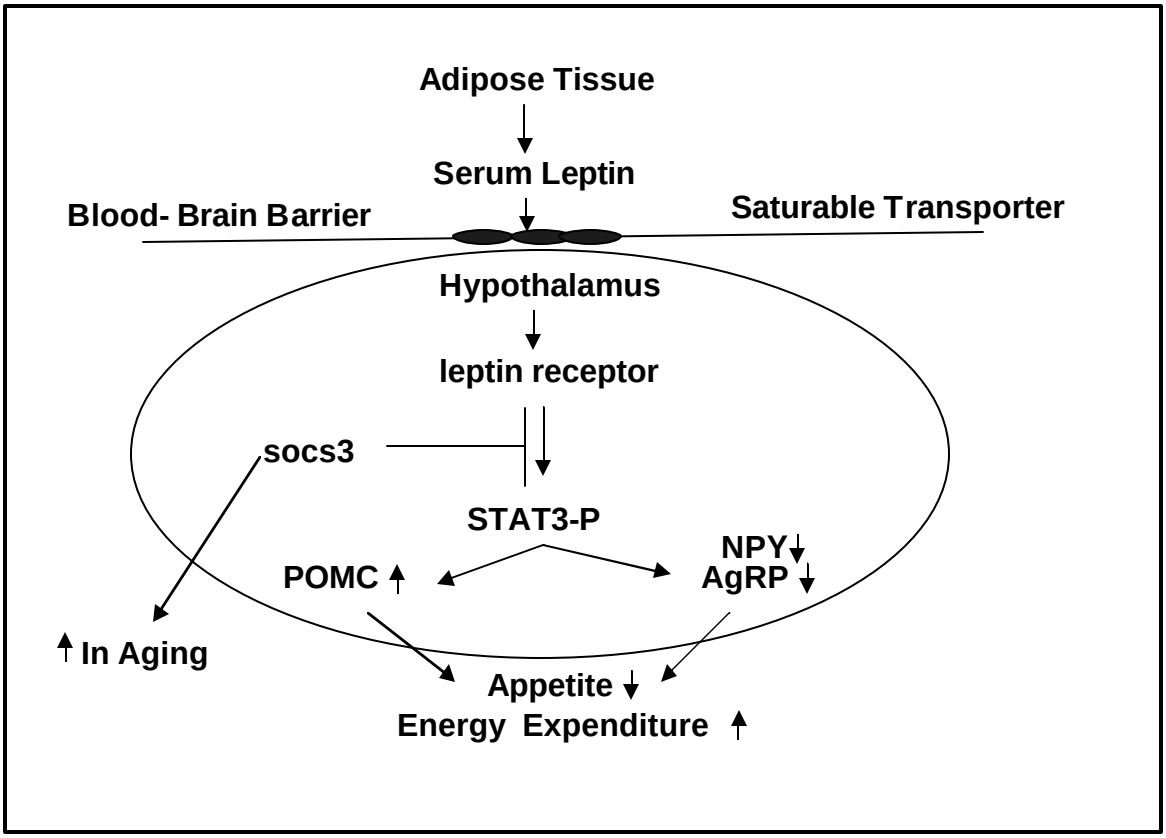

Figure 2.1 Mechanism of Leptin Signaling (25)

Leptin Receptor Polymorphism

Genetic, environmental and life style factors are believed to influence the changes in body composition and risk of metabolic diseases. The leptin receptor gene is a candidate gene for obesity and is located close to the marker (DIS198) of insulin secretion on human chromosome (11). Expression of the leptin receptor gene in pancreatic beta-cells is believed to play an important role in leptin-induced inhibition of insulin secretion (65), resulting in hyperinsulinemia (65 and 66). Both leptin receptor and SOCS3 are expressed in peripheral

tissues such as liver, pancreas, and kidney and increased levels of SOCS3 in these peripheral tissues may result in decreased leptin receptor signaling and leptin resistance (12 and 28). Recently, pentanucleotide insertion in the 3'-untranslated region of the leptin receptor gene has been shown to be associated with weight change (70) and serum insulin levels (67). Similarly, Lakka et al., in a prospective case-control study on middle-aged men, reported lower serum insulin levels and type 2 diabetes (T2D) risk among participants homozygous or heterozygous for insertion allele (I/I or I/D) as compared to those who were homozygous for deletion allele (D/D) (68 and 69). Furthermore, in a study on overweight postmenopausal women, leptin receptor gene polymorphisms were shown to be associated with abdominal fat (71), while variations in DNA sequence in the leptin receptor gene was suggested to play a role in body fat distribution (71). Thus, the association of leptin receptor (I/D) polymorphism with body adiposity and serum insulin level suggests a potential role of this genotype on T2D risk.

Inflammation

Serum leptin has been shown to play a critical role in inflammatory activity (7) and has been linked to serum levels of markers of systemic inflammation such as C reactive protein (CRP), tumor necrosis factor – a (TNF- a), and interleukin 6 (IL6) (33, 34, and 35). In vitro, leptin has been shown to play a critical role in the inflammatory response (7) by augmenting the proliferation of leukocytes and controlling the humoral inflammatory responses via its effect on T-cells, endothelial cells, monocytes, and neutrophils (35). Gainsford et al., demonstrated that leptin is associated with increased production of pro-inflammatory cytokines such as TNF-a, IL-6 and IL-12 in monocytes and macrophages (75). Similarly, several previous studies have shown that there is a concomitant increase in the serum levels of leptin, TNF- a, IL-6, CRP and Serum Amyloid A (34, 37, 39, and 51) during aging, sepsis,

dementia and atherosclerosis (33, 35, 38, 39, and 51). Furthermore, increased levels of serum leptin (49), IL-6 (30), TNF- a, (31), elevated acute phase proteins (32), and increased levels of markers of endothelial cell dysfunction and activation (29) have all been linked to human obesity (29 and 32).

Metabolic Syndrome

Both adipokines and pro-inflammatory cytokines have been implicated in the etiology of metabolic syndrome (40 and 41). Leptin directly inhibits insulin secretion from pancreatic ß- cells (46). Elevated serum leptin levels are associated with fasting insulin, insulin resistance (HOMA-IR), and total cholesterol (40, 42, 43, 45, 47 and 136). Leptin mediated dysregulation of body fat distribution has a cause-effect relationship between age-associated increase in visceral fat and the decrease in hepatic insulin action (44), which are major determinants of the metabolic syndrome (137 and 144). Recombinant human leptin given by subcutaneous injection (0.01 mg/kg and 0.03 mg/kg twice daily) for successive 3 month periods has been shown to lower visceral fat, improve dyslipidemia and insulin sensitivity in patients with HIV-associated lipoatrophy (142). In a crossectional study on healthy male subjects, Francisco et al. found that plasma leptin concentration was associated with the metabolic disturbances that constitute the metabolic syndrome, including over all and central obesity, elevated blood pressure, insulin resistance, hyperinsulinemia, high plasma triglycerides, and elevated serum uric acid (40). Ruige JB et al, in a large population study, confirmed a positive correlation between serum leptin and basal insulin and found relationships between serum leptin and triacylglycerols independent of gender and BMI (48). Similarly among postmenopausal women, Lichnovska et al. showed the positive association between serum leptin and insulin resistance independent of increase in BMI (17). Furthermore, Buettener et al. proposed that relative hyperleptinemia (leptin levels above the 95th percentile of leptin by BMI) may be a

new component of the metabolic syndrome among subjects aged 49.4 ±0.3. They also suggested that subjects with relative hyperleptinemia develop the metabolic syndrome at an earlier age (50).

Survival

Serum leptin has been implicated in development of several age-associated chronic diseases such as atherosclerosis, insulin resistance, diabetes, and osteoporosis (17, 36, 52, 53, and 54). Leptin also plays a critical role in energy intake (55 and 56), inflammatory response (55) and is strongly correlated with body fat (17, 57, 58, and 59), all of which are linked to mortality (34). These correlations suggest a potential role of serum leptin in mortality. Several previous studies have implied that serum leptin is associated with all-cause mortality and CVD mortality (60 and 61). In Framingham Heart Study, Lieb et al. found a U-shaped association between serum leptin and all-cause mortality with elevated risk of death at both low and high levels of leptin (60). Additionally, hypoleptinemia has been shown to elevate the risk of cancer mortality (63), while hyperleptinemia increased the risk of T2D mortality (62). Similarly in a longitudinal study on middle age women, moderately elevated serum leptin was inversely associated with CVD mortality after adjusting for adiposity (64). Furthermore in a PROspective Study of Pravastatin in the Elderly at Risk trial (PROSPER), Welsh et.al. found an independent association between T2D risk and serum leptin after adjusting for potential confounders and BMI (62).

B. Rationale

Although, leptin was shown to be associated with the increased production of pro-inflammatory cytokines (34, 35, and 59) that are linked to obesity (29 and 32), the process of

aging (37 and 39), atherogenic activity (31 and 54), metabolic syndrome (40 and 50), and mortality (34), the association of serum leptin with metabolic syndrome and mortality independently of pro-inflammatory compounds has not been explored in the previous studies. Therefore, the current study examines the associations of serum leptin with metabolic syndrome, all-cause mortality, and mortality from CVD, as well as with diabetes as a contributing cause of death before and after adjusting for inflammatory factors and body adiposity among functionally well older adults. While leptin receptor gene polymorphism has been shown to be associated with serum insulin levels (67 and 69) and body adiposity (70 and 71), none of the previous studies explored the multiplicative effect of leptin gene polymorphism and body adiposity on the risk of T2D. Because the leptin receptor has structural and functional similarity with other members of cytokine family such as IL-6 (7), the influence of the proinflammatory cytokines on the association of leptin receptor genotype with diabetes as a contributing cause of death, was additionally explored in the present study.

Chapter 3: Methods

A. The Health, Aging and Body Composition (Health ABC) Study Design

The Health, Aging and Body Composition (Health ABC) study is a longitudinal cohort study of community dwelling, well functioning older adults initiated by the Laboratory of Epidemiology, Demography, and Biometry of the National Institute on Aging (NIA) of the National Institutes of Health (NIH). The objective of Health ABC is to investigate the associations between changes in body composition, incident functional limitation, behavioral factors, and weight-related health conditions.

At baseline, between April 1997 and June 1998, 3075 participants aged 70-79 years were recruited in the study from a random sample of white residents receiving Medicare benefits and all age-eligible black residents of Pittsburgh, Pennsylvania and Memphis, Tennessee. The sample included 48% men and 52% women at baseline. Of the men, 33% were African Americans and 67% Caucasians, while among women, 46% were African Americans and 54% Caucasians. Subjects were considered eligible to participate in the study if they reported no difficulty walking a quarter of a mile, climbing 10 steps without resting, or performing basic activities of daily living, were free of life-threatening illness, and planned to remain in the geographic area for at least 3 years. Those who reported active treatment for cancer or participation in diet or exercise intervention were excluded from the study. Participants gave written informed consent, and protocols were approved by the Institutional Review Boards at the Universities involved in the two study sites. A home interview was administered to collect information on demographic, socioeconomic factors, and health behaviors. A clinic visit was conducted at baseline to collect blood samples, gather information on measures of body composition, and obtain weight-related health conditions,

and physical function. Subsequently, follow-up clinical examinations were conducted annually, and telephone interviews semi-annually.

B. Data Preparation

Data obtained at baseline and through year 10 were analyzed for this study. The Health ABC study consists of extensive information on anthropometric, biochemical, clinical, demographic and genetic variables. Data on specific prevalent diseases at baseline, indicators of the incidence of these same diseases during follow-up, and mortality are also available. Reliability and reproducibility of anthropometric, biochemical, dietary and clinical data collection methods were evaluated by Health ABC study. The Health ABC data set is sorted by a unique enrollment identification number (HABCID) that is assigned to each participant. In our study, we used the HABCID to extract the variables of interest from each data set and merge the files. In the final data set, main variables of interest were recoded to either reduce the number of groups within categorical variables, or to exclude missing values from the analysis. The cytokines, included in the study, were not normally distributed and were log-transformed. All the analyses were performed using SAS (version 9.1; SAS Institute Inc., Cary, NC).

C. Statistical Methods

Analysis of Baseline Characteristics

Because both low and high levels of serum leptin have been shown to be associated with metabolic disorders (47 and 48), participants were grouped either in quintiles or quartiles of serum leptin depending upon the sample size of the analysis. Grouping observations according to serum leptin quintiles and getting separate parameter estimates for each level not only mitigated the lack of linearity between leptin and logit of response variable but, also provided more information on the association of leptin with the response variable. Since serum

leptin levels have been shown to vary by gender (72), all the analyses were performed separately between men and women. Baseline characteristics of the participants in quintile 2, 3, 4 and 5 were compared with those in quintile 1 by Dunett's test for continuous variables and chi-square test for categorical variables. Potential confounders included in the study were age, gender, race, study site, education, smoking status, alcohol use, physical activity, calorie intake, hormonal replacement therapy, fasting insulin, number of hours fasted before blood was drawn, markers of systemic inflammation (CRP, TNF- a, IL-6 and PAI-1) and body adiposity.

Multivariate Logistic Regression Model

Logistic regression allows prediction of discrete variables by a combination of continuous and discrete predictors. It is frequently used by researchers because it is not based on any assumption regarding linear relationship between response and predictor variable, normal distribution of response variables or error terms, and homogeneity of variance for response variable. However, it does assume a linear relationship among the explanatory variable and the logit of the response variable. When the assumption of linearity in the logits is violated, then logistic regression underestimates the degree of relationship between the explanatory and the response variable and thus, lacks power. All the explanatory variables were checked for multicolinearity.

Multivariate logistic regression models were constructed to determine the association between baseline serum leptin quintiles and risk of developing metabolic syndrome at year 6 after adjusting for potential confounders. Trend analyses were performed to explore the linear relationship between metabolic syndrome and serum leptin after adjustment for potential confounders.

Cox Proportional Hazard Regression Model

Cox proportional hazard regression model is used for analysis of time until an event or time between events. One or more predictor variables are used to predict an event (response) variable. The event variable is analyzed in relation to a time variable and a hazard or survival rate is the main outcome. A hazard rate at a specified time is the probability of an event occurring in that time period, given survival through all prior time intervals. A hazard ratio is the estimate of the ratio of the hazard rate in one group to the hazard rate in another group. For continuous variables, hazard ratio is the ratio of the hazard rate given one unit increase in the covariate to the hazard rate without such an increase. The proportional hazard model is not based on any assumptions concerning the nature or shape of the underlying survival distribution. However, its main assumption is the proportionality assumption which specifies that the ratio of the estimated hazards over time will be constant. To check the proportionality assumption, the association between covariates and the survival time variable was investigated by computing and plotting the estimates of the survival distribution function. Test of equality across strata was also considered to select the predictors for the final model.

Cox proportional hazard regression analysis was utilized to assess the relationship between serum leptin and all-cause mortality, and mortality from cardiovascular disease. The same statistical method was applied to examine the association between leptin, leptin receptor gene polymorphism and diabetes as a contributing cause of death. Trend analyses were performed to explore the linear relationship between serum leptin and mortality after adjustment for potential confounders.

Receiver Operating Characteristic (ROC) Curves

ROC curves are useful for assessing the accuracy of a diagnostic test in discriminating healthy and diseased individuals. Measures that are commonly used for the

performance of a test are the sensitivity and specificity. Sensitivity, also called the true positive rate (TPR), is the probability of diagnosing the presence of disease when it is actually present. Specificity, also called true negative rate (TNR) is the probability of identifying the absence of disease when it is not present. The ROC curve is a graphical plot of the sensitivity (along the vertical axis) against 1 minus the specificity (along the horizontal axis).The ideal sensitivity and specificity is one. A diagonal line on the graph from the lower left hand corner to the upper right hand corner reflects a test with no discriminating power. The closer the graph gets to the upper left hand corner, the better the test is at discriminating between disease cases and noncases. ROC curve enables us to select a specific value of an indicator variable to use as a threshold that provides a desired trade off in sensitivity and specificity. A lower threshold cut-off results in a higher sensitivity and lower specificity. In other words, the number of false-negatives decreases and the number of false-positives increases. Inversely, a higher threshold cut-off value results in a lower sensitivity and higher specificity.

The ROC curve was used to determine optimal cut off point of serum leptin concentration for diagnosis of metabolic syndrome cross-sectionally. The gold standard for the ROC curve was based on the presence of the metabolic syndrome at year 6. Multivariate logistic regression analysis was used to assess the relative risks of developing metabolic syndrome at year 6 among persons below the cut off value as compared to those above the cut off value, after adjustment for potential confounders.

Chapter 4: Hyperleptinemia, Adiposity and Risk of Metabolic Syndrome in Older Adults

Abstract

Background: Total fat mass and particularly abdominal adiposity increase with aging, and as does insulin resistance which is frequently associated with leptin resistance and high leptin levels.

Objectives: This study investigates the prospective association between serum leptin and the risk of metabolic syndrome and examines this association in relation to measures of body adiposity and proinflammatory cytokines.

Design: The Health, Aging and Body Composition (HABC) study is a prospective cohort of 3,075 older adults aged 70 to 79 years. Partcipants' body mass index, total percent fat, visceral fat, and subcutaneous fat were assessed. Plasminogen activator inhibitor I, interleukin-6, C-reactive protein, and tumor necrosis factor-a were measured by enzyme linked immunosorbant assay (ELISA).

Results: Serum leptin quintile 2 (p=.008), 3 (p=.009), 4 (p=.0002), and 5 (p<.0001) were found to be significantly associated with the risk of metabolic syndrome as compared to quintile 1 after adjusting for potential confounders (age, gender, race, study site, education, smoking status, alcohol use, physical activity, and calorie intake) among women. Serum leptin remained independently associated with the risk of metabolic syndrome after adjusting for fat depots (p=.0024) and proinflammatory cytokines (p=.0098), in women. Among men, association between serum leptin and metabolic syndrome risk was not independent on body adiposity.

Conclusion: Among older adult women, elevated concentrations of serum leptin may increase the risk of developing metabolic syndrome independent of body adiposity and proinflammatory cytokines.

Introduction:

Leptin is an adipocyte derived polypeptide hormone that influences feeding behavior through its direct effect on the hypothalamus (6). Serum levels of leptin reflect the amount of energy stored in adipose tissues (7). Previous studies have reported that serum leptin levels decrease with aging and that older adults have lower levels of circulating leptin as compared to younger adults (9, 76, and 38). However, recent studies refuted these previous findings and demonstrated that aging is accompanied by an increase in serum leptin levels (36 and 37). Leptin plays a critical role in the inflammatory response (35), apparently due to the structural and functional similarities of leptin and its receptor with the IL-6 family of cytokines (7). A concomitant increase in TNF- a and serum leptin with aging has been associated with obesity and atherosclerosis (7 and 38).Both adipokines and pro-inflammatory cytokines are implicated in the etiology of insulin resistance (40) and metabolic syndrome (40 and 41). Leptin directly inhibits insulin secretion from pancreatic ß- cells (46) and elevated serum leptin levels are shown to be associated with fasting insulin, insulin resistance (HOMA-IR), and total cholesterol (40, 42, 43, 45, 136 and 137), that are the core metabolic disturbances of the metabolic syndrome. It has been also demonstrated in elegant experiments on rodent models that failure of leptin to "cross talk" with other fat depots to regulate fat distribution has a cause-effect relationship between the age-related increase in visceral fat and decrease in hepatic insulin action (44), major determinants of the metabolic syndrome (137 and 144).

Although the proinflammatory cytokines are associated with serum leptin and are implicated in the development of metabolic syndrome, the prospective association of serum

leptin with metabolic syndrome was not explored in light of proinflammatory cytokines and body fat depots among older adults.

The primary objective of this study is to examine the association between serum leptin and the development of metabolic syndrome over a 6-year follow-up among a cohort of older adults and examine whether such an association may be independent of markers of inflammation and body fat.

Subjects and Methods

Study Design

The Health, Aging and Body Composition (Health ABC) study is a longitudinal cohort study of 3,075 community dwelling, well functioning white and black men and women aged 70 to 79 years at the commencement of the study. Participants were recruited for the study from a random sample of white residents receiving Medicare benefits and all age-eligible black residents of Pittsburgh, Pennsylvania, and Memphis, Tennessee. Subjects were considered eligible to participate in the study if they reported no difficulty walking a quarter of a mile, climbing 10 steps without resting, or performing basic activities of daily living, were free of life-threatening illness, and planned to remain in the area for at least 3 years. Those who reported active treatment for cancer or participation in diet or exercise intervention were excluded from the study. Participants gave written informed consent, and protocols were approved by the Institutional Review Boards at the two study sites. A home interview was administered to collect information on demographic, socioeconomic factors, and health behaviors. A clinic visit at baseline was conducted for examination of biological variables, body composition, weight-related health conditions, and physical function between April 1997 and June 1998. Thereafter, follow-up clinical examinations have been conducted annually, and interviews semi-annually.

Subjects

In the present study data from baseline and year 6 of the Health ABC study were used. The exclusion criteria were having prevalent metabolic syndrome at baseline (n = 773); having an energy intake of less than 800 kcal/day or more than 4,000 kcal/day among men (n = 88) and an energy intake of less than 500 kcal/day or more than 3,500 kcal/day (n = 64) among women; and if the participants had missing or implausible values for the metabolic syndrome at year 6 (n = 850) or missing control variables such as serum leptin and BMI (n = 180).

Biochemical Variables

Participants underwent venipuncture at baseline visit after an overnight fast, and serum samples were frozen at -70°C. Plasma glucose levels were measured using an automated glucose oxidase reaction (YSI 2300 Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH). Triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) levels were measured using a chemical analyzer (Vitros 950; Johnson & Johnson, Raritan, NJ). Both interleukin-6 (IL-6) and tumor necrosis factor- a (TNF- a) levels were measured in duplicate using an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The limit of detection was 0.10 pg/ml for IL-6 and 0.18 pg/ml for TNF- a. The level of plasminogen activator inhibitor-1 (PAI-1) was measured in citrated plasma samples using a 2-site enzyme-linked immunosorbent assay according to previously published methods (91). The assay had coefficient of variation of 3.5%. Serum levels of C-reactive protein (CRP) were also measured in duplicate by enzyme-linked immunosorbent assay based on purified protein and polyclonal anti-CRP antibodies (Calbiochem, San Diego, California) with a coefficient of variation (CV) of 8.0%. Serum concentrations of leptin were measured in duplicate by means of radioimmunoassay (Linco Research Inc, St Charles, Mo). The minimum concentration detectable in serum with use of this assay is 0.05 ng/ml.

Intraassay CVs ranged from 3.7% to 7.5%, and interassay CVs ranged from 3.2% to 8.9%. The assay is highly specific for human leptin and shows minimal reactivity with mouse or rat leptin.

Anthropometric and Clinical Variables

Weight was measured using a standard balance beam scale in kilograms. Height was measured twice using a Harpenden stadiometer (Holtain Ltd., Crosswell, U.K.) in centimeters and the average of the two measurements was used. BMI [weight (kg)/height (m^2)] was calculated. At baseline, and year 6 of the Health ABC study, axial computerized tomography (CT) scanning of abdomen of the participants was performed. Abdominal visceral and subcutaneous fat were quantified from scans, performed on a General Electric 9800 Advantage (Milwaukee, WI) in Pittsburgh and a Siemens Somatom Plus (Iselin, NJ) and Picker PQ2000S (Cleveland, OH) in Memphis. CT scans were analyzed by the CT Scan Reading Center (CTSRC) of the University of Colorado Health Sciences Center according to a standardized protocol (77). Dual energy x-ray absorptiometry (DXA) (Hologic QDR 4500A, software version 8.21, Hologic, Waltham, MA) was used to assess the total fat mass and total percentage of body fat was calculated (77). Systolic and diastolic blood pressures were measured by manual sphygmomanometer in a seated position.

Demographic and Life Style Variables

A standardized questionnaire was administered at baseline to collect information on socio-demographic variables including age, gender, self-identified race and years of education, and lifestyle variables including smoking status and average alcohol consumption (0,1-2 or >2 drinks/day) during the past year. Cigarette packs smoked per day were multiplied by the number of years of smoking to calculate the pack-years over a lifetime of cigarette smoking. Level of physical activity was obtained using a standardized questionnaire specifically

designed for the Health ABC study. The frequency, duration, and intensity level of activities such as self-report of walking and exercise were recorded, and approximate values of metabolic equivalent unit (MET) were assigned to each activity category to estimate weekly energy expenditure in kcal/kg/week (78).

Outcome Variable

In Health ABC metabolic syndrome at year 6, was defined according to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) as the presence of 3 or more of the 5 risk components as follows: 1) waist circumference >102 cm for men and >88 cm for women; 2) TG =150 mg/dl; 3) HDL-C <40 mg/dl for men and <50 mg/dl for women; 4) blood pressure (BP) = 130/85 mmHg or currently on antihypertensive medicine(s); 5) fasting glucose =110 mg/dl or currently using anti-diabetic (insulin or oral agents) medication.

Statistical Analysis

Serum leptin values and body fat vary by gender, therefore, the analysis were performed separately by gender. Observations were grouped according to the quintiles of serum leptin level. Baseline characteristics of men and women were examined by quintiles of serum leptin levels. For continuous variables Dunnett's test and for categorical variables chi-square test was used to compare the means of quintiles 2 through 5 to those of quintile 1. All adipokines were log transformed because they were not normally distributed. The interaction of serum leptin with race, pro-inflammatory cytokines, and adiposity measures were tested and found to be non significant. Multivariate logistic regression modeling was used to determine the association between baseline serum leptin and risk of developing metabolic syndrome at year 6 follow up. Persons in quintiles 2 through 5 of serum leptin were compared with those in quintile 1, in 3 different models after adjustment for descriptive variables (age, gender, race,

study site, education, smoking status, alcohol use, physical activity, calorie intake, hormonal replacement therapy and number of hours fasted when blood was drawn), markers of systemic inflammation (CRP, TNF- a, IL-6 and PAI-1) and body fat (abdominal fat, total fat percent, BMI). The final model included the main predictor variable and covariates that were significantly associated with the outcome. Log transformed leptin was used in regression model for trend analysis. To further study the effect of body adiposity on the association of serum leptin with metabolic syndrome, stratified by two BMI categories (BMI = 25 and BMI>25), multivariate logistic regression analysis were run by BMI categories adjusting for potential confounders. Statistical significance was set at $p = 0.05$, and analysis was performed using SAS (version 9.1; SAS Institute Inc., Cary, NC).

Results

There was a significant difference ($p<0.0001$) between the mean serum leptin concentrations among men 7.1 (±5.8) and women 18.7 (±13.3). Baseline characteristics indicate that among men, those in quintile 1 had significantly lower BMI, total fat percent, abdominal visceral and subcutaneous fat as compared to those in quintile 2, 3, 4, and 5 **(Table 4.1)**. Individuals with leptin in quintile 1 also had significantly lower abdominal circumference than those in quintile 3, 4, 5 and lower fasting glucose, C- reactive protein and PAI-1 than quintile 4 and 5. Additionally, men in quintile 1 had significantly lower IL-6 than men in quintile 4 and lower TNF-a than men in quintile 5.

The baseline characteristics of women are shown in **Table 4.2**. Women in quintile 1 had significantly lower BMI, total fat percent, abdominal visceral and subcutaneous fat than women in all other quintiles. The number of whites in quintile 1 was significantly higher as compared to quintile 3 and 4. Those in quintile 3 and 4 were less likely to complete high school than those in quintile 1. Women in quintile 1 also had significantly lower IL-6 than women in quintile 4 and lower TNF-a than women in quintile 5. Additionally, quintile 1 had significantly lower systolic blood pressure than quintile 2 and significantly lower fasting glucose, C- reactive protein and PAI-1 than quintile 4 and 5.

In multivariate analysis, men in quintile 5 of serum leptin were at higher odds (OR = 2.69; 95% CI = 1.29 – 5.64) of developing metabolic syndrome at year 6 as compared to those in quintile 1 after adjusting for potential confounders. However, when the analysis was additionally adjusted for BMI and cytokines (model 2 and 3, respectively), serum leptin was no longer associated with the risk of metabolic syndrome **(Table 4.3)**.

Among women, those in quintile 2 (OR = 0.46; 95% CI = 0.24 - 0.85) and 3 (OR = 0.45; 95% CI = 0.24 - 0.83) of serum leptin were at lower risk of metabolic syndrome than women in quintile 5 in model 1. When the analysis was adjusted for BMI, women in quintile 2

(OR = 0.61; 95% CI = 0.30 - 1.22) and 3 (OR = 0.55; 95% CI = 0.28 - 1.07) of serum leptin were no longer at lower risk of metabolic syndrome in model 2 compared to women in quintile 5. With the further adjustment for proinflammatory cytokines in model 3, women in quintile 2 (OR = 0.65; 95% CI = 0.32 - 1.32) and 3 (OR = 0.58; 95% CI = 0.29 - 1.14) of serum leptin were still not at lower risk of metabolic syndrome than women in quintile 5 of serum leptin. Women in quintiles 2 (OR = 3.29; 95% CI = 1.36 - 7.95), 3 (OR = 3.25; 95% CI = 1.33 - 7.93), 4 (OR = 5.21; 95% CI = 2.16 - 12.56), and 5 (OR = 7.97; 95% CI = 3.30 - 19.24) of serum leptin were at higher odds of developing metabolic syndrome at year 6 as compared to those in quintile 1 after adjusting for potential confounders. In model 2, when the analysis was additionally adjusted for BMI individuals whose leptin values were in quintiles 2-5, were still at higher odds of developing metabolic syndrome at year 6 as compared to quintile 1 (OR = 3.04; 95% CI = 1.21 - 7.62, OR = 2.94; 95% CI = 1.12 - 7.71, OR = 4.56; 95% CI = 1.65 - 12.61, and OR = 6.38; 95% CI = 2.09 - 19.42), respectively. Adjusting for cytokines in addition to BMI and other potential confounders in model 3, did not affect the significance of the positive association between serum leptin and the risk of metabolic syndrome at year 6. There was a significant positive trend between metabolic syndrome risk and the serum leptin quintiles in model 1 (p=0.0001), 2 (p=0.0024), and 3 (p=0.0098) **(Table 4.4)**.

Association of serum leptin with metabolic syndrome may be confounded by body adiposity due to its correlation with serum leptin. Therefore, to examine the independent relationship between serum leptin and risk of metabolic syndrome, the sample was stratified by 2 BMI categories (BMI = 25 and BMI > 25). Among women, serum leptin was significantly associated with the risk of metabolic syndrome at year 6 in both BMI groups after adjusting for potential confounders. Among men, no association was found in either BMI category between serum leptin and metabolic syndrome after adjusting for potential confounders **(Table 4.5)**.

Discussion

In the present study, serum leptin concentrations were associated with the risk of developing metabolic syndrome among older women independent of age, race, site, education, physical activity, smoking, drinking, hormonal replacement therapy, and hours of fasting. These associations remained significant when the measures of body adiposity and the pro-inflammatory cytokines were controlled for. Women in quintile 1 of serum leptin levels were at lower risk for developing metabolic syndrome as compared to women in all other quintiles.

Some longitudinal studies of serum leptin and metabolic disease have found a significant positive association. In middle-age men, serum leptin formed a part of the cluster that constituted insulin resistance and hyperinsulinemia independent of measures of body fat (40). Franks et al., in the Medical Research Council Ely Study of middle age subjects, found leptin to be positively related to the metabolic syndrome risk after adjusting for obesity (79). Several cross-sectional studies have linked serum leptin levels to individual components of the metabolic syndrome (40, 42, 17, 18, 19, 80, 81 and 132). High blood pressure and serum insulin have been shown to be positively associated with hyperleptinemia, while serum triglyceride level was negatively associated (18, 17, 19, 80, and 81). A study on hypertensive older adults reported that serum leptin and systolic blood pressure are positively associated among women while serum leptin is associated with diastolic blood pressure among men (81). Although, leptin has been shown to stimulate the production of the pro-inflammatory cytokines (39) that are linked to obesity (44), process of aging (7), atherogenic activity (76) and metabolic syndrome (7 and 76), contribution of the pro-inflammatory molecules to the association of serum leptin and metabolic syndrome was not explored in the aforementioned studies.

Due to the structural and functional similarities of leptin and its receptor with the IL-6 family of cytokines (7), leptin appears to play a critical role in the inflammatory response

(38). Enhanced production of pro-inflammatory cytokines, IL-6, TNF- a, CRP, has been implicated in the development of metabolic syndrome (40, 89 and 90) and diabetes (135). CRP and IL-6 predict the future coronary artery disease (40 and 51) and serum leptin has been suggested to be independently associated with production of C- reactive protein (39 and 139). Although serum leptin has been linked to proinflammatory cytokines, we found a significant association between hyperleptinemia and metabolic syndrome risk independent of proinflammatory cytokines among women, suggesting the potential role of leptin resistance in the development of metabolic disorders.

In the current study, serum leptin remained a potential predictor of metabolic syndrome among individuals with BMI below 25 and those above it, after adjusting for potential confounder among women. In men, the association between serum leptin and the risk of metabolic syndrome appeared to be dependent on body adiposity.

Previous studies on the role of adiposity on the association of serum leptin and metabolic disorders have shown inconsistent results. The Atherosclerosis Risk in Communities (ARIC) study showed the protective effect of elevated leptin on incident diabetes after adjusting for potential confounders and adiposity among both men and women (45), whereas, an inverse association between elevated leptin and the risk for subsequent diabetes was found to be mediated by adiposity and insulin insensitivity among Japanese men (82). A cross-sectional study on Iranian population found an association between serum leptin, insulin resistance and metabolic syndrome to be dependent on central obesity (138). However, analysis was not performed by gender in this study. A recent study from National Health and Nutrition Examination Survey (NHANES) on United States population found a positive association between higher serum leptin and mayocardial infarction or stroke independent of obesity (83).

In addition to the current study, several other studies have suggested that there may be gender differences in the association of serum leptin levels with disease outcome and body

adiposity. As compared to men, elevations in serum leptin and the association between serum leptin and insulin resistance was shown to be independent of total fat mass (84) and BMI (17) among women. Furthermore, in a study on older men, the association of serum leptin with diabetes was mediated by obesity and insulin resistance (49) and the association of serum leptin with lipid parameters was mediated by BMI (40). Compared to older men, higher serum leptin concentrations in older women were not fully explained by visceral and subcutaneous adipose tissues measured by magnetic resonance imaging (MRI) (87) and computed tomography (88).Women have been consistently shown to have higher serum leptin concentrations independent of total body fat mass or percent fat (85, 86, and 72) and it has been proposed that elevated levels of serum leptin can lead to the development of leptin resistance (74 and 129) suggesting that women may be more leptin resistant as compared to men. Thus, the sex difference in the association of serum leptin with metabolic syndrome in our study may be explained by relatively greater leptin insensitivity in women.

Leptin exerts its effects both centrally and peripherally, as it has receptors in the hypothalamus as well as in peripheral organs. Centrally, it affects energy intake and expenditure, body weight and neuro-endocrine functions. In liver and muscle it activates 5' - Adenosine Mono Phosphate – activated Protein Kinase (AMPK), which inhibits acetyl-CoA carboxylase and increases fatty acid oxidation (145). Activated AMPK also decreases glucose regulated transcription, protein synthesis, cholesterol synthesis, triglyceride synthesis and increases glucose transport and glycolysis (74). Several theories have been proposed to explain the mechanism for leptin resistance. Leptin stimulates the production of cytokine signaling 3 suppressors (SOCS-3), which inhibits the leptin signaling by its effect on leptin receptor OBRb. The leptin receptor activates Janus kinase–signal transducers and activators of transcription (JAKSTAT) pathway via STAT3 proteins to provide intracellular signaling (143). The leptin stimulated SOCS-3 down regulates the JAKSTAT pathways and inhibits leptin signaling (13). Expression of SOCS-3 amplifies along with the increase in fat mass and

abdominal adiposity during aging, as well as an increase in insulin resistance, suggesting that it could be a mediator of leptin resistance in aging individuals (9, 10, 11, 12, 129 and 130). Leptin resistance in brain leads to excess triglyceride accumulation in adipose tissue, liver, muscles and pancreas, resulting in impaired insulin sensitivity and secretion (146). Thus, the relatively greater leptin insensitivity among women in our study may be explained by elevated serum leptin levels among women (85, 86, and 72), which may impair leptin signaling due to increased production of SOCS-3 resulting in leptin resistance (143) and accumulation of body fat.

The strength of this study includes the evaluation of the prospective association between the serum leptin and the metabolic syndrome as the data were collected in several waves in Health ABC. Because in the HABC study extensive information was collected on life style and biochemical variables, it was possible to adjust for many potential confounders such as the use of hormonal replacement therapy, number of hours fasted when the blood was drawn, total calorie intake, physical activity unlike the previous studies which adjusted for few confounders. Also, analysis was limited to the variables available in Health ABC. Information on metabolic syndrome variable was available only for baseline and year 6, so metabolic syndrome at year 6 was included in the analysis. Due to the unavailability of longitudinal data on serum insulin it was not possible to explore the prospective association of serum leptin and insulin resistance.

In conclusion, the current study suggests that serum leptin is prospectively associated with the development of metabolic syndrome independent of body adiposity and pro-inflammatory markers among older women. Because the prevalence of metabolic syndrome increases with aging and hyperleptinemia has been linked to metabolic syndrome and its components including blood pressure, adiposity and insulin sensitivity, elevated serum leptin levels may become useful as an indicator of the risk for metabolic syndrome among

older women. If leptin resistance occurs due to hyperleptinemia, leptin sensitizing drugs may be useful in ameliorating the features of metabolic syndrome.

Tables

Table 4.1 Baseline Characteristics of Men by Serum Leptin Quintile [1]

	Serum Leptin Quintile				
	1	**2**	**3**	**4**	**5**
Men (n (%))	110 (19.9)	110 (19.9)	111 (20.1)	110 (19.9)	111 (20.1)
Mean Serum Leptin (ng/ml) [3]	2.14 (±0.05)	3.78 (±0.05) [2]	5.61 (±0.06) [2]	8.13 (±0.09) [2]	15.81 (±0.75) [2]
Demographic and behavioral variables					
Age (years) [3]	75.4 (±0.29)	75.1 (±0.25)	74.9 (±0.29)	75.1 (±0.28)	75.2 (±0.28)
Race (% white)	68	70	74	65	61
Drinking (% any consumption)	65	69	63	58	61
Education (% completed high school)	74	79	75	76	81
Smoking (lifetime pack-years) [3]	18.87 (±2.41)	19.1 (±2.44)	23.9 (±3.05)	20.9 (±3.15)	22.87 (±2.91) [2]
Physical activity (kcal/kg/week) [3]	10.2 (±1.87)	12.89 (±2.69)	8.08 (±1.48)	9.97 (±2.10)	8.55 (±1.43)
Dietary and anthropometric variables					
Total calorie intake (kcal) [4]	2197 (±90.4)	1966 (±79.4)	2055 (±75.7)	2014 (±78.2)	2124 (±96.1)
BMI (kg/m^2) [3]	23.76 (±0.22)	25.16 (±0.20) [2]	26.15 (±0.25) [2]	27.44 (±0.27) [2]	29.11 (±0.38) [2]
Abdominal circumference (cm) [3]	91.29 (±0.67)	95.26 (±0.59)	98.3 (±0.69) [2]	101.9 (±0.67) [2]	105.32 (±0.94) [2]
Abdominal visceral fat (cm^2) [3]	103.2 (±3.9)	123.9 (±4.4) [2]	140.6 (±4.5) [2]	154.57 (±5.5) [2]	183.98 (±7.5) [2]
Abdominal subcutaneous fat (cm^2) [3]	149 (±4.21)	190 (±4.1) [2]	213.61 (±5.4) [2]	248.8 (±6.7) [2]	276.91 (±7.9) [2]
Total body fat (%) [3]	24.4 (±0.26)	27.5 (±0.30) [2]	28.82 (±0.27) [2]	31.05 (±0.32) [2]	32.86 (±0.42) [2]
Clinical and biochemical variables					
Diastolic Blood pressure (mmHg) [3]	71.9 (±1.23)	71.8 (±1.07)	71.97 (±1.25)	74.2 (±1.09)	74.1 (±1.24)
Systolic Blood pressure (mmHg) [3]	132 (±1.81)	133 (±2.19)	133.2 (±1.9)	135.8 (±2.03)	132.1 (±2.29)
Fasting glucose (mg/dl) [3]	95.4 (±2.63)	97 (±2.58)	99.02 (±2.5)	97.4 (±1.92) [2]	99.6 (±1.7) [2]
HDL cholesterol (mg/dl) [3]	52.2 (±1.24)	50.6 (±1.2)	48.3 (±1.1)	48.5 (±1.1)	50.2 (±0.98)
Triglycerides (mg/dl) [3]	104.5 (±4.1)	102.6 (±3.97)	115.3 (±4.3)	114.4 (±5.1)	116.2 (±4.2)
C-reactive protein (µg/ml) [3]	1.74 (±0.23)	2.03 (±0.3)	1.94 (±0.23)	2.26 (±0.24) [2]	2.07 (±0.16) [2]
PAI-1 (ng/ml) [3]	17.93 (±1.55)	17.9 (±1.25)	24.6 (±1.8)	22.29 (±1.5) [2]	29.8 (±2.0) [2]
Clinical and biochemical variables					
IL-6 (pg/ml) [3]	1.98 (±0.17)	2.08 (±0.14)	2.14 (±0.18)	2.15 (±0.15) [2]	2.01 (±0.12)
TNF-alpha (pg/ml) [3]	2.98 (±0.10)	3.05 (±0.12)	3.45 (±0.30)	3.37 (±0.13)	3.36 (±0.17) [2]

[1] Means (±SEM) are provided in the table unless specified.
[2] Significantly different from leptin quintile 1, P=0.05 (Dunnett's test for continuous variables and chi-square test for categorical variables).
[3] Values from baseline of the Health ABC study
[4] Values from year 2 of the Health ABC study.

Table 4.2 Baseline Characteristics of Women by Serum Leptin Quintile [1]

	Serum Leptin Quintile				
	1	**2**	**3**	**4**	**5**
Women (n (%))	113 (19.9)	114 (20.7)	113 (19.9)	114 (20.7)	114 (20.7)
Mean Serum Leptin (ng/ml) [3]	5.24 (± 0.21)	10.01 (± 0.11) [2]	15.28 (± 0.18) [2]	21.97 (± 0.23) [2]	39.67 (± 1.1) [2]
Demographic and behavioral factors					
Age (years) [3]	75.35 (±0.30)	75.35 (±0.30)	75.05 (±0.29)	74.76 (±0.27)	75.27 (±0.28)
Race (% white)	80	73	71	45[2]	41[2]
Drinking (% any consumption)	44	49	50	45	42
Education (% completed high school)	88	87	84	71[2]	78[2]
Smoking (lifetime pack-years) [3]	13.5 (±2.5)	8.29 (±1.70)	12.7 (±2.3)	11.3 (±2.27)	5.75[2] (±1.43)
Physical activity (kcal/kg/week) [3]	5.52 (±1.04)	5.75 (±1.05)	5.72 (±1.24)	2.83 (±0.65)	7.31 (±2.76)
Dietary and anthropometric variables					
Total calorie intake (kcal) [4]	1612 (±63.6)	1793 (±85.4)	1650 (±68.0)	1690 (±67.3)	1654 (±64.9)
BMI (kg/m^2) [3]	22.16 (±0.32)	24.17 (±0.31)[2]	25.66 (±0.34)[2]	27.9 (±0.41)[2]	30.3 (±0.46)[2]
Abdominal circumference (cm) [3]	84.74 (±1.06)	88.9 (±0.98)	95.5 (±1.08)[2]	99.7 (±1.56)[2]	103.88 (±1.33)[2]
Abdominal visceral fat (cm^2) [3]	78.6 (±3.8)	104.3 (±4.6)[2]	109.3 (±4.56)[2]	121 (±4.2)[2]	139.45 (±4.74)[2]
Abdominal subcutaneous fat (cm^2) [3]	219.9 (±8.3)	267 (±7.8)[2]	306.2 (±9.1)[2]	370 (±9.5)[2]	418.12 (±11.3)[2]
Total body fat (%)[3]	34.18 (±0.52)	38 (±0.43)[2]	39.76 (±0.39)[2]	42.3 (±0.35)[2]	44.7 (±0.37)[2]
Clinical and biochemical variables					
Diastolic Blood pressure (mmHg) [3]	69 (±1.1)	71 (±1.13)	66.94 (±1.2)	71 (±1.09)	68.5 (±1.1)
Systolic Blood pressure (mmHg) [3]	130 (±2.14)	137 (±2.01)[2]	131.1 (±1.9)	135 (±2.1)	132.2 (±2.19)
Fasting glucose (mg/dl) [3]	87 (±0.84)	91 (±1.6)	91.2 (±1.8)	92.8 (±1.1)[2]	92.0 (±0.78)[2]
HDL cholesterol (mg/dl) [3]	67 (±1.37)	68 (±1.7)	64.9 (±1.48)	63.7 (±1.49)	64.3 (±1.44)
Triglycerides (mg/dl) [3]	101.45 (±3.7)	113 (±4.99)	113 (±5.0)	110.4 (±4.20)	111.7 (±4.31)
C-reactive protein (µg/dl) [3]	1.52 (±0.16)	2.33 (±0.31)	2.27 (±0.18)	3.18 (±0.33)[2]	3.3 (±0.56)[2]
PAI-1 (ng/ml) [3]	17.5 (±1.43)	24.3 (±2.57)	22.2 (±2.10)	28.9 (±2.10)[2]	27.5 (±2.18)[2]
IL-6 (pg/ml) [3]	1.78 (±0.16)	1.74 (±0.16)	1.96(±0.21)	2.67 (±0.28)[2]	2.17 (±0.18)
TNF-alpha (pg/ml) [3]	2.76 (±0.11)	2.98 (±0.11)	2.94 (±0.11)	3.13 (±0.12)	3.31 (±0.25)[2]

[1] Means (±SEM), unless otherwise specified.
[2] Significantly different from leptin quintile 1, P=0.05 (Dunnett's test for continuous variables and chi-square test for categorical variables).
[3] Values from baseline of the Health ABC study
[4] Values from year 2 of the Health ABC study.

Table 4.3 Adjusted Odd Ratios (OR) of the Metabolic Syndrome by Serum Leptin Quintile Among Men

	Serum Leptin Quintile					
	1	**2**	**3**	**4**	**5**	**p value**
Men (n)	110	110	111	110	111	
Serum leptin quintile (mean ± SD)	2.11 (±0.511)	3.74(±0.49)	5.55 (±0.65)	8.09(±0.90)	15.82(±7.35)	<0.0001
Metabolic syndrome cases (n)	13	11	18	23	29	0.0076
Model 1						
OR (95% CI)	1	0.83 (0.35 - 1.98)	1.36 (0.62 - 2.99)	1.88 (0.87 - 4.05)	2.69 (1.29 - 5.64)	0.0002
Model 2						
OR (95% CI)	1	0.63 (0.25 - 1.58)	0.90 (0.38 - 2.14)	1.01 (0.40 - 2.54)	1.03 (0.37 - 2.86)	0.1435
Model 3						
OR (95% CI)	1	0.65 (0.26 - 1.63)	0.73 (0.30 - 1.76)	0.77 (0.29 - 2.01)	0.68 (0.23 - 2.01)	0.5034

[1]: Tests for linear trend used leptin as a continuous variable in logistic regression.
Model 1: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity (testosterone level and numbers of hours fasted were not significant and did not affect the association between predictor and outcome, so they were not included in the model)
Model 2: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, and BMI (Effect of total percent fat, visceral fat, subcutaneous fat on the association between main predictor and the outcome were similar to BMI, so they were not included in the final analysis)
Model 3: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, BMI, CRP, and PAI-1 (TNF-alpha and IL-6 were not significant and did not affect the association between main predictor and the outcome, so they were not included in the final analysis)

Table 4.4 Adjusted Odd Ratios (OR) of the Metabolic Syndrome by Serum Leptin Quintile Among Women

	Serum Leptin Quintile					
	1	**2**	**3**	**4**	**5**	**p value**
Women (n)	113	114	113	114	114	
Serum leptin quintile (mean ± SD)	5.34 (± 2.02)	10.06 (± 1.12)	15.18 (± 1.71)	21.77 (± 2.15)	38.88 (± 10.45)	<0.0001
Metabolic syndrome cases (n)	8	21	20	27	34	0.0003
Model 1						
OR (95% CI)	1	3.29 (1.36 - 7.95)	3.25 (1.33 - 7.93)	5.21 (2.16 - 12.56)	7.97 (3.30 - 19.24)	<0.0001
Model 2						
OR (95% CI)	1	3.04 (1.21 - 7.62)	2.94 (1.12 - 7.71)	4.56 (1.65 - 12.61)	6.38 (2.09 - 19.42)	0.0024
Model 3						
OR (95% CI)	1	2.85 (1.12 - 7.22)	2.88 (1.091 -7.59)	4.23 (1.53 - 11.7)	5.96 (1.95 - 18.13)	0.0098

1: Tests for linear trend used leptin as a continuous variable in logistic regression.
Model 1: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity (hormonal replacement therapy and numbers of hours fasted were not significant and did not affect the association between predictor and outcome, so they were not included in the model)
Model 2: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, and BMI (Effect of total percent fat, visceral fat, subcutaneous fat on the association between main predictor and the outcome were similar to BMI, so they were not included in the final analysis)
Model 3: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, BMI, CRP, and PAI-1 (TNF-alpha and IL-6 were not significant and did not affect the association between main predictor and the outcome, so they were not included in the final analysis)

Table 4.5 Adjusted Odd Ratios (OR) of Metabolic Syndrome by BMI Category

	BMI <=25 (Normal)	BMI > 25 (High)
Men (n)	210	372
Mean Serum Leptin (mean ± SD)	4.61 (±3.5)	8.44 (±6.3)
Metabolic Syndrome Cases (n)	18	91
OR (95% CI)	1.08 (0.49 - 2.40)	1.32 (0.90 - 1.94)
Women (n)	267	329
Mean Serum Leptin (mean ± SD)	11.44 (±8.8)	24.50 (±13.6)
Metabolic Syndrome Cases (n)	37	91
OR (95% CI)	2.10 (1.20 - 3.67)	2.00 (1.22 - 3.26)

Adjusted for age, race, site, years of education, alcohol use, smoking, and physical activity.

Chapter 5: Hyperleptinemia, Adiposity and Mortality in Older Adults

Abstract

Background: Elevated levels of serum leptin are associated with measures of adiposity and increased production of pro-inflammatory cytokines. Both cytokines and body adiposity have been shown to predict cardiovascular events and mortality.

Objectives: The primary objective of the present study was to explore the associations between serum leptin and all-cause mortality and mortality from CVD over a span of 10 years controlling for body adiposity and proinflammatory cytokines. A secondary objective was to examine the association between serum leptin and diabetes as a contributing cause of mortality stratified by leptin receptor genotype.

Design: The Health, Aging and Body Composition (HABC) study is a prospective cohort of 3,075 older adults aged 70 to 79 years. All-cause mortality and incident cardiac events (CHD and HF) were detected from baseline through year 10. Participants, genetic variation in the leptin receptor gene were identified. Variables of body adiposity and markers of systemic inflammation were assessed in detail.

Results: As compared to women in quartile 1, those in quartile 3 were at lower risk of all-cause mortality and those in quartile 2 and 3 were at lower risk of mortality from CVD independent of proinflammatory cytokines and total percent fat. A significant trend for diabetes as a contributing cause of mortality was found after adjusting for potential confounders (p=0.01), total percent fat (p=0.0006) and proinflammatory cytokines (p=0.02) among women.

Additionally, there was a significant interaction between leptin receptor genotype and total percent fat in association with diabetes as a contributing cause of mortality among older women ($p=0.03$).

Conclusion: The present study suggests a paradoxical association between leptin, cardiovascular risk factors and mortality from CVD. Intermediary concentrations of serum leptin are associated with lower risk of all-cause mortality and mortality from CVD, while elevated levels increase the risk of diabetes as a contributing cause of death among older women. An interaction was found between leptin receptor genotype and body fat, which may lower the risk of diabetes as a contributing cause of mortality among older women.

Introduction

Serum leptin and proinflammatory cytokines have been implicated in the development of several age-associated chronic conditions such as atherosclerosis, insulin resistance, diabetes and osteoporosis (34, 51, 52, 53 54, 84, 89 and 96). Leptin plays a critical role in energy intake (55 and 56), inflammatory response (35) and is strongly correlated with body fat (9, 35, 58 and 59), all of which are linked to mortality (34). This suggests a potential association between serum leptin and mortality.

There is a concomitant increase in the levels of serum leptin, proinflammatory cytokines (34, 35, and 95) and prevalence of metabolic diseases with aging (6, 47, 55, 92 and 96). Leptin and its receptor have structural and functional similarities with the IL-6 family of cytokines (54). Leptin has been shown to be associated with increased production of the pro-inflammatory cytokines (61 and 95) that are linked to obesity (55), the process of aging (40), atherogenic activity (97) and metabolic syndrome (40, 79 and 97). High levels of IL-6 were associated with cardiovascular and other causes of mortality among healthy older adults (34). Additionally, a

concomitant increase in TNF- a and serum leptin with aging has been associated with obesity and atherosclerosis (7 and 38).

Although serum levels of leptin have been consistently shown to be associated with increased inflammation and morbidity, previous studies have not explored the association of leptin with survival while controlling for inflammatory factors and body adiposity among functionally well older adults.

The primary objective of this study was to examine the association between serum leptin and the risk of all-cause mortality and mortality from CVD over a 10-year follow-up and to investigate whether these associations are mediated by markers of inflammation and body fat. The other objective was to examine the association between serum leptin and diabetes as a contributing cause of mortality stratified by leptin receptor genotype.

Subjects and Methods

Study Design

The Health, Aging and Body Composition (Health ABC) study is a longitudinal cohort study of 3,075 community dwelling, well functioning white and black men and women aged 70 to 79 years at the commencement of the study. This study was described in details elsewhere (91). Briefly, participants were recruited for the study from a random sample of white residents receiving Medicare benefits and all age-eligible black residents of Pittsburgh, Pennsylvania, and Memphis, Tennessee. The subjects were considered eligible to participate in the study if they reported no difficulty walking a quarter of a mile, climbing 10 steps without resting, or performing basic activities of daily living, were free of life-threatening illness, and planned to remain in the geographic area for at least 3 years. Those who reported active treatment for cancer or participation in diet or exercise intervention were excluded from the study. Participants gave written informed consent, and protocols were approved by the Institutional Review Boards at the

two study sites. A home interview was administered to the study participants to collect information on demographic, socioeconomic factors, and health behaviors. A clinic visit at baseline was conducted for examination of biological variables, body composition, weight-related health conditions, and physical function between April 1997 and June 1998. Thereafter, follow-up clinical examinations have been conducted annually, and interviews semi-annually.

Subjects

In the present study data from baseline through year 10 of the Health ABC study were used. The sample size for the present study was 2,919 (men = 1,419; women = 1,500) after excluding those with incomplete information for vital status (n =101) and missing values for serum leptin (n = 55).

Biochemical Variables

Participants underwent venipuncture at baseline visit after an overnight fast, and serum samples were frozen at -70°C. Plasma glucose was measured using an automated glucose oxidase reaction (YSI 2300 Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH). Both IL-6 and TNF- a were measured in duplicate using an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn). The limit of detection was 0.10 pg/ml for IL-6 and 0.18 pg/ml for TNF- a. The level of plasminogen activator inhibitor-1 (PAI-1) was measured in citrated plasma samples using a 2-site enzyme-linked immunosorbent assay according to previously published methods (91). Serum levels of CRP were also measured in duplicate by enzyme-linked immunosorbent essay based on purified protein and polyclonal anti-CRP antibodies (Calbiochem, San Diego, California) with a coefficient of variation of 8.0%. Serum concentrations of leptin were measured in duplicate by means of radioimmunoassay (Linco Research Inc, St Charles, Mo). The assay range is 0.05 to 100 ng/ml leptin in serum or plasma. Intraassay CVs ranged from 3.7% to 7.5%, and interassay CVs ranged from 3.2% to

8.9%. The assay is highly specific for human leptin and shows minimal reactivity with mouse or rat leptin (91).

Anthropometric Variables

Weight was measured using a standard balance beam scale in kilograms. Height was measured twice using a Harpenden stadiometer (Holtain Ltd., Crosswell, U.K.) in centimeters and the average of the two measurements was used. Dual energy x-ray absorptiometry (DXA) (Hologic QDR 4500A, software version 8.21, Hologic, Waltham, MA) was used to assess the total fat mass. The total percentage of body fat was calculated (77).

Demographic and Life Style Variables

A standardized questionnaire was administered at baseline to collect information on socio-demographic variables including age, gender, self-identified race and years of education, and lifestyle variables including smoking status and average alcohol consumption during the past year (0,1-2 or >2 drinks/day). Cigarette packs smoked per day were multiplied by the number of years of smoking to calculate the pack-years over a lifetime of cigarette smoking. Level of physical activity was ascertained by a standardized questionnaire specifically designed for the Health ABC study. The frequency, duration, and intensity level of activities such as self-report of walking and exercise were recorded, and approximate values of metabolic equivalent unit (MET) were assigned to each activity category to estimate weekly energy expenditure in kcal/kg/week (78).

Genotyping

TaqMan polymerase chain reaction was used to genotype the entire Health ABC cohort, for identifying 3'untranslated region pentanucloetide insertion/deletion polymorphism of leptin receptor gene (91).

Outcome Variable

In Health ABC, mortality outcomes were assessed from baseline through November 26, 2007. The study outcome measures were all-cause mortality, mortality from CVD and from diabetes as a contributing cause of mortality. Immediate and underlying causes of death were adjudicated by a central committee using established criteria. Deaths were ascertained through the review of the death certificate, hospital records, proxy interviews and Social Security Death Index data. Survival time was defined as the time from the baseline visit until the date of first event (death or cardiac event) and/or date of last contact. Mortality from CVD was defined as any death where the underlying cause of death was confirmed as atherosclerotic cardiovascular disease (definite fatal myocardial infarction, or definite or possible fatal coronary artery disease), cerebrovascular disease, and atherosclerotic disease other than cardiovascular, and other cardiovascular disease. The sample size was too low for analysis of mortality from T2D by serum leptin quartile. Therefore, diabetes as a contributing cause of mortality was defined as any death where the participant was suffering from T2D (previously and newly diagnosed diabetes). Participants were contacted every 6 months through in-person examinations or telephone interviews during which time their health status was assessed.

Statistical Analysis

Serum leptin values and body fat vary by gender; therefore, the analysis was performed separately by gender (72). Baseline characteristics of men and women were examined by quartiles of serum leptin. For continuous variables Dunnett's test and for categorical variables chi-square test were used to compare the means of quartile 2 through 4 to those of quartile 1. All adipokines were log transformed because they were not normally distributed. The interaction of serum leptin with race, pro-inflammatory cytokines, and adiposity measures were tested and found to be non significant.

Cox proportional hazard regression analysis was used to assess the risk of all-cause mortality and mortality from cardiovascular for persons in quartiles 2 through 4 of serum leptin compared with those in quartile 1. Three regression models were run which sequentially adjusted for potential confounders (age, gender, race, study site, education, smoking status, alcohol use, and physical activity), total percent fat and markers of systemic inflammation (CRP, TNF- a, and IL-6). Final model included the main predictor variable and covariates that were significantly associated with the outcome. Log transformed leptin was used in regression model for trend analysis. All the covariates met the assumptions for proportional hazard regression.

The association between the 3' un-translated region (UTR) insertion/deletion polymorphism of the leptin receptor gene and the risk of diabetes as a contributing cause of mortality were examined before and after addition of the total percent fat to the model. The leptin receptor genotype was dichotomized for deletion/deletion (D/D), and for I allele (insertion/ deletion and insertion/insertion genotypes combined). An interaction term was created to check the interaction between leptin receptor genotype and total percent fat after adjustment for potential confounders. The frequency of leptin receptor (I/I) and (I/D) genotype differed significantly by race in the present study and gender dimorphism has been reported in the expression of leptin receptor in skeletal muscles in the previous study (107), therefore, analysis was performed separately by race and gender. Statistical significance was set at $p = 0.05$, and analysis was performed using SAS (version 9.1; SAS Institute Inc., Cary, NC).

Results

There was a significant difference ($p<0.0001$) between the mean serum leptin concentrations among men 7.9 (±6.9) and women 21.3 (±14.6). Baseline characteristics indicate that among men and women, those in quartile 1 had significantly lower serum leptin levels, BMI and total percent fat as compared to those in quartile 2, 3, and 4 (**Table 5.1** and **5.2)**. Individuals

with leptin in quartile 1 had significantly lower TNF-a than those in quartile 4. Women in quartile 1 also had significantly lower CRP and IL-6 levels than those in quartile 3 and 4. Both, men and women in quartile 4 of serum leptin had lower percent of alcohol consumption and higher T2D mortality than those in quartile 1. Men in quartile 3 were more likely to be white, complete high school and had lower all-cause mortality as compared to those in quartile 1. Women in quartile 2, 3, and 4 were less likely to be white and those in quartile 3 and 4 and were less likely to complete high school as compared to those in quartile 1.

Tables 5.3 and 5.4 show characteristics of the men and women, respectively, according to leptin receptor genotype and race. White men with leptin receptor (D/D) genotype had significantly higher CRP than those with leptin receptor (I/I) and (I/D) genotype. White women with leptin receptor (D/D) genotype were more likely to consume alcohol than those with leptin receptor (I/I) and (I/D) genotype. Black women with leptin receptor (D/D) genotype had significantly lower diabetes as a contributing cause of mortality than those with leptin receptor (I/I) and (I/D) genotype.

The average follow-up time from baseline was 8.4 years, with a range of 1.1 to 10.4 years. During the follow-up time, 556 (39.2%) men and 401 (26.7%) women died due to all-cause mortality. **Table 5.5** shows the relative risk of all-cause mortality according to the serum leptin quartile among men and women. Association between risk of all-cause mortality and serum leptin levels among men was not statistically significant. Among women, those in quartile 2 (RR = 0.71; 95% CI = 0.53, 0.95) and 3 (RR = 0.73; 95% CI = 0.55, 0.98) had significantly lower risk of mortality as compared to those in quartile 1 after adjusting for potential confounders in model 1. When the analysis was additionally adjusted for total percent fat in model 2, the associations were no longer significant. With further adjustment with proinflammatory cytokines in model 3, association between quartile 3 of serum leptin and mortality risk became marginally significant (RR = 0.68; 95% CI = 0.47, 0.99). Analysis of trend for the risk of mortality across quartiles of serum leptin was not significant in any of the models among men and women.

During the follow-up time, 197 (13.9%) men and 154 (10.3%) women died due to mortality from CVD. (**Table 5.6)** There was no significant association between risk for mortality from CVD and serum leptin levels among men. Women in quartile 2 of serum leptin (RR = 0.60; 95% CI = 0. 37, 0.97) were at lower risk for mortality from CVD as compared to women in quartile 1 after adjustment for potential confounders in model 1. This association was no longer significant once adjusted for percent fat in model 2. In model 3, when the analysis was additionally adjusted for proinflammatory cytokines women in quartile 2 (RR = 0.48; 95% CI = 0. 27, 0.86) and 3 (RR = 0.50; 95% CI = 0. 27, 0.92) were at lower risk for mortality from CVD as compared to those in quartile 1. No significant trend for the risk of mortality from CVD across serum leptin quartiles was shown among men and women.

During the follow-up time, 171 (12.1%) men and 115 (7.6%) of the women who died had diabetes as a contributing cause of mortality. (**Table 5.7)** Men in quartile 4 of serum leptin had a significantly higher risk of mortality from diabetes as a contributing cause of death as compared to those in quartile 1 after sequential adjustment for potential confounders and total percent fat in model 1 (RR = 2.07; 95% CI = 1.33, 3.25), and 2 (RR = 2.79; 95% CI = 1.49, 5.25). These associations were no longer significant once the model was further adjusted for proinflammatory cytokines in model 3. There was a significant positive trend for the risk of diabetes as a contributing cause of mortality across the serum leptin quartiles in model 1 (p=0.0006) and 2 (p=0.0003) among men. Among women, those in quartile 4 of serum leptin were at significantly higher risk of diabetes as a contributing cause of mortality as compared to those in quartile 1 after adjustment for potential confounders and total percent fat in model 2 (RR = 2.42; 95% CI = 1.09, 5.35). With further adjustment for proinflammatory cytokines the association was no longer significant. However, there was a significant positive trend for the risk of diabetes as a contributing cause of mortality across the serum leptin quartiles in model 1 (p=0.01), 2 (p=0.0006), and 3 (p=0.02).

Table 5.8 and 5.9 shows the relative risk of diabetes as a contributing cause of mortality stratified by genotype for leptin receptor among black and white men and women. Black women with I/I and I/D genotype were at higher risk for diabetes as a contributing cause of mortality (p= 0.04) as compared to black women in the reference group (D/D genotype) after adjustment for potential confounders and total percent fat in model 2. There was a significant interaction between leptin receptor genotype and total percent fat in model 3 (p= 0.001) among black women. The interaction term remained significantly negatively associated (p= 0.003) with diabetes as a contributing cause of mortality after adjusting for proinflammatory cytokines among black women in model 4. There was no significant association between leptin receptor genotype and risk of mortality from diabetes as a contributing cause of death among white men and women and black men.

Discussion

In the present study intermediate levels of serum leptin were associated with lower risk of all-cause mortality and mortality from CVD, while elevated levels were associated with increased risk of mortality among individuals with diabetes as a contributing cause of death among women. These associations remained significant after controlling for measures of body adiposity and the pro-inflammatory cytokines.

Results of this study are supported by previous studies that have shown an association between serum leptin and all-cause and CVD mortality (60 and 61). Lieb et al., in Framingham Heart Study found a U-shaped association between serum leptin and all-cause mortality with elevated risk of death at both low and high levels of leptin (60). Furthermore, they showed that the U-shape association was mainly contributed by increased risk of death due to non-cardiovascular diseases (60). Hypoleptinemia has been shown to elevate the risk of cancer mortality (63), while hyperleptinemia increased the risk of mortality from T2D (62). Additionally, in a longitudinal study on middle age women moderately elevated serum leptin was inversely associated with CVD mortality after adjusting for adiposity (64). Although leptin has been shown to stimulate the production of the pro-inflammatory cytokines (61 and 102) that are linked to obesity (100) and chronic diseases (7, 38, and 89), the association of serum leptin, all-cause mortality and CVD mortality was not explored in context of pro-inflammatory cytokines among functionally well older adults in above studies.

Previous studies have consistently showed protective effect of leptin on all-cause mortality and CVD mortality, however the mechanisms for such associations remain unclear. It has been shown that hyperleptinemia can cause impaired leptin signaling and leptin insensitivity leading to storage of fat in muscles and liver (14 and 94). On the other hand, hypoleptinemia has been associated with complete or partial absence of subcutaneous fat (58 and 98). Leptin regulates actions of anorexigenic and orexigenic peptide in hypothalamus to control appetite (55

and 56) and facilitate accumulation of adipose tissue in body (55 and 93). Body fat forms a U-shaped association with mortality (103) and being slightly overweight has been associated with lower risk of mortality among older adults (103 and 109). Therefore, moderate levels of serum leptin may help to maintain optimal body fat and thus, lower mortality risk. Furthermore, regulation of energy balance is different among men and women (105 and108) and brains of women are relatively more sensitive to leptin as a strategy for weight maintenance (108). Thus, the gender difference in the association of leptin with all-cause mortality and CVD mortality may be explained by greater sensitivity among women to the effects of leptin for maintaining appropriate body fat.

Previous studies have shown positive association between elevated serum leptin and the risk factors for cardiovascular disease among healthy and diabetic individuals (6, 38, 51, 54, and 110). Paradoxically, elevated leptin has been also shown to reduce cardiomyocyte lipotoxicity by promoting fatty acid oxidation (111 and 112) and provides an environment that compensates for the reduced cardiac output and improves survival (112). Leptin has receptors on cardiac myocytes and it exerts direct antihypertrophic effects in heart by stimulating nitric oxide signaling (113). Leptin deficiency or impaired leptin signaling, as in state of leptin resistance, may lead to development of left ventricular hypertrophy (113), suggesting that both very low and very high levels of serum leptin may lead to cardiac dysfunction.

Due to the structural and functional similarities of leptin and its receptor with the IL-6 family of cytokines, leptin appears to play a critical role in the immune response (7). Enhanced production of CRP and IL-6 has been shown to predict coronary artery disease and it has been suggested that serum leptin is associated with increased production of C- reactive protein (39, 40, and 51). Although serum leptin has been linked to proinflammatory cytokines, the association of leptin with all-cause mortality and mortality from CVD was stronger after controlling for inflammatory factors, suggesting that the protective effect of leptin may be independent of the proinflammatory state.

In addition to the current study, several previous studies have shown hyperleptinemia to be associated with the risk of T2D. Elevated levels of serum leptin was associated with insulin resistance independent of total fat mass (84) and BMI (17) among women, while serum leptin formed a part of the cluster that constituted insulin resistance and hyperinsulinemia independent of measures of body fat (40 and 101). Recently, elevated leptin levels have been found to predict glucose intolerance in middle-aged white adults (45). Additionally Welsh et.al., in the PROSPER study found an independent association between risk of T2D and serum leptin after adjusting for potential confounders and BMI (62). These studies, however, did not examine inflammatory markers, such as TNF-a, PAI-1, and IL-6 which have been shown to be related to the risk of type 2 diabetes (89) and serum leptin (7),

Several previous studies have also shown gender differences in the association of serum leptin and risk of T2D. Among women but not men, studies have shown that the association between serum leptin and insulin resistance are independent of total fat mass and BMI (17 and 84). Furthermore, in a study among older men, the association of serum leptin with diabetes was mediated by obesity and insulin resistance (49 and 99). Women have been consistently shown to have higher serum leptin concentrations independent of total body fat mass or percent fat (85) and it has been proposed that elevated levels of serum leptin can lead to the development of leptin resistance (74) suggesting that women may be more leptin resistant as compared to men. Thus, the sex difference in the association of serum leptin with diabetes as a contributing cause of mortality in our study may be explained by relatively greater leptin resistance in women.

Several mechanisms have been postulated by which leptin exerts its direct (16) and indirect (14, 15, and 104) effect on the risk of T2D. It is suggested that leptin stimulates the oxidation of fatty acids, increases the uptake of glucose in muscle and liver by activating 5' - Adenosine Mono Phosphate – activated Protein Kinase (AMPK) and thwarts lipotoxicity by preventing the spilling of fat to non-adipose tissue (14 and 104). Elevated levels of leptin may lead to accumulation of lipid in non-adipose tissue such as liver, pancreas, and muscles and are

linked to impaired insulin action (14) and insulin resistance (15). Serum leptin directly affects the synthesis and release of insulin from pancreatic ß- cells and in turn, insulin stimulates secretion of leptin from adipose tissue creating a regulatory feedback loop, which is called adipoinsular axis (16). Leptin resistance in pancreatic ß - cells contributes to dysregulation of adipoinsular axis leading to hyperinsulinemia and T2D (16).

In the current study, a significant interaction between leptin receptor (I/D) genotype and total percent fat was found among black women. The interaction term was inversely associated with diabetes as a contributing cause of mortality suggesting that total percent fat may modulate the association of leptin receptor I allele with diabetes as a contributing cause of mortality among black women independent of inflammatory factors.

Several studies have examined the association between leptin receptor genotype and risk of T2D. Expression of leptin receptor gene in pancreatic beta-cells may lead to leptin-induced inhibition of insulin secretion (65). Thus, defect in leptin receptor gene may result in hyperinsulinemia due to leptin resistance (66). Lakka et al., and Nannipieri et al., reported lower serum insulin levels and risk of T2D among homozygous or heterozygous carriers of insertion allele than those who were homozygous for deletion allele (68 and 69). Although, Lakka et al., and Nannipieri et al., have reported protective effect of LEPR I/I and I/D genotype as compared to D/D genotype on diabetes risk (68 and 69), black women with LEPR I/I and I/D genotype were found to be at higher risk of diabetes as contributing cause of mortality after adjusting for total percent fat in our study. In the above studies, either combined analysis was performed on both genders (69) or the study sample included only men (68). The studies also lacked information on the race of the participants of the study (68 and 69). Thus, it is difficult to compare their finding with our results among black women. The racial difference observed in the association of leptin receptor (I/D) genotype with diabetes as a contributing cause of mortality, in the present study, may be because the associated genetic variants were not directly affecting the conditions and

possibly be explained by different linkage disequilibrium patterns or the different population histories of blacks and whites (147).

Previous studies have shown the association between body leptin receptor genotype and body adiposity (71, 106, 126 and 127). Variants at 3' region of leptin receptor gene have been associated with obesity in Pima Indians (127). In a study on overweight postmenopausal women, leptin receptor gene polymorphisms were shown to be associated with abdominal fat (71), while variations in DNA sequence in the leptin receptor gene was suggested to play a role in body fat distribution (71). Individuals with leptin receptor (I/I) (I/D) genotype had lower body weight than those with leptin receptor (D/D) genotype during 3 year follow up among impaired glucose tolerant individuals in Finnish Diabetes Prevention Study (126) and in STOP-NIDDM study (106), suggesting the potential role of this polymorphism in regulating body adiposity (106). However, in our sample of older adults, no difference was found in total percent fat and BMI among the two genotypes in white and black men and women.

The protective effect of leptin receptor (I/I) (I/D) genotype on the risk of T2D has been reported frequently among obese subjects in the previous studies (67 and 128). Francke et al., reported that obese carriers of insertion allele have lower insulin concentrations compared with carriers of the D allele (128) and obese heterozygous women had lower insulin values at 30 minutes in the oral glucose tolerance test (128). Additionally, better insulin sensitivity was found in obese subjects carrying the I allele because of lower insulin and insulin-to-glucose ratio (67). The protective effect of insertion allele in obese phenotype in the above studies (67 and 128) and interaction between leptin receptor (I/I) (I/D) and body fat in the present study on the risk of diabetes may imply that insertion allele lowers the risk of diabetes in presence of some amount of body fat, perhaps by reducing body weight (106 and 126). However, the reason behind the elevated risk of diabetes as a contributing cause of mortality in black women with (I/I) (I/D) genotype as compared to those with (D/D) genotype in the present study is unclear.

The mechanism underlying the association between the 3'-UTR polymorphism and the risk of type 2 diabetes remains to be elucidated. A pentanucleotide insertion at 3'-UTR of LEPR generates a putative stem-loop motif in the leptin receptor mRNA (67). Stem-loop sequences affect mRNA stability by binding to the regulatory proteins, which in turn could affect the rate of degradation and/or translation of mRNA (148). However, there are no data on the direct effects of this polymorphism on leptin action or insulin secretion.

Recently ethnic differences in the frequencies of leptin receptor Q223R (LEPRQ223R) genotype has been reported (149). The highest proportion of heterozygous variant was observed in African subjects, while Caucasian subjects had the lowest frequency of heterozygous variants (149). Because race was self reported in the present study, it is difficult to evaluate the admixture present in this population. Genetic admixture has been previously reported to lead to inappropriate classification of groups by genotype (150). Thus, the large study with complete epidemiological information and the frequencies of leptin receptor (I/D) genotypes on admixture is warranted.

The strength of this study includes the longitudinal design of Health ABC study. Data were collected in several waves over a period of 10 years. HABC's extensive information on life style and biochemical variables made it possible to control several potential confounders unlike previous studies. A limitation of this study is that there were not enough cases of mortality from cancer which did not permit us to examine the association between serum leptin and cancer mortality. This might have enabled us to provide a complete examination of the suggested U-shape association between leptin and mortality from chronic conditions. Another limitation is the unavailability of data on other candidate leptin receptor genotypes for metabolic diseases among older adults such as Gln223Arg (134), which have been shown to be associated with serum insulin and obesity (71 and 151).

In conclusion, the results of the present study suggest that although elevated levels of serum leptin are associated with increased metabolic risk and risk of diabetes as a contributing

cause of mortality, moderately elevated leptin levels may protect from the risk of mortality from CVD among older women. Because women are exposed to relatively elevated levels of leptin throughout their life, these effects of leptin were observed exclusively among women. The current finding of paradoxical association of leptin with cardiovascular risk factors and mortality from CVD would contribute to the understanding of pathophysiological implications that are elicited by hypo and hyperleptinemia in cardiovascular disease. Additionally, a significant interaction between leptin receptor genotype and body fat, if confirmed in functional study of genetic variants on admixture, may imply that leptin receptor genotype in relation to body adiposity may lower the risk of diabetes as a contributing cause of mortality among older black women.

Tables

Table 5.1 Baseline Characteristics of Men by Serum Leptin Quartile [1]

	Serum Leptin Quartile			
	1	**2**	**3**	**4**
Men (n (%))	354 (24.9)	355 (25)	355 (25)	355 (25)
Mean Serum Leptin (ng/ml) [3]	2.11 (±0.93)	4.77 (±0.76) [2]	7.92 (±1.13) [2]	16.97 (±8.13) [2]
Demographic and behavioral variables				
Age (years) [3]	75.4 (±2.9)	75.1 (±2.5)	75.4 (±3.0)	75.2 (±2.8)
Race (% white)	62	67	69[2]	56
Drinking (% any consumption)	64	60	55	53[2]
Education (% completed high school)	69	73	76[2]	74
Smoking (lifetime pack-years) [3]	25.2 (±29.6)	25.2 (±29.5)	25.4 (±31.9)	27.1 (±31.1)
Physical activity (kcal/kg/week) [3]	9.35 (±20.6)	8.8 (±20.9)	7.7 (±17.1)	7.4 (±15.5)
Dietary and anthropometric variables				
Total calorie intake (kcal) [4]	2128 (±867)	2084 (±849)	1991 (±798)	1875 (±775)
BMI (kg/m2) [3]	23.75 (±2.7)	26.1 (±2.8) [2]	28.1 (±2.9) [2]	30.2 (±3.9) [2]
Total body fat (%)[3]	24.1 (±3.8)	28.2 (±3.0) [2]	31.2 (±3.4) [2]	33.3 (±4.2) [2]
Biochemical variables				
C-reactive protein (µg/ml)[3]	2.44 (±4.3)	2.7 (±5.0)	2.7(±5.4)	2.8 (±3.1)
IL-6 (pg/ml) [3]	2.46 (±2.1)	2.27 (±1.62)	2.55 (±2.1)	2.6 (±1.8)
TNF-alpha (pg/ml) [3]	3.5 (±1.8)	3.5 (±2.1)	3.6 (±1.5)	3.9 (±1.9) [2]
Survival				
CVD mortality (%)	12.7	14.9	12.4	15.5
T2D mortality (%)	9.94	9.01	11.3	18.9[2]
All-cause mortality (%)	43	39	35[2]	40
Genetic variable				
LEPR (I/D) or (I/I) genotype (%)	44	38	42	48

[1] Means (±SEM), unless otherwise specified.
[2] Significantly different from leptin quintile 1, P=0.05 (Dunnett's test for continuous variables and chi-square test for categorical variables).
[3] Values from baseline of the Health ABC study
[4] Values from year 2 of the Health ABC study.

Table 5.2 Baseline Characteristics of Women by Serum Leptin Quartile [1]

	Serum Leptin Quartile			
	1	**2**	**3**	**4**
Women (n (%))	375 (25)	375 (25)	375 (25)	375 (25)
Mean Serum Leptin (ng/ml) [3]	6.4 (±2.6)	14.0 (±2.2) [2]	22.7 (±2.9) [2]	42.1 (±11.1) [2]
Demographic and behavioral factors				
Age (years) [3]	75.3 (±2.9)	75.3 (±2.9)	75.0 (±2.8)	75.0 (±2.8)
Race (% white)	71	59[2]	51[2]	61[2]
Drinking (% any consumption)	45	47	44	35[2]
Education (% completed high school)	84	78	77[2]	70[2]
Smoking (lifetime pack-years) [3]	14.2 (±25.2)	10.6 (±18.7)	13.4 (±25.6)	11.2 (±21.8)
Physical activity (kcal/kg/week) [3]	5.1 (±11.8)	4.6 (±10.3)	2.9 (±7.5) [2]	3.9 (±15.6)
Dietary and anthropometric variables				
Total calorie intake (kcal) [4]	1731 (±627)	1717 (±684)	1701 (±657)	1677 (±629)
BMI (kg/m2) [3]	23.1 (±3.7)	26.4 (±3.9) [2]	29.0 (±4.3) [2]	32.3 (±4.9) [2]
Total body fat (%) [3]	35.0 (±5.4)	39.5 (±3.9) [2]	42.2 (±3.7) [2]	45.0 (±4.1) [2]
Biochemical variables				
C-reactive protein (µg/ml) [3]	2.6 (±5.6)	2.6 (±2.4)	3.7 (±4.6) [2]	4.3 (±5.2) [2]
IL-6 (pg/ml) [3]	2.0 (±1.8)	1.9 (±1.5)	2.5 (±2.1) [2]	2.8 (±2.0) [2]
TNF-alpha (pg/ml) [3]	3.3 (±1.6)	3.3 (±1.5)	3.3 (±1.3)	3.7 (±1.9) [2]
Survival				
CVD mortality (%)	11.7	9.6	9.3	10.4
T2D mortality (%)	5.6	6.4	7.2	11.5[2]
All-cause mortality (%)	30	25	25	27
Genetic variable				
LEPR (I/D) or (I/I) genotype (%)	39	41	37	45

[1] Means (±SEM), unless otherwise specified.
[2] Significantly different from leptin quintile 1, P=0.05 (Dunnett's test for continuous variables and chi-square test for categorical variables).
[3] Values from baseline of the Health ABC study
[4] Values from year 2 of the Health ABC study

.

Table 5.3 Baseline Characteristics of Men by Race and Leptin Receptor Genotype [1]

	Whites		Blacks	
	(D/D)	**(I/I) or (I/D)**	**(D/D)**	**(I/I) or (I/D)**
Men (n (%))	562 (62.4)	338 (37.5)	246 (47.3)	273 (52.6)
Mean Serum Leptin (ng/ml) [3]	7.75 (±0.29)	7.8 (± .36)	7.9 (± 0.29)	8.5 (± .36)
Demographic and behavioral variables				
Age (years) [3]	75.2 (±0.1)	75.5 (±0.1)	75 (±0.1)	75.1 (±0.1)
Drinking (% any consumption)	64	64.2	46	48
Education (% completed high school)	87	85	49	51
Smoking (lifetime pack-years) [3]	27.1 (±1.4)	27.7(±1.8)	23.6 (±1.7)	22.3(±1.5)
Physical activity (kcal/kg/week) [3]	11.0 (±.9)	9.2 (±.9)	5.1 (±1)	4.4 (±.9)
Anthropometric variables				
BMI (kg/m2) [3]	27 (±.13)	26.8 (±.20)	27 (±.26)	27.3 (±.27)
Total body fat (%)[3]	30 (±0.2)	29.7 (±0.3)	28 (±0.3)	28.1 (±0.3)
Biochemical variables				
C-reactive protein (µg/ml) [3]	2.7 (±.2)	1.94 (±.12)[2]	2.9 (±.3)	3.1 (±.2)
IL-6 (pg/ml) [3]	2.4 (±.08)	2.3 (±.09)	2.5 (±.1)	2.7 (±.1)
TNF-alpha (pg/ml) [3]	3.7 (±.08)	3.7 (±.09)	3.4 (±.2)	3.3 (±.1)
Survival				
CVD mortality (%)	13	12	16.3	16.5
T2D mortality (%)	7.8	9.5	18.3	18.3
All-cause mortality (%)	33	36	47	49

[1] Means (±SEM), unless otherwise specified.
[2] Significantly different from leptin quintile 1, P=0.05 (t test for continuous variables and chi-square test for categorical variables).
[3] Values from baseline of the Health ABC study

Table 5.4 Baseline Characteristics of Women by Race and Leptin Receptor Genotype [1]

	Whites		Blacks	
	(D/D)	**(I/I) or (I/D)**	**(D/D)**	**(I/I) or (I/D)**
Women (n (%))	537 (65.5)	281 (34.3)	357 (52.3)	325 (47.6)
Mean Serum Leptin (ng/ml) [3]	18.3 (± 0.6)	18.5 (± .8)	25 (± 0.8)	24.5 (± .8)
Demographic and behavioral variables				
Age (years) [3]	75.1 (±0.1)	75.3 (±0.2)	75 (±0.2)	75 (±0.2)
Drinking (% any consumption)	56	44[2]	34	27
Education (% completed high school)	90	89	65	59
Smoking (lifetime pack-years) [3]	13.1 (±1.1)	12.7(±1.5)	12 (±1.1)	11 (±1.2)
Physical activity (kcal/kg/week) [3]	5 (±.6)	7 (±.9)	2.4 (±.3)	2.5 (±.4)
Anthropometric variables				
BMI (kg/m2) [3]	26 (±.2)	26 (±.3)	30 (±.3)	29.5 (±.3)
Total body fat (%)[3]	40 (±0.2)	40.3(±0.3)	41 (±0.3)	40 (±0.3)
Biochemical variables				
C-reactive protein (µg/ml) [3]	2.8 (±.2)	3.0 (±.3)	3.7 (±.3)	3.8 (±.3)
IL-6 (pg/ml) [3]	2.2 (±.08)	2.0 (±.09)	2.6 (±.1)	2.6 (±.1)
TNF-alpha (pg/ml) [3]	3.4 (±.06)	3.5 (±.1)	3.3 (±.1)	3.2 (±.1)
Survival				
CVD mortality (%)	9.3	7.8	11	13
T2D mortality (%)	3.9	4.2	9.5	14.8[2]
All-cause mortality (%)	22	23	30	34

[1] Means (±SEM), unless otherwise specified.
[2] Significantly different from leptin quintile 1, P=0.05 (t test for continuous variables and chi-square test for categorical variables).
[3] Values from baseline of the Health ABC study

Table 5.5 Adjusted Relative Risk (RR) of All-Cause Mortality by Serum Leptin Quartile

	Serum Leptin Quartile				
	1	**2**	**3**	**4**	**p for logleptin[1]**
Men (n)	354	355	355	355	
Serum leptin quartile (mean ± SD)	2.10 (±0.93)	4.76 (±0.76)	7.92 (±1.34)	16.97 (±8.13)	<0.0001
All-Cause mortality cases (n)	151	139	125	141	0.241
Model 1					
RR (95% CI)	1	0.97 (0.76 - 1.23)	0.79 (0.61 - 1.01)	0.88 (0.69 - 1.12)	0.44
Model 2					
RR (95% CI)	1	1.04 (0.79 - 1.37)	0.85 (0.62 - 1.17)	1.03 (0.72 - 1.46)	0.30
Model 3					
RR (95% CI)	1	0.93 (0.70 - 1.23)	0.74 (0.53 - 1.03)	0.82 (0.56 - 1.94)	0.06
Women (n)	375	375	375	375	
Serum leptin quartile (mean ± SD)	6.43 (± 2.64)	13.95 (± 2.27)	22.72 (± 2.91)	42.13 (± 11.17)	<0.0001
All-Cause mortality cases (n)	111	92	95	103	0.0003
Model 1					
RR (95% CI)	1	0.71 (0.53 - 0.95)	0.73 (0.55 - 0.98)	0.84 (0.63 - 1.13)	0.50
Model 2					
RR (95% CI)	1	0.78 (0.56 - 1.09)	0.82 (0.58 - 1.17)	1.06 (0.71 - 1.58)	0. 25
Model 3					
RR (95% CI)	1	0.72 (0.51 - 1.02)	0.68 (0.47 - 0.99)	0.76 (0.50 - 1.67)	0.89

[1]: Tests for linear trend used leptin as a continuous variable in logistic regression.
Model 1: Adjusted by age, race, site, years of education, alcohol use, smoking, and physical activity (total calorie intake, hormonal replacement therapy and numbers of hours fasted were not significant and did not affect the association between predictor and outcome, so they were not included in the model)
Model 2: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity and TOTPF
Model 3: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, TOTPF, CRP, PAI-1, TNF, and IL6

Table 5.6 Adjusted Relative Risk (RR) of CVD Mortality by Serum Leptin Quartile

	Serum Leptin Quartile				
	1	**2**	**3**	**4**	**p for logleptin[1]**
Men (n)	354	355	355	355	
Serum leptin quartile (mean ± SD)	2.10 (±0.93)	4.76 (±0.76)	7.92 (±1.34)	16.97 (±8.13)	<0.0001
CVD mortality cases (n)	45	53	44	55	0.48
Model 1					
RR (95% CI)	1	1.96 (0.79 - 1.83)	0.99 (0.64 - 1.53)	1.09 (0.71 - 1.67)	0.21
Model 2					
RR (95% CI)	1	1.16 (0.69 - 1.78)	0.88 (0.51 - 1.50)	0.70 (0.49 - 1.38)	0.24
Model 3					
RR (95% CI)	1	0.99 (0.61 - 1.60)	0.71 (0.40 - 1.23)	0.67 (0.35 - 1.27)	0.14
Women (n)	375	375	375	375	
Serum leptin quartile (mean ± SD)	6.43 (± 2.64)	13.95 (± 2.27)	22.72 (± 2.91)	42.13 (± 11.17)	<0.0001
CVD mortality cases (n)	44	36	35	39	0.543
Model 1					
RR (95% CI)	1	0.60 (0.37 - 0.97)	0.71 (0.45 - 1.25)	0.76 (0.48 - 1.21)	0.71
Model 2					
RR (95% CI)	1	0.64 (0.38 - 1.11)	0.77 (0.43 - 1.35)	0.97 (0.51 - 1.85)	0.26
Model 3					
RR (95% CI)	1	0.48 (0.27 - 0.86)	0.50 (0.27 - 0.92)	0.59 (0.29 - 1.19)	0.85

[1]: Tests for linear trend used leptin as a continuous variable in logistic regression.
Model 1: Adjusted by age, race, site, years of education, alcohol use, smoking, and physical activity (total calorie intake, hormonal replacement therapy and numbers of hours fasted were not significant and did not affect the association between predictor and outcome, so they were not included in the model)
Model 2: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity and TOTPF
Model 3: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, TOTPF, CRP, PAI-1, TNF, and IL6

Table 5.7 Adjusted Relative Risk (RR) of Diabetes as a Contributing Cause of Mortality by Serum Leptin Quartile

	Serum Leptin Quartile				
	1	**2**	**3**	**4**	**p for logleptin [1]**
Men (n)	354	355	355	355	
Serum leptin quartile (mean ± SD)	2.10 (±0.93)	4.76 (±0.76)	7.92 (±1.34)	16.97 (±8.13)	<0.0001
T2D mortality cases (n)	32	32	40	67	0.48
Model 1					
RR (95% CI)	1	0.99 (0.59 - 1.67)	1.26 (0.78 - 2.06)	2.07 (1.33 - 3.25)	0.0006
Model 2					
RR (95% CI)	1	1.18 (0.67 - 2.09)	1.57 (0.86 - 2.85)	2.79 (1.49 - 5.25)	0.0003
Model 3					
RR (95% CI)	1	0.55 (0.27 - 1.14)	0.84 (0.45 - 1.58)	0.94 (0.51 - 1.73)	0.19
Women (n)	375	375	375	375	
Serum leptin quartile (mean ± SD)	6.43 (± 2.64)	13.95 (± 2.27)	22.72 (± 2.91)	42.13 (± 11.17)	<0.0001
T2D mortality cases (n)	21	24	27	43	0.484
Model 1					
RR (95% CI)	1	0.82 (0.43 - 1.54)	0.92 (0.51 -1.68)	1.46 (0.84 - 2.55)	0.01
Model 2					
RR (95% CI)	1	1.01 (0.49 - 2. 08)	1.16 (0.55 - 2.44)	2.42 (1.09 - 5.35)	0.0006
Model 3					
RR (95% CI)	1	1.03 (0.49 - 2.17)	0.90 (0.41 - 2.01)	1.56 (0.67 - 3.60)	0.02

[1]: Tests for linear trend used leptin as a continuous variable in logistic regression.

Model 1: Adjusted by age, race, site, years of education, alcohol use, smoking, and physical activity (total calorie intake, hormonal replacement therapy and numbers of hours fasted were not significant and did not affect the association between predictor and outcome, so they were not included in the model)

Model 2: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity and TOTPF

Model 3: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, TOTPF, CRP, PAI-1, TNF, and IL6

Table 5.8 Adjusted Relative Risk (RR) of Diabetes as a Contributing Cause of Mortality by Leptin Receptor Polymorphism and Race Among Men

	Leptin Receptor (I/D)		
	(D/D)	**(I/D) or (I/I)**	**p value**
White Men (n)	562	338	<0.0001
T2D mortality cases (n)	44	32	
Model 1			
RR (95% CI)	1	1.25 (0.78 - 2.0)	0.36
Model 2			
RR (95% CI)	1	1.28 (0.79 - 2.1)	0.31
Model 3 (model 2 + totpf*lepr2id)			
RR (95% CI)	1	1.01 (0.91 - 1.12)	0.79
Model 4 (Model3 + inflammation)			
RR (95% CI)	1	1.03 (0.92 - 1.17)	0.52
Black Men (n)	246	273	<0.0001
T2D mortality cases (n)	45	50	
Model 1			
RR (95% CI)	1	0.97 (0.64 - 1.47)	0.88
Model 2			
RR (95% CI)	1	0.97 (0.64 - 1.49)	0.89
Model 3 (model 2 + totpf*lepr2id)			
RR (95% CI)	1	0.95 (0.87 - 1.03)	0.21
Model 4 (Model3 + inflammation)			
RR (95% CI)	1	0.91 (0.89 - 1.01)	0.05

[1]: Tests for linear trend used leptin as a continuous variable in logistic regression.
Model 1: Adjusted by age, race, site, years of education, alcohol use, smoking, and physical activity
Model 2: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, and TOTPF
Model 3: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, and TOTPF*LEPR2ID
Model 4: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, TOTPF*LEPR2ID, CRP, PAI-1, TNF and IL6

Table 5.9 Adjusted Relative Risk (RR) of Diabetes as a Contributing Cause of Mortality by Leptin Receptor Polymorphism and Race Among Women

	Leptin Receptor (I/D)		
	(D/D)	**(I/D) or (I/I)**	**p value**
White Women (n)	537	281	<0.0001
T2D mortality cases (n)	21	12	
Model 1			
RR (95% CI)	1	1.17 (0.57 - 2.39)	0.66
Model 2			
RR (95% CI)	1	1.01 (0.47 - 2.15)	0.98
Model 3 (Model2 + totpf*lepr2id)			
RR (95% CI)	1	0.98 (0.86 - 1.12)	0.81
Model 4 (Model3 + inflammation)			
RR (95% CI)	1	1.06 (0.91 - 1.22)	0.46
Black Women (n)	357	325	<0.0001
T2D mortality cases (n)	34	48	
Model 1			
RR (95% CI)	1	1.25 (0.78 - 2.0)	0.36
Model 2			
RR (95% CI)	1	1.64 (1.01 - 2.68)	0.046
Model 3 (model 2 + totpf*lepr2id)			
RR (95% CI)	1	0.86 (0.79 - 0.94)	0.001
Model 4 (Model3 + inflammation)			
RR (95% CI)	1	0.87 (0.79 - 0.95)	0.003

[1]: Tests for linear trend used leptin as a continuous variable in logistic regression.
Model 1: Adjusted by age, race, site, years of education, alcohol use, smoking, and physical activity
Model 2: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, and TOTPF
Model 3: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, and TOTPF*LEPR2ID
Model 4: Adjusted by age, race, site, years of education, alcohol use, smoking, physical activity, TOTPF*LEPR2ID, CRP, PAI-1, TNF and IL6

Chapter 6: Serum Leptin Cut Off Level Suggesting Risk of Metabolic Syndrome Among Older Adults

Abstract

Background: Serum leptin is suggested as an independent risk factor for coronary heart disease. Elevated levels of serum leptin are associated with insulin resistance and increased risk of metabolic syndrome independent of measures of adiposity.

Objectives: The objective of the present study was to determine the serum leptin cut off level that predicts the risk of metabolic syndrome among older men and women. A secondary aim was to compute and compare the cut off values of serum leptin, serum leptin per kilogram of body fat and serum leptin per unit of BMI for determining incidence of metabolic syndrome at year 6.

Design: The Health, Aging and Body Composition (HABC) study is a prospective cohort of 3,075 older adults aged 70 to 79 years. Metabolic syndrome at year 6 was defined by the NCEP ATPIII definition as presence of 3 or more of the 5 risk components as follows: 1) waist circumference >102 cm for men and >88 cm for women; 2) triglyceride (TG) =150 mg/dl; 3) high-density lipoprotein cholesterol (HDL-C) <40 mg/dl for men and <50 mg/dl for women; 4) blood pressure (BP) = 130/85 mmHg or currently on antihypertensive medicine(s); 5) fasting glucose =110 mg/dl or currently using anti-diabetic (insulin or oral agents) medication. Anthropometric measures were evaluated in detail.

Results: The serum leptin cut off value for diagnosis of metabolic syndrome was 6.45 ng/ml with 60% sensitivity and 63% specificity among men and 18.25 ng/ml with 55% sensitivity

and 62% specificity among women. The area under curve (AUC) was 0.62 for serum leptin, 0.58 for leptin per kilogram of body fat, and 0.59 for leptin per unit of BMI among men and women.

Conclusion: The prediction of metabolic syndrome was better by serum leptin as compared to leptin per kilogram of body fat and leptin per unit of BMI among older adults. The serum leptin cut off value may be included among risk factors of metabolic syndrome for identifying people at risk.

Introduction

Leptin is an adipocyte derived polypeptide hormone that influences feeding behavior through its direct effect on the hypothalamus (6). Serum levels of leptin reflect the amount of energy stored in adipose tissues (7). Previous studies have reported that serum leptin levels decrease with aging and that older adults have lower levels of circulating leptin as compared to younger adults (9, 38, and 76). However, recent studies refuted these previous findings and demonstrated that aging is accompanied by increase in serum leptin levels (36 and 37). A concomitant increase in serum leptin and TNF- a with aging has been associated with obesity and atherosclerosis (7 and 38).

Leptin plays a critical role in the inflammatory response (35) and has been implicated in the etiology of insulin resistance (40) and metabolic syndrome (76, 40, and 49). Leptin directly inhibits insulin secretion from pancreatic ß- cells (46) and elevated serum leptin levels are shown to be associated with fasting insulin, insulin resistance (HOMA-IR), and total cholesterol (40, 42, 43, 45 and 140), that are the core metabolic disturbances of the metabolic syndrome (141). It has been also demonstrated by elegant experiments on rodent models that failure of leptin to "cross talk" with other fat depots to regulate fat distribution has a cause-effect relationship between the age-related increase in visceral fat and the decrease in hepatic insulin action (44), which are major determinants of the metabolic syndrome (137 and 144).

Although serum leptin levels have been consistently shown to be associated with metabolic syndrome and its components, none of the studies have determined the serum leptin cut off level that suggests the risk of metabolic syndrome among older adults.

The primary objective of this study was to find out the serum leptin cut off value that suggests the risk of metabolic syndrome among older men and women. In addition, the other objective was to compute and compare the performance of cut off points for serum leptin, leptin per kilogram of body fat and leptin per unit of BMI.

Subjects and Methods

Study Design

The Health, Aging and Body Composition (Health ABC) study is a longitudinal cohort study of 3,075 community dwelling, well functioning white and black men and women aged 70 to 79 years at the commencement of the study. Participants were recruited for the study from a random sample of white residents receiving Medicare benefits and from all age-eligible black residents of Pittsburgh, Pennsylvania, and Memphis, Tennessee. Subjects were considered eligible to participate in the study if they reported no difficulty walking a quarter of a mile, climbing 10 steps without resting, or performing basic activities of daily living, were free of life-threatening illness, and planned to remain in the geographic area for at least 3 years. Those who reported active treatment for cancer or participation in diet or exercise intervention were excluded from the study. Participants gave written informed consent, and protocols were approved by the Institutional Review Boards at the two study sites. A home interview was administered to participants to collect information on demographic, socioeconomic factors and health behaviors between April 1997 and June 1998. A clinic visit was conducted for examination of biochemical variables, body composition, weight-related health conditions, and physical function. Thereafter,

follow-up clinical examinations are being conducted annually, and telephone interviews semi-annually.

Subjects

In the present study, data from baseline and year 6 of the Health ABC study were used. The data of individuals with prevalent metabolic syndrome at baseline (n = 768) and at year 6 (n=143) were excluded from the analysis. Also excluded were the data of individuals with missing or implausible values for leptin (n = 180) and total body fat (n = 775).

Biochemical Variables

Participants underwent venipuncture at baseline visit after an overnight fast, and serum samples were frozen at -70°C. Plasma glucose was measured using an automated glucose oxidase reaction (YSI 2300 Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH). Triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured using a chemical analyzer (Vitros 950; Johnson & Johnson, Raritan, NJ). Serum concentrations of leptin were measured in duplicate by means of radioimmunoassay (Linco Research Inc, St Charles, Mo). The assay range is 0.05 to 100 ng/ml leptin in serum or plasma. Intraassay coefficient of variations (CVs) ranged from 3.7% to 7.5%, and interassay CVs ranged from 3.2% to 8.9%. The assay is highly specific for human leptin and shows minimal reactivity with mouse or rat leptin (91).

Anthropometric and Clinical Variables

Weight was measured using a standard balance beam scale in kilograms. Height was measured twice using a Harpenden stadiometer (Holtain Ltd., Crosswell, U.K.) in centimeters and the average of the two measurements was used. Body mass index (BMI) [weight (kg)/height (m^2)] was calculated. Dual energy x-ray absorptiometry (DXA) (Hologic QDR 4500A, software

version 8.21, Hologic, Waltham, MA) was used to assess the total fat mass (77). Systolic and diastolic blood pressures were measured by manual sphygmomanometer in a seated position on left arm.

Demographic and Life Style Variables

A standardized questionnaire was administered at baseline to collect information on socio-demographic variables including age, gender, race and years of education, and; on lifestyle variables including smoking status and average alcohol consumption during the past year (0, 1-2 or >2 drinks/day). Cigarettes smoked per day were multiplied by the number of years of smoking to calculate the pack-years over a lifetime of cigarette smoking. Level of physical activity was obtained using a standardized questionnaire specifically designed for the Health ABC study. The frequency, duration, and intensity level of activities were recorded, and the approximate values of metabolic equivalent unit (MET) were assigned to each activity category to estimate weekly energy expenditure in kcal/kg/week (78).

Outcome Variable

In Health ABC, metabolic syndrome at year 6, was defined according to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) as the presence of 3 or more of the 5 risk components as follows: 1) waist circumference >102 cm for men and >88 cm for women; 2) TG =150 mg/dl; 3) HDL-C <40 mg/dl for men and <50 mg/dl for women; 4) blood pressure (BP) = 130/85 mmHg or currently on antihypertensive medicine(s); 5) fasting glucose =110 mg/dl or currently using anti-diabetic (insulin or oral agents) medication.

Statistical analysis

Multivariate logistic regression analysis was used to assess the association of serum leptin, leptin per kilogram of body fat and leptin per unit of BMI with risk of metabolic syndrome

at year 6 after adjustment for potential confounders (age, race, site, and years of education, alcohol use, smoking, and physical activity) in both genders. Serum leptin values and body fat vary by gender, therefore, the cut off value of serum leptin levels were determined separately by gender. Receiver operating characteristic (ROC) curve was used to find out the serum leptin cut off value for determining risk of metabolic syndrome. The "gold standard" for the ROC curves was based on the presence of metabolic syndrome at year 6. Sensitivity and specificity were calculated for several cut off points of serum leptin among men and women. Because serum leptin has been shown to be associated with total body fat, sensitivity and specificity of the cut off values of serum leptin per kilogram of body fat and serum leptin per unit of BMI were also computed for determining the risk of metabolic syndrome at year 6. To evaluate the cut off point for the above three tests, the shortest distance on the sex-specific ROC curve was computed and searched. A distance on the ROC curve is equal to v $(1\text{-sensitivity})^2 + (1\text{-specificity})^2$ (43). Younden index (J) was also calculated for all three tests. Youden index is defined as sensitivity plus specificity minus 1. When the test is perfect J is 1, while its equal to 0 when the performance of test is poor. The value with shortest distance and highest J was selected as the cut off point. Area under curve (AUC) was estimated by logistic regression analysis and the test with highest AUC was considered to be the best among three tests for determining risk of metabolic syndrome. Statistical significance was set at $p = 0.05$, and analyses performed using SAS (version 9.1; SAS Institute Inc., Cary, NC).

Results

There was a significant difference ($p<0.0001$) between the mean serum leptin concentrations among men (7.1 ±5.8) ng/ml) and women (18.7 ±13.3 ng/ml). Baseline characteristics indicate that among men (leptin cut off value = 6.45 ng/ml) and women (leptin cut off value = 18.25 ng/ml), those with serum leptin levels below the cut off point had significantly lower, BMI, abdominal circumference, total fat mass, abdominal visceral fat, subcutaneous fat, and triglycerides as compared to those with serum leptin levels above the cut off point. Additionally, men with serum leptin levels below the cut off value had significantly lower diastolic blood pressure and women with serum leptin levels below the cut off value had significantly lower fasting glucose and HDL cholesterol as compared to men and women with serum leptin levels above the cut off point. Women with serum leptin levels below the cut off point were more likely to be white, complete high school and were older as compared to those with serum leptin levels above the cut off point **(Table 6.1 and 6.2)**.

In multivariate analysis, serum leptin and serum leptin per unit of BMI were significantly associated with the risk of developing metabolic syndrome after adjusting for potential confounders among men (1.71; 95% CI = 1.26 – 2.32), (OR = 1.58; 95% CI = 1.14 – 2.2), respectively. Among women, serum leptin (OR = 2.02; 95% CI = 1.49 – 2.74), serum leptin per kg of body fat (OR = 1.89; 95% CI = 1.3 – 2.75), and serum leptin per unit of BMI (OR = 1.98; 95% CI = 1.4 –2.8) were positively associated with increased risk of developing metabolic syndrome after adjusting for potential confounders **(Table 6.3)**.

Among men, area under ROC curve (AUC) for serum leptin was higher (0.62) as compared to AUC of 0.58 and 0.59 for serum leptin per kg of body fat and serum leptin per unit of BMI, respectively. The serum leptin cut off values for determining metabolic syndrome was found to be 6.45 ng/ml with shortest distance on ROC curve, highest Youden's index (J) of 0.23 and sensitivity and specificity of 60% and 63 %, respectively. The cut off points for serum leptin per kg of body fat and serum leptin per unit of BMI were also determined. Serum leptin level of

0.27 ng/ml per kg body fat and 0.22 ng/ml per unit of BMI were found to predict metabolic syndrome risk with sensitivity of 54% and 65%, respectively and specificity of 60% and 55%, respectively. The appropriate value of J for leptin per kg body fat and leptin per unit of BMI were 0.14 and 0.20, respectively **(Table 6.4)**. The ROC curve for serum leptin concentrations predicting metabolic syndrome among men is shown in **Figure 6.1**.

Among women, area under ROC curve (AUC) for serum leptin was higher (0.62) as compared to AUC of 0.58 and 0.59 for serum leptin per kg of body fat and serum leptin per unit of BMI, respectively. The serum leptin cut off values for predicting metabolic syndrome was found to be 18.25 ng/ml with shortest distance on ROC curve, highest Youden's index (J) of 0.17 and a sensitivity and specificity of 55% and 62 %, respectively. Serum leptin level of 0.61 ng/ml per kg body fat and 0.71 ng/ml per unit of BMI were found to predict metabolic syndrome risk with sensitivity of 61% and 51%, respectively and specificity of 54% and 65%, respectively. The appropriate value of J for leptin per kg body fat and leptin per unit of BMI were 0.15 and 0.16, respectively **(Table 6.4)**. The ROC curve for serum leptin predicting metabolic syndrome among women is shown in **Figure 6.2**.

Discussion

In the present study, compared to men, women had higher serum leptin concentrations and cut off values for the three tests (18.25 ng/ml for leptin, 0.61 ng/ml for leptin per kg body fat, and 0.71 ng/ml for leptin per unit of BMI among women; 6.45 ng/ml for leptin, 0.27 ng/ml for leptin per kg body fat, and 0.22 ng/ml for leptin per unit of BMI among men). Serum leptin had the highest Youden's index (J), AUC and sensitivity and specificity in association with the risk of metabolic syndrome as compared to serum leptin per unit body fat and serum leptin per unit of BMI, among men and women.

Few longitudinal (40 and 79) and several cross-sectional (17, 18, 19, 40, 42, 81, and 125) studies have shown a significant association between serum leptin concentrations, the metabolic syndrome and its components. Franks et al., in the Medical Research Council Ely Study of subjects aged 50-59 years, found leptin to be positively related to the risk of metabolic syndrome after adjusting for obesity (79). In men aged 45±1.3 years, when risk factors of the metabolic syndrome were considered together in factor analysis, serum leptin formed a part of the cluster that constituted insulin resistance and hyperinsulinemia independent of measures of body fat (40). In addition, serum leptin has been shown to be positively associated with systolic blood pressure among women and with diastolic blood pressure among men (81). Hyperleptinemia was positively associated with serum insulin levels while a negative association has been reported between serum leptin and triglyceride levels (17, 19, 40, 81, and 125). Recently Zhuo et al., in a cross-sectional study on Chinese older population, showed that association of leptin to adiponectin ratio and leptin alone with metabolic syndrome was stronger than adiponectin alone after adjusting for BMI (132), while Kumagai et al., in glucose intolerant and diabetic subjects found that relationship of leptin to adiponectin ratio, leptin and adiponectin alone with metabolic syndrome is dependent on abdominal adiposity and cardio-respiratory fitness (131). Although previous studies consistently showed serum leptin to be associated with insulin resistance and

components of metabolic syndrome, none of them have determined a cut off value of serum leptin for determining the risk of metabolic syndrome.

Previous studies have consistently shown a correlation between circulating levels of leptin and variables of body adiposity, such as BMI, percent body fat and total fat mass (48, 88, 115, and 116). BMI and waist circumference were found to be the strongest determinants of serum leptin levels among middle aged women and men, respectively (48), while percent body fat was shown to be the best adiposity-related predictor of serum leptin among post menopausal women (116). Recently, the adipocyte was recognized as an endocrine organ that plays a prominent role in metabolic disorders (114). Leptin per kg of fat mass is an indicator of adipocyte function (117), and BMI have been shown to be a good indicator of body fat (115). Thus, the cut off values for both, serum leptin per kg of body fat and serum leptin per unit of BMI, were identified in the present study.

Although serum leptin has been shown to be related to body adiposity, several studies have shown an independent association between serum leptin and metabolic diseases (40, 79, and 83). The Atherosclerosis Risk in Communities (ARIC) study showed an independent association between elevated leptin and diabetes after adjusting for potential confounders and adiposity among both men and women (45). A recent study from National Health and Nutrition Examination Survey (NHANES) found a positive association between higher serum leptin and myocardial infarction or stroke independent of obesity (86). Similarly, a study on type 2 diabetic men showed a concomitant increase in the serum leptin and insulin resistance independent of body adiposity (124). Thus, higher predictive value of serum leptin levels (shown by higher AUC and J value for serum leptin) as compared to serum leptin per kg of body fat and serum leptin per unit of BMI supports the adiposity independent association between serum leptin and metabolic syndrome found in the above studies. The AUC for the prediction of metabolic syndrome by serum leptin levels (~0.62) was significantly higher than what would be expected by chance, indicating that leptin predicts metabolic syndrome. However, as compared to the maximum value

of AUC (1.0 for perfect prediction), these AUC values suggested that other risk factors also contributed to the prediction of metabolic syndrome. Therefore, it may be useful to include serum leptin cut off values among other risk factors of metabolic syndrome.

Several previous studies have shown higher serum leptin concentrations among women as compared to men (72, 85, and 86). There is a concomitant increase in serum leptin and body fat among men and women (88, 115 and 116). But, for any given measure of obesity, serum leptin levels are higher in women than men because of relative hyperleptinemia among women (88 and 115). Thus, to achieve similar biological effect, higher levels of serum leptin are required among women (115), which may explain our finding of the relatively higher cut off values among women.

In the current study, the sensitivities and specificities of the cut off points for all the three tests were moderate. Several studies have shown that a subject's clinical and pathological characteristics may lead to the variation in sensitivity and specificity of the diagnostic test (118, 119 and 122). Leeflang et al., with a series of examples, demonstrated that prevalence of a disease is a marker of variations in the sensitivity and specificity in different clinical populations (123). Studies that were performed on a population with higher disease prevalence or disease severity have shown higher sensitivity and specificity (120, 121 and 122). Thus, the low prevalence of metabolic syndrome (21.7 % in women and 19 % in men) in our study population of well-functioning older adults may explain the moderate sensitivities and specificities of the three cut off values in the present study.

The strength of this study includes the use of longitudinal data for determining the cut-off value of serum leptin. The HABC study collected extensive information on life style and anthropometric variables, which made it possible to adjust for many potential confounders in the multivariate analysis. A limitation of this study is that the sensitivities and specificities of the cut off values are moderate, which may have been due to lower prevalence of metabolic syndrome in

the Health ABC study cohort. Future studies on older population with higher prevalence of metabolic syndrome are needed to confirm the diagnostic value of serum leptin.

In conclusion, the current study suggests that serum leptin value of 6.45ng/ml in men and 18.25 ng/ml in women may be cut- off values for determining the risk of metabolic syndrome among older adults. Measurement of body fat mass requires expensive and invasive techniques (88), limiting its use in large population studies. BMI has been shown to be a good indicator of percent body fat (88), but a natural decease in height among older adults may lead to the overestimation in the number of overweight older adults (133). Serum leptin is relatively reliable and feasible to measure and has been shown to be predictive of metabolic syndrome risk (40 and79). Thus, the inclusion of serum leptin among the other risk factors of metabolic syndrome may improve the diagnosis of individuals at risk of chronic disease and may play an important role in public health.

Tables and Figures:

Table 6.1 Baseline Characteristics of Men by Serum Leptin cutoff value[1]

	Leptin =6.45 (ng/ml)	Leptin >6.45 (ng/ml)
Men (n (%))	343 (58)	251 (42)
Mean Serum Leptin (ng/ml) [3]	3.7 (± 0.8)	11.6 (± 0.39) [2]
Demographic and behavioral variables		
Age (years) [3]	75.2 (±0.15)	75.2 (±0.18)
Race (% white)	69	63
Drinking (% any consumption)	65	61
Education (% completed high school)	76	78
Smoking (lifetime pack-years) [3]	19.9 (±1.36)	22.8 (±1.94)
Physical activity (kcal/kg/week) [3]	10 (±1.2)	9.9 (±17.8)
Anthropometric variables		
BMI (kg/m^2) [3]	25 (±0.13)	29 (±0.21) [2]
Abdominal circumference (cm) [3]	95 (±0.45)	103 (±0.55) [2]
Abdominal visceral fat (cm^2) [3]	123 (±2.6)	167 (±4.2) [2]
Abdominal subcutaneous fat (cm^2) [3]	181 (±2.8)	262 (±4.7) [2]
Total body fat mass (kg) [3]	20 (±0.21)	27 (±0.38) [2]
Biochemical and clinical variables		
Diastolic Blood pressure (mmHg) [3]	71 (±0.63)	74 (±0.73) [2]
Systolic Blood pressure (mmHg) [3]	132 (±1.05)	135 (±1.32)
Fasting glucose (mg/dl) [3]	97 (±1.4)	99 (±1.4)
HDL cholesterol (mg/dl) [3]	51 (±0.71)	50 (±0.67)
Triglycerides (mg/dl) [3]	106 (±2.2)	115 (±2.9) [2]

1 Means (±SEM), unless otherwise specified.
2 Significant difference between the two groups 1, P=0.05 (t test for continuous variables and chi-square test for categorical variables)..
3 Values from baseline of the Health ABC study

Table 6.2 Baseline Characteristics of Women by Serum Leptin cutoff value [1]

	Leptin =18.25 (ng/ml)	Leptin >18.25 (ng/ml)
Women (n (%))	358 (58.2)	257 (41.8)
Mean Serum Leptin (ng/ml) [3]	9.7 (± 0.15)	31.4 (± 0.45) [2]
Demographic and behavioral variables		
Age (years) [3]	75.3 (±0.11)	74.7 (±0.09) [2]
Race (% white)	74	42[2]
Drinking (% any consumption)	48	43
Education (% completed high school)	84	75[2]
Smoking (lifetime pack-years) [3]	12.3 (±0.84)	12.03 (±0.81)
Physical activity (kcal/kg/week) [3]	4.9 (±0.44)	3.5 (±0.41)
Anthropometric variables		
BMI (kg/m^2) [3]	24.5 (±0.15)	30.4 (±0.17)[2]
Abdominal circumference (cm) [3]	91.1(±0.41)	105.2 (±0.43) [2]
Abdominal visceral fat (cm^2) [3]	108 (±2.05)	151 (±2.03) [2]
Abdominal subcutaneous fat (cm^2) [3]	266 (±3.52)	401 (±3.99)[2]
Total body mass(kg) [3]	23 (±0.34)	33 (±0.46)[2]
Biochemical and clinical variables		
Diastolic Blood pressure (mmHg) [3]	70 (±0.45)	70.4 (±0.41)
Systolic Blood pressure (mmHg) [3]	136 (±0.77)	137 (±0.76)
Fasting glucose (mg/dl) [3]	98 (±1.2)	105 (±1.22)[2]
HDL cholesterol (mg/dl) [3]	62 (±0.69)	59 (±0.57)[2]
Triglycerides (mg/dl) [3]	135 (±2.9)	146 (±3.1)[2]

1 Means (±SEM), unless otherwise specified.
2 Significant difference between the two groups, P=0.05 (t test for continuous variables and chi-square test for categorical variables).
3 Values from baseline of the Health ABC study

Table 6.3 Adjusted Odd Ratios (OR) of Metabolic Syndrome at Year 6 by Serum Leptin, Leptin per kg Body Fat Mass, and Leptin per unit of BMI Among Men and Women

	Serum Leptin	Leptin/ kg Fat Mass	Leptin / BMI
Men (n)	479	479	479
Metabolic Syndrome Cases (n)	115	115	115
OR (95% CI)	1.71 (1.26 – 2.32)	1.42 (0.99 – 2.1)	1.58 (1.14 – 2.2)
Women (n)	481	481	481
Metabolic Syndrome Cases (n)	134	134	134
OR (95% CI)	2.02 (1.49 – 2.74)	1.89 (1.3 – 2.75)	1.98 (1.4 – 2.8)

Adjusted for age, race, site, years of education, alcohol use, smoking, and physical activity

Table 6.4 Comparison of Optimal Value, AUC, Sensitivity, Specificity, and Youden's Index (J) of Leptin, Leptin per kg Body Fat Mass, and Leptin per unit of BMI for Diagnosis of Metabolic Syndrome Among Men and Women

Men					
Potential biomarker	**Cutoff**	**AUC**	**Sensitivity**	**Specificity**	**J**
Leptin	5.72 ng/ml	0.62	0.65	0.56	0.21
	6.45 ng/ml	0.62	0.60	0.63	0.23
Leptin / Total Fat Mass	0.24 ng/ml /kg body fat	0.58	0.62	0.51	0.13
	0.27 ng/ml / kg body fat	0.58	0.54	0.60	0.14
Leptin/BMI	0.21 ng/ml / kg/m^2	0.59	0.65	0.53	0.18
	0.22 ng/ml / kg/m^2	0.59	0.65	0.55	0.20
Women					
Leptin	15.68 ng/ml	0.62	0.61	0.54	0.15
	18.25 ng/ml	0.62	0.55	0.62	0.17
Leptin / Total Fat Mass	0.58 ng/ml / kg body fat	0.58	0.62	0.51	0.13
	0.61 ng/ml / kg body fat	0.58	0.61	0.54	0.15
Leptin/BMI	0.59 ng/ml / kg/m^2	0.59	0.58	0.53	0.11
	0.71 ng/ml / kg/m^2	0.59	0.51	0.65	0.16

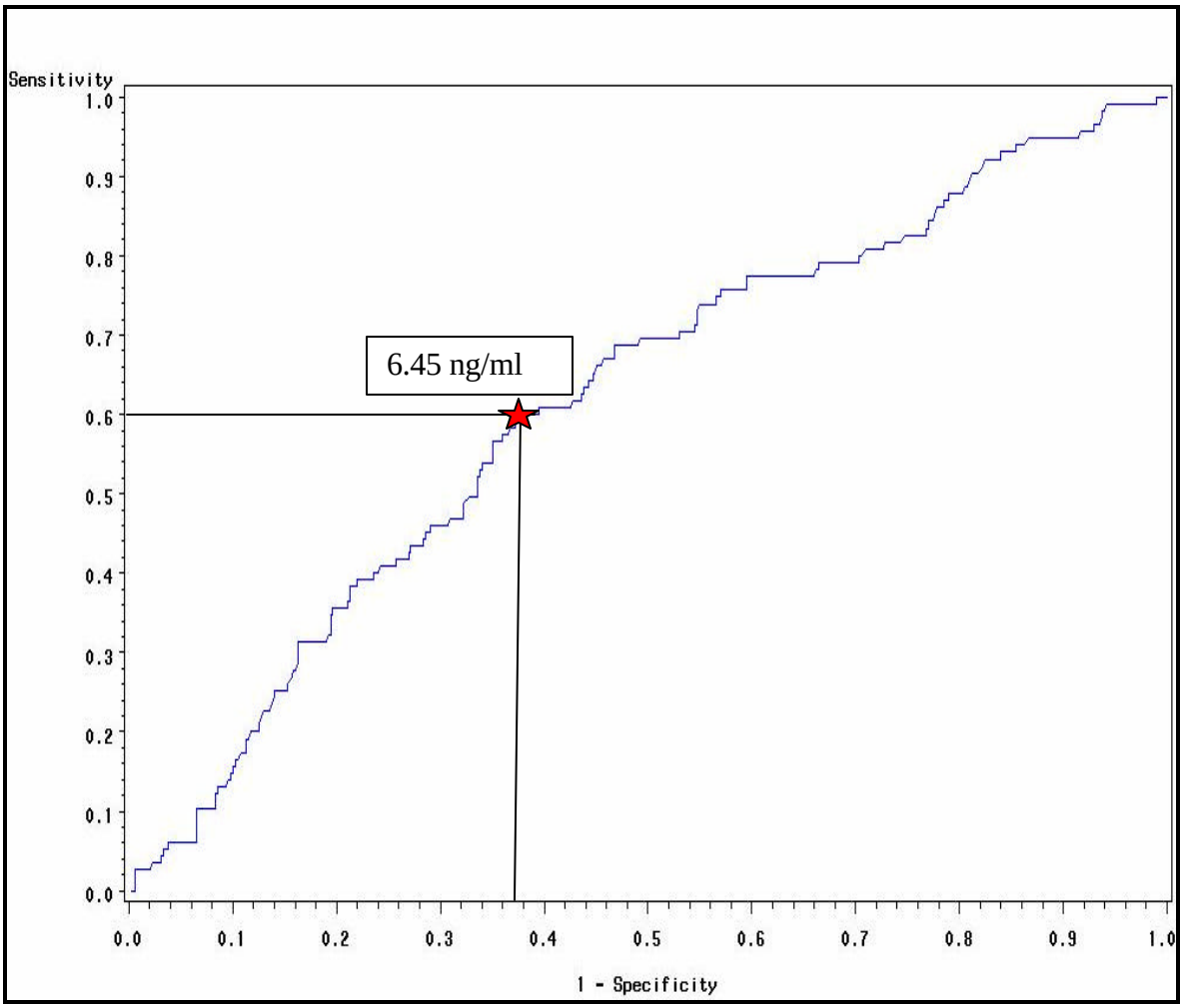

Figure 6.1 Roc Curve for Diagnosis of Metabolic Syndrome among Men

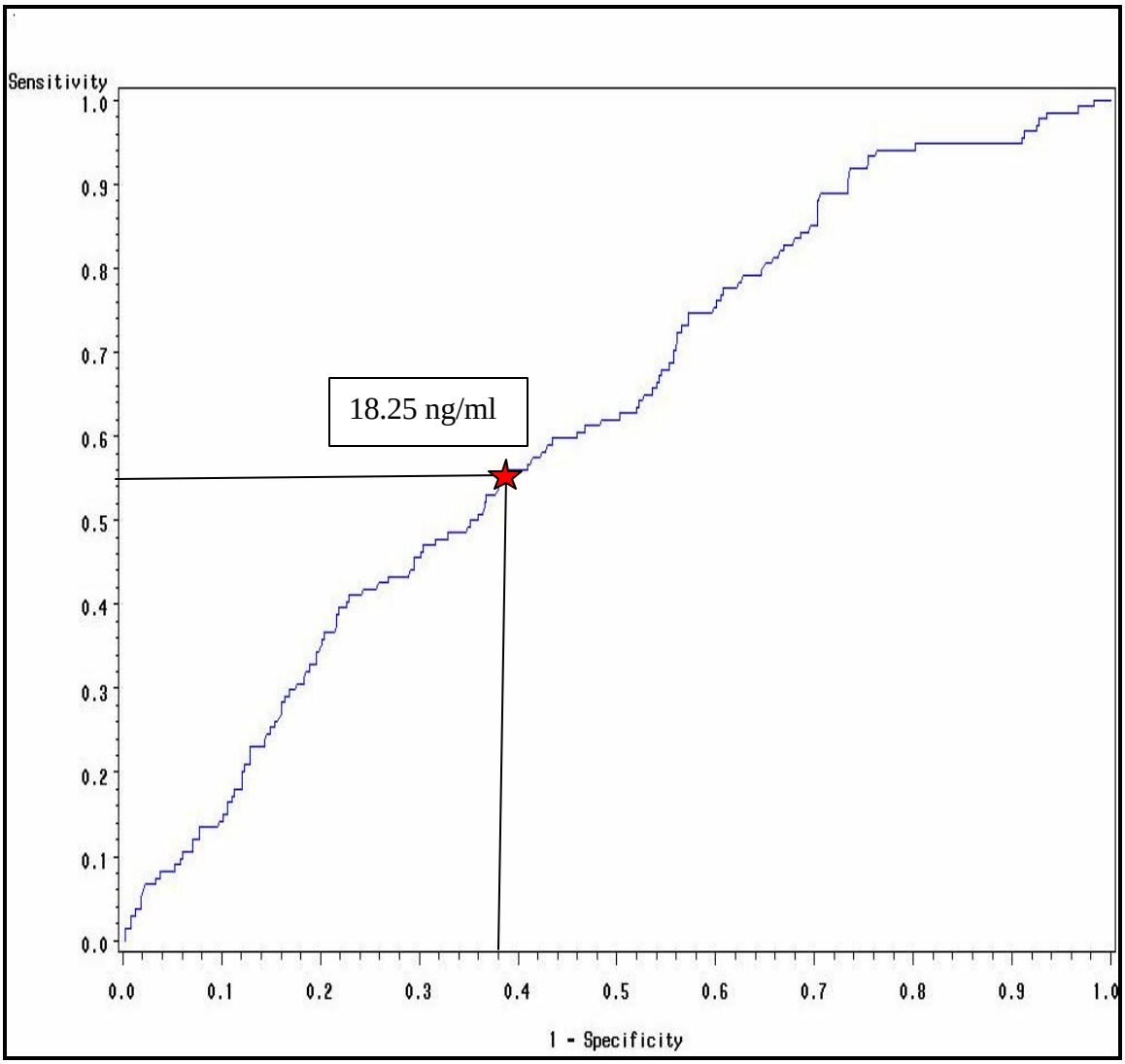

Figure 6.2 Roc Curve for Diagnosis of Metabolic Syndrome among Women

Chapter 7: Summary and Implications

A. Summary

In this study the prospective association of serum leptin with risk of metabolic syndrome, all-cause mortality and mortality from CVD was examined. Association between serum leptin and diabetes as a contributing cause of death and leptin receptor (I/D) genotype was also investigated. The influence of proinflammatory cytokines and markers of adiposity on these associations were explored. In addition, cut off values of serum leptin, leptin per kg of body fat and leptin per unit of BMI for determining risk of metabolic syndrome were identified.

Women were found to have significantly higher serum leptin levels as compared to men. Men and women in quintile 1 of serum leptin had significantly lower BMI, abdominal visceral fat, abdominal subcutaneous fat and total fat percent as compared to men and women in all other quintiles. Additionally, individuals in quintile 1 had significantly lower C- reactive protein and PAI-1 than those in quintile 4 and 5. Women in quintile 1 of serum leptin were found to be at significantly lower risk of developing metabolic syndrome at year 6 than women in all other quintiles. The positive association of serum leptin with metabolic syndrome was independent of proinflammatory cytokines and body adiposity among women. In contrast to the results in women, the association between serum leptin and metabolic syndrome was dependent on body adiposity among men.

Women in intermediate levels of serum leptin were at lower risk of all-cause mortality and mortality from CVD as compared to those with low levels of leptin after adjusting for body fat and proinflammatory cytokine. Additionally, women with elevated levels of serum leptin were at increased risk for diabetes as a contributing cause of death.

Among men, no association was found between serum leptin and all-cause mortality and mortality from CVD, while the association between leptin and risk for diabetes as a contributing cause of death was explained by proinflammatory cytokines among men.

An interaction was found between leptin receptor (I/D) genotype and total percent fat in relation to risk of diabetes as a contributing cause of death among black women. White men who were homozygous for deletion allele at the 3'UTR region of leptin receptor gene had higher C- reactive protein as compared to those who were heterozygous or homozygous for insertion allele. Black women who were homozygous for deletion allele had significantly lower risk of diabetes as a contributing cause of mortality as compared to those who were heterozygous or homozygous for insertion allele.

Cut off values of serum leptin, leptin per kg of body fat and leptin per unit of BMI were found to be 18.25 ng/ml, 0.61 ng/ml, and 0.71 ng/ml, respectively, among women. Among men, the cut off values of serum leptin, leptin per kg of body fat and leptin per unit of BMI were found to be 6.45 ng/ml, 0.27 ng/ml, and 0.22 ng/ml, respectively. The area under curve (AUC), Youden Index (J) and sensitivities and specificities were highest for serum leptin in identifying risk of metabolic syndrome compared to serum leptin per unit of body fat mass and serum leptin per unit of BMI among men and women. Men and women with serum leptin values below the cut off point had significantly lower BMI, total fat mass, abdominal visceral and subcutaneous fat than those with serum leptin concentrations above the cut off point.

Direct comparison of the results of this study with previous research is difficult because most of the studies were conducted on younger individuals. However, in the current and previous studies, association between serum leptin and metabolic disorders was consistently independent of body adiposity. Elevated levels of serum leptin have been shown to be associated with insulin resistance, myocardial infarction and T2D independent of adiposity. In the current study only intermediate quartile of serum leptin was associated with

all-cause mortality and mortality from CVD, suggesting an optimal intermediary range of serum leptin for improved survival among older adults.

The strength of this study includes the longitudinal design of Health ABC study and detailed assessment of life style, anthropometric, biochemical and genetic variables. Data were collected in several waves over a period of 10 years. Studies on hyperleptinemia, inflammation, adiposity and metabolic syndrome have tended to include younger individuals. In this study, the population was 70 years of age and older. The sampling frame for Health ABC study, however, includes functionally well older adults from Tennessee and Pennsylvania. Therefore, it is not possible to extrapolate the results from this study to the general population of older adults in the United States.

B. Implications

Previous studies of the prospective association of serum leptin with metabolic syndrome and mortality did not explore these associations in context of proinflammatory cytokines such as IL-6, TNF-a, CRP, and PAI-1. Proinflammatory cytokines have been associated with serum levels of leptin and also play a crucial role in the etiology of metabolic syndrome, and thus could bias the association between serum leptin and metabolic diseases. Therefore in the present study, it was possible to control for proinflammatory cytokines and body fat to provide a comprehensive picture of the association of serum leptin with metabolic syndrome and mortality. On average, women are more hyperleptinemic and leptin resistant than men. The independent association between serum leptin and metabolic syndrome among older women suggests that the etiology of metabolic syndrome among men and women could be somewhat different and may require separate approaches for preventing metabolic syndrome. Additionally, leptin resistance may play an important role in the development of

metabolic syndrome and, therefore, leptin sensitizing drugs may be helpful in ameliorating the components of metabolic syndrome among leptin insensitive older women.

Although serum leptin has been shown to be positively associated with cardiovascular disease risk factors, our study indicates that moderately elevated leptin is associated with reduced risk of all-cause mortality and mortality from CVD among older women. The current finding of paradoxical association of leptin with cardiovascular disease risk factors and mortality from CVD would help in understanding the diverse pathophysiological effects elicited by leptin in cardiovascular disease among older women.

The current study suggests a need to explore the rationale behind the interaction between leptin receptor (I/D) genotype and percent body fat and its association with higher risk of diabetes as a contributing cause of mortality. Genetic variation plays an important role in metabolic diseases that are associated with changes in body composition. Technological advances for identification of single-nucleotide polymorphisms (SNPs) makes it possible to identify and study the role of gene polymorphisms on disease risk. If percent body fat interacts with leptin receptor genotype to alter the risk of death from diabetes, targeting percent body fat may be useful particularly in a subpopulation of older adults.

Identification of serum leptin cut off value for the diagnosis of metabolic disorders may need to be studied further. Measurement of body fat mass requires expensive and invasive techniques, limiting its use in large population studies (88). BMI has been shown to be a good indicator of percent body fat (88), but a natural decrease in height among older adults may limit its use in this population (133). Serum leptin is relatively reliable and has been shown to predict body fat (88) and risk of metabolic syndrome (40 and 79). Therefore, the inclusion of serum leptin as a risk factor of metabolic syndrome could become an easy and feasible biomarker utilized at the population level, and may also have important implications in the search for effective therapies to prevent and treat metabolic syndrome.

References

1. Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003–2006. National Health Statistics Report. 2009; 13: 1-8

2. Ford ES, Giles WH and Dietz WH. Prevalence of the metabolic syndrome among US adults findings from the third national health and nutrition examination survey. JAMA. 2002; 287(3): 356-359

3. U.S. Census Bureau. An Older and More Diverse Nation by Midcentury. http://www.census.gov/Press- release/www/releases/archives/population/012496.html (accessed December 13, 2009).

4. Foot DK, Lewis RP, Pearson TA, Beller GA. Demographics and cardiology, 1950-2050. J Am Coll Cardiol. 2000; 35,1067-1081.

5. U.S. Census Bureau. 2008 National Population Projections Tables and Charts. http://www.census.gov/population/www/projections/tablesandcharts.html (accessed December 13, 2009).

6. Ren J. Leptin and hyperleptinemia: from friend to foe for cardiovascular function. J Endocrinol. 2004;181:1–10.

7. Miguel Oteroa, Roci´o Lago, Francisca Lago, Felipe F. Casanueva, Carlos Dieguez, Juan Jesu´s Go´mez-Reino, Oreste Gualillo. Mini review: Leptin, from fat to inflammation: old questions and new insights. FEBS Letters 579 (2005) 295–301.

8. Rajala MW and Scherer PE. Minireview: The Adipocyte—At the Crossroads of Energy Homeostasis, Inflammation, and Atherosclerosis. Endocrinology 144: 3765–3773, 2003

9. Moller N, O'Brien P, Nair KS. Disruption of the relationship between fat content and leptin levels with aging in humans. J Clin Metab. 1998;83:931–934.

10. Gabriely I, Ma HX, Yang XM, Rossetti and Barzilai N. Leptin resistance during aging is independent of fat mass. Diabetes. 51:1016–1021, 2002.

11. Peralta S, Carrascosa JM, Gallardo N, Ros M, Arribas C. Ageing increases SOCS-3 expression in rat hypothalamus: effects of food restriction. Biochem Biophys Res Comm. 2002; 296: 425–428.

12. Bjorbaek C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. J Biol Chem. 1999;274:30059–30065.

13. La Fleur SE. The effects of glucocorticoids on feeding behavior in rats. Physiology & Behavior 89. 2006.110–114.

14. Unger, R. H., Zhou, Y.-T. & Orci, L. Regulation of fatty acid homeostasis in cells: novel role of leptin.Proc. Natl Acad. Sci. 1999; 96: 2327-2332.

15. Ruderman, N. B., Saha, A. K., Vavvas, D. & Witters, L. A. Malonyl-CoA, fuel sensing, and insulin resistance. Am. J. Physiol. 1999; 276: E1-E18.

16. Koh KK, Park SM, and Quon MJ. Leptin and cardiovascular disease: response to therapeutic interventions. Circulation. 2008; 117: 3238-3249.

17. Lichnovská R, Gwozdziewiczová S, Chlup R, Hrebícek J. Serum Leptin in the development of insulin resistance and other disorders in the metabolic syndrome. Biomed. Papers. 2005; 149(1), 119–126.

18. Maggio M, Lauretani F, Ceda GP, Bandinelli S, Basaria S, Ble A, Egan J, Paolisso G, Najjar S, Metter EJ, Valenti G, Guralnik JM, FerrucciL. Association Between Hormones and Metabolic Syndrome in Older Italian Men. Journal of the American Geriatrics Society. 2006; 54 (12): 1832–1838.

19. Henriksen JH, Holst JJ, Moller S, Andersen UB, Bendtsen F, Jensen G. Elevated circulating leptin levels in arterial hypertension: relationship to arteriovenous overflow and extraction of leptin. Clin Sci. 2000;99:527–534.

20. Venner AA, Lyon ME, and Doyle-Baker PK. Leptin: A potential biomarker for childhood obesity? Clinical Biochemistry. 2006; 39: 1047–1056.

21. Mantzoros CS. The Role of Leptin in Human Obesity and Disease: A Review of Current Evidence. Ann Intern Med. 1999;130:671-680.

22. Ahima RS & Flier JS. Adipose tissue as an endocrine organ. Trends in endocrinology and Metabolism. 2006; 11: 327–332.

23. Sahu A. Minireview: A Hypothalamic Role in Energy Balance with Special Emphasis on Leptin. Endocrinology. 2004;145(6):2613–2620.

24. Bouret SG and Simerly RB. Minireview: Leptin and Development of hypothalamic Feeding Circuits. Endocrinology. 2004; 145(6):2621–2626.

25. Schwartz MW. Brain pathways controlling food intake and body weight. Experimental Biology of Medicine. 2001; 226: 978–981.

26. Speakman JR. Obesity: the integrated roles of environment and genetics. J Nutr 2004;134(8 Suppl):2090S.–105S

27. Winick, J.D., Stoffel, M. and J.F. Friedman. Identification of microsatellites linked to the human leptin receptor gene on chromosome 1. Genomics. 1996; 36: 221-222.

28. Baratta M. Leptin—from a signal of adiposity to a hormonal mediator in peripheral tissues. Med Sci Monit 2002; 8: RA282–92.

29. Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. J Am Soc Nephrol. 2004; 31: 2792–2800.

30. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW. Subcutaneous adipose tissue releases interleukin- 6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab. 1997; 82: 196–200.

31. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest. 1995; 95:2409–2415.

32. Bays H, Mandarano L, and DeFronzo RA. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J Clin Endocrinol Metab. 2004; 89(2): 463–478.

33. Arnalich F, Lopez J, Codoceo R, Jimenez M, Madero R, Montiel C. Relationship of plasma leptin to plasma cytokines and human survival in sepsis and septic shock. The Journal of Infectious Diseases.1999; 180: 908–911.

34. Bruunsgard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokine. Curr Opin Hematol. 2001; 8:131–136.

35. Hukshorn CJ, Lindeman JHN, Toet KH, Saris WHM, Eilers PHC, Plantenga MSW and Kooistra T. Leptin and the proinflammatory state associated with human obesity. J Clin Endocrinol Metab. 2004; 89 (4):1773-1778

36. Sánchez-Rodríguez M, García-Sánchez A, Retana-Ugalde R, Mendoza- Núñez VM. Serum leptin levels and blood pressure in the overweight elderly. Arch Med Res. 2000 Jul-Aug;31(4):425-8.

37. Gabriely I, Ma HX, Yang XM, Rossetti and Barzilai N. Leptin resistance during aging is independent of fat mass. Diabetes. 51:1016–1021, 2002.

38. Mendoza-Nunez VM, Garcia AS, Rodriguez MS, Duarte REG, and Yerena MEF. Overweight, waist circumference, age, gender, and insulin resistance as risk factors for hyperleptinemia. Obes Res. 2002;10:253–259.

39. Alessandro B, Windham GB, Bandinelli S, Taub DD, Volpato S, Bartali B, Tracy RP, Guralnik JM, Ferrucci L. Relation of plasma leptin to C-reactive protein in older adults (from the Invecchiare nel Chianti Study). The American Journal of Cardiology. 2005; 96(7): 991-995.

40. Francisco L, Godsland IF, Mohammed G, Proudler AJ, Aldis S, Walton C, Bloom S and Stevenson JC. Hyperleptinemia as a component of a metabolic syndrome of cardiovascular risk. Arterioscler Thromb Vasc Biol. 1998;18: 928-933

41. Gannage´-Yared MH, Khalife S, Semaan M, Fares F, Jambart S and Halaby G. Serum adiponectin and leptin levels in relation to the metabolic syndrome, androgenic profile and somatotropic axis in healthy non-diabetic elderly men. European Journal of Endocrinology (2006) 155 167–176.

42. Zamboni M, Zoico E, Fantin F, Panourgia MP, Francesco VD, Tosoni P, Solerte B, Vettor R and Bosello O . Relation between leptin and the metabolic syndrome in elderly women. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2004; 59:M396-M400.

43. Anker SD, Leyva F, Godsland IF, Lees B, Stevenson JC, Coats AJ. Leptin in cardiac cachexia: possible regulatory effects of insulin and insulin resistance. Eur Heart J. In press.

44. Barzilai N and Gupta. Interaction between aging and syndrome X: new insights on the pathophysiology of fat distribution. Annals of the New York Academy of Sciences. 1999; 892:58-72.

45. Schmidt MI, Duncan BB, Vigo A, Pankow JS, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Leptin and incident type 2 diabetes: risk or protection? Diabetologia. 2006; 49(9): 2086-2096.

46. Ronaldo BC, Heikki A K, Juleen RZ, and Gary S. Analysis of paradoxical observations on the association between leptin and insulin resistance. FASEB J. 2002; 16, 1163–1176.

47. Wallace AM, McMahon AD, Packard CJ, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation. 2001;104: 3052– 6.

48. Ruige JB, Dekker JM, Blum WF, Stehouwer CD, Nijpels G, Mooy J, Kostense PJ, Bouter LM, Heine RJ. Leptin and variables of body adiposity, energy balance, and insulin resistance in a population-based study.The Hoom Study. Diabetes Care. 1999; 22: 1097–104.

49. Yared MHG, Simon Khalife S, Michelle Semaan M, Fares F , S Jambart Sand Halaby G. Serum adiponectin and leptin levels in relation to the metabolic syndrome, androgenic profile and somatotropic axis in healthy non-diabetic elderly men. European Journal of Endocrinology. 2006; 155: 167–176.

50. Buettner R, Bollheimer LC, Zietz B, DrobnikW, Lackner K, Schmitz G, Scholmerich J & Palitzsch KD. Definition and characterization of relative hypo- and hyperleptinemia in a large Caucasian population. Journal of Endocrinology. 2002; 175 745–756.

51. Claudia L, Bergstrom J, Scheidt C, Pfeilschifter J, Barrett E. Cardiovascular death and the metabolic syndrome: Role of adiposity-signaling hormones and inflammatory markers. Diabetes Care. 2006; 29:1363–1369.

52. National Cholesterol Education Program Expert Panel: ATP III final report: third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection,

evaluation, and treatment of high blood cholesterol in adults' final report. I. Background and introduction. Circulation. 2002; 106:3157–3160.

53. Havel PJ: Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol. 2002; 13:51–59.

54. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzman M, Brownlee M: Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem. 2001; 276:25096–25100.

55. Shimokawa I, Higami Y. Leptin signaling and ageing: insight from caloric restriction. Mech Ageing Dev. 2001;122:1511–1519.

56. Yeh S, Schuster MW. Geriatric cachexia: the role of cytokines. Am J Clin Nutr. 1999;70:183–197.

57. Moller N, O'Brien P, Nair KS. Disruption of the relationship between fat content and leptin levels with aging in humans. J Clin Metab. 1998;83:931–934.

58. Javor ED, Cochran EK, Musso C, et al. Long-term efficacy of leptin replacement in patients with generalized lipodystrophy. Diabetes 2005; 54:1994–2002.

59. Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL, Stern JS Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. J Clin Endocrinol Metab. 1996; 81:4406–4413.

60. Lieb W, Sullivan LM, Harris TB, Ronenn Roubenoff R, Levy D, Fox CS, Wang TJ, Wilson PW, Kannel WB, and Vasan RS. Plasma Leptin Levels and Incidence of Heart Failure, Cardiovascular Disease, and Total Mortality in Elderly Individuals. Diabetes Care. 2009; 32: 612-616.

61. Scholze A, Rattensperger D, Zidek W and Tepel M. Low Serum Leptin Predicts Mortality in Patients with Chronic Kidney Disease Stage 5. Obesity. 2007;31, 1617–1622.

62. Welsh P, Murray HM, Buckley BM, Craen AJMD, Ford I, Jukema JW, Macfarlane PW, Packard CJ , Stott DJ , FRCPI1, Westendorp RGJ, Shepherd J, and Naveed Sattar N. Leptin predicts diabetes but not cardiovascular disease: Results from a large prospective study in an elderly population. Diabetes Care. 2009; 32 (2): 308-310.

63. Dalamaga M, Migdalis I, Fargnoli JL, Papadavid E, Bloom E, Mitsiades N, Karmaniolas K, Pelecanos N, Tseleni-Balafouta S, Dionyssiou-Asteriou A and Christos S. Mantzoros CS. Pancreatic cancer expresses adiponectin receptors and is associated with hypoleptinemia and hyperadiponectinemia: a case–control study. Cancer Causes and Control. 2008; 20: 625-633.

64. Piemonti L, Calori G, Mercalli A, Lattada G, Monti P, Garancini MP, Costantino F, Ruotolo G, Luzi L, Ghin G. Fasting Plasma Leptin, Tumor Necrosis Factor-a Receptor 2,

and Monocyte Chemoattracting Protein 1 Concentration in a Population of Glucose-Tolerant and Glucose-Intolerant Women. Diabetes Care .2003; 26: 2883–2889.

65. Kieffer TJ, Heller RS, Haberner JF. Leptin receptors expressed on pancreatic b-cells. Biochem Biophys Res Commun 1996; 224: 522 - 527.

66. Kieffer TJ, Heller SR, Leech CA, Holz GG, Habener JF. Leptin suppression of insulin secretion by activation of ATPsensitive K channels in pancreatic beta-cells. Diabetes 1997; 46: 1087 - 1093.

67. Oksanen L, Kaprio J, Mustajoki P, Kontula K: A common pentanucleotide olymorphism of the 3'-untranslated part of the leptin receptor gene generates a putative stem-loop motif in the mRNA and is associated with serum insulin levels in obese individuals. Int J Obes Relat Metab Disord. 1998; 22: 634–640

68. Lakka HM, Oksanen L, Tuomainen TP, Kontula K, Salonen JT: The common pentanucleotide polymorphism of the 3'-untranslated region of the leptin receptor gene is associated with serum insulin levels and the risk of type 2 diabetes in nondiabetic men: a prospective case-control study. J Intern Med. 2000; 248:77– 83.

69. Nannipieri M, PosadasR, Bonotti A, Williams K, Gonzalez-Villapando C, Stern MP, Ferrannini E. Polymorphism of the 3-untranslated region of the leptin receptor gene, but not the adiponectin SNP45 polymorphism, predicts type 2 diabetes. Diabetes Care. 2006; 29 (11):2509-2511.

70. Zacharova J, Chiasson, JL and Laakso M. Leptin receptor gene variation predicts weight change in subjects with impaired glucose tolerance. Obesity research. 2005; 13 (3): 501-506.

71. Wauters M, Mertens I, Chagnon M, Rankinen T, Considine RV, Chagnon YC, Van Gaal LF and Bouchard C. Polymorphisms in the leptin receptor gene, body composition and fat distribution in overweight and obese women. International Journal of Obesity. 2001; 25: 714-720.

72. Rosenbaum M, Pietrobelli A, Vasselli JR, Heymsfield SB, Leibel RL. Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. Int J Obes Relat Metab Disord 2001;25:1365–71

73. Dotsch J, Rascher W, and Meibner U. New insights into leptin resistance by modifying cytokine receptor signal transduction**.** Eur J Endocrinol. 2005; 152: 333-334.

74. Rajala MW and Scherer PE. Minireview: The Adipocyte—At the Crossroads of Energy Homeostasis, Inflammation, and Atherosclerosis. Endocrinology 144: 3765–3773, 2003.

75. Gainsford, T. et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc. Natl Acad. Sci. USA. 1996; 93, 14564–14568.

76. Vanden Saffele JK, Goemaere S, DeBacquer D, Kaufman JM. Serum leptin levels in healthy ageing men: are decreased serum testosterone and increased adiposity in elderly men the consequence of leptin deficiency? Clin Endocrinol. 1999;51: 81–8.

77. Menke A, Muntner P, Wildman RP, Reynolds K, He J. Measures of adiposity and cardiovascular disease risk factors. Obesity (Silver Spring). 2007; 15(3): 785-95.

78. Lindqvist P, Andersson K, Sundh V, Lissner L, Björkelund C, Bengtsson C. Concurrent and separate effects of body mass index and waist-to-hip ratio on 24-year mortality in the Population Study of Women in Gothenburg: evidence of age-dependency. Eur J Epidemiol. 2006;21(11):789-94.

79. Franks PW, Brage S, Luan J, Ekelund U, Rahman M, Farooq S, Halsall I, O'Rahilly S, and Wareham NJ. Leptin Predicts a Worsening of the Features of the Metabolic Syndrome Independently of Obesity. Obesity Research. 2005;13(8):1476-1484.

80. Canatan H, Bakan I, Akbulut M, Baydas G, Halifeoglu I, Gursu MF. Comparative analysis of plasma leptin levels in both genders of patients with essential hypertension and healthy subjects. Endocr Res. 2004;30:95–105.

81. Mendoza-Nunez VM, Munoz EC, Cruz EAG, Rodriguez MAS, Duarte REG, Ugalde RR. Hyperleptinemia as a risk factor for high blood pressure in the elderly. Arch Pathol Lab Med. 2006;130:170–175.

82. Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, Weigle DS: Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. Diabetes.1998;47:239 –243.

83. Justo SJ, Abel RC, Francisco LJ, Apoor SG, Fatima HSK, Robert W, and Virend KS. Relation of Increased Leptin Concentrations to History of Myocardial Infarction and Stroke in the US Population. Am J Cardiol. 2007;100(2): 234–239.

84. Couillard C, Lamarche B, Mauriege P, et al. Leptinemia is not a risk factor for ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. Diabetes Care. 1998;21:782–786

85. Ruhl CE, Everhart JE. Leptin concentrations in the United States: relations with demographic and anthropometric measures. Am J Clin Nutr. 2001;74:295–301.

86. Ongphiphadhanakul B, Rajatanavin R, Chanprasertyothin S, Piaseu N, Chailurkit L. Serum leptin concentrations in relation to body fat, gender, sex hormones and metabolic covariates in Thais. J Med Assoc Thai 1999;82:862–7.

87. Baumgartner RN, Ross RR, Waters DL, et al. Serum leptin in elderly people: associations with sex hormones, insulin, and adipose tissue volumes. Obes Res 1999;7:141–9.

88. Ruhl CE, Harris TB, Ding J, Goodpaster BH, Kanaya M, Kritchevsky SB, Simonsick EM, Tylavsky FA, and Everhart JE. Body mass index and serum leptin concentration independently estimate percentage body fat in older adults. Am J Clin Nutr. 2007; 85: 1121–1126.

89. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C- reactive protein, interleukin 6 and risk of developing type 2 diabetes mellitus. JAMA. 2001; 286(3): 327-334.

90. Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM: Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS).Circulation. 2000;102:42– 47.

91. Health, Aging and Body Composition Study Operations Manual. Internet: https://psg-mac43.ucsf.edu (accessed October, 2009).

92. Gorina Y, Hoyert D, Lentzner H, Goulding M. Trends in causes of death among older persons States. Aging Trends, No 6. Hyattsville, Maryland: National Center for Health Statistics. 2006.

93. Shahar BY, Houston DK, Kritchevsky SB, Lee JS, Rubin SM, Sellmeyer DE, Tylavsky FA, and Harris TB. Dietary Factors in Relation to Daily Activity Energy Expenditure and Mortality among Older Adults. Journal of Nutrition Health & Aging, 2009; 13 (5): 414-20.

94. Myers MG, Cowley MA, Mu¨nzberg H. Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 2008;70:537–56.

95. Gualillo O, Eiras S, Lago F, Dieguez C, Casanueva FF. Evaluated serum leptin concentrations induced by experimental acute inflammation. Life Sci. 2000;67:2433–2441.

96. Beltowski J, Wo´ jcicka G, Marciniak A, Jamroz A. Oxidative stress, nitric oxide production, and renal sodium handling in leptin-induced hypertension. Life Sci. 2004;74:2987–3000.

97. Brennan AM, Li TY, Kelesidis I, Gavrila A, Hu FB and Mantzoros CS. Circulating leptin levels are not associated with cardiovascular morbidity and mortality in women with diabetes: a prospective cohort study. Diabetologia. 2007; 50: 1178–1185.

98. Oral EA, Simha V, Ruiz E, et al. Leptin-replacement therapy for lipodystrophy. N Engl J Med 2002;346:570–8.

99. Wannamethee SG, Lowe GDO, Rumley A, Cherry L, Whincup PH, Sattar N. Adipokines and Risk of Type 2 Diabetes in Older Men. Diabetes Care. 2007; 30:1200–1205.

100. Perfetto F, Manuso F, Tarquini R 2002 Leukocytosis and hyperleptinemia in obesity: is there a link. Haematologica 87:ELT25.

101. Zimmet PZ, Collins VR, de Courten MP, Hodge AM, Collier GR, Dowse GK, Alberti KG, Tuomilehto J, Hemraj F, Gareeboo H, Chitson P, Fareed D. Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean nation of Mauritius. Mauritius NCD Study Group. Int J Obes Relat Metab Disord. 1998; 22:171–177.

102. Van Dielen FM, van't Veer C, Schols AM, Soeters PB, Buurman WA, Greve JW. Increased leptin concentrations correlate with increased concentrations

of inflammatory markers in morbidly obese individuals. Int J Obes Relat Metab Disord. 2001; 25:1759–1766.

103. Avons P, Ducimetiere P, Rakotovao R: Weight and mortality. Lancet. 1983;2: 1104.

104. Minokoshi Y, Kim YB, Peroni OD, Fryer LGD, Mueller C, Carling D and Barbara B. Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature. 2002; 415: 339-343.

105. Ross R, Shaw KD, Rissanen J, Martel Y, Guise J de and Avruch L. Sex differences in lean and adipose tissue distribution by magnetic resonance imaging: anthropometric relationships. Am J Clin Nutr. 1994; 59: 1277-85.

106. Zacharova J, Chiasson, JL and Laakso M. Leptin receptor gene variation predicts weight change in subjects with impaired glucose tolerance. Obesity research. 2005; 13 (3): 501-506.

107. Guerra B, Fuentes T, Delgado-Guerra S, Guadalupe-Grau A, Olmedillas H, et al. Gender dimorphism in skeletal muscle leptin receptors, serum leptin and insulin sensitivity. PLoS ONE. 2008; 3(10): e3466.

108. Woods SC, Gotoh K and Clegg DJ. Gender Differences in the Control of Energy Homeostasis. 2003. Exp Biol Med 228:1175–1180.

109. Lavie CJ, Osman AF, Milani RV, Mehta MR. Body composition and prognosis in chronic systolic heart failure: the obesity paradox. Am J Cardiol. 2003; 91: 891-894.

110. Patel JV, Lim HS, Hughes EA, Lip GY. Adiponectin and hypertension: a putative link between adipocyte function and atherosclerotic risk? J Hum hypertens; 2007; 21: 1-4.

111. McGaffin KR, Sun CK, Rager JJ et al. leptin signaling reduces the severity of cardiac dysfunction and remodeling after chronic ischemic injury. 2008; 77: 4-5.

112. Patel JV, Tracy I, Hughes EA, Lip GY. Unraveling the paradoxical link between obesity and heart failure: the role of adipocytokines. Expert Rev Cardiovasc Ther. 2009; 7(4): 337-340.

113. Barouch LA, Berkowitz DE, Harrison RW, O' Donnell CP, Hare JM. Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. Circulation. 2003; 108: 754-759.

114. Berggren JR, Hulver MW, Houmard JA. Fat as an endocrine organ: influence of exercise. J Appl Physiol. 2005. 99: 757–764.

115. Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q, and Garwey WT. The Metabolic Significance of Leptin in Humans: Gender-Based Differences in Relationship to Adiposity, Insulin Sensitivity, and Energy Expenditure. J Clin Endocrinol Metab. 1997. 82: 1293–1300.

116. Mahabir S, Baer D, Johnson LL, Roth M, Campbell W, Clevidence B and Taylor PR. Body Mass Index, percent body fat, and regional body fat distribution in relation to leptin concentrations in healthy, non-smoking postmenopausal women in a feeding study. Nutrition Journal. 2007. 6; 3: 1475-2891.

117. Bulow B, Ahren Bo and Erfurth EM. Increased leptin and tumour necrosis factor a per unit fat mass in hypopituitary women without growth hormone treatment. European Journal of Endocrinology. 2001; 146: 737–742.

118. Whiting P, Rutjes AW, Reitsma JB, Glas AS, Bossuyt PM, Kleijnen J. Sources of variation and bias in studies of diagnostic accuracy: a systematic review. Ann Intern Med. 2004;140:189–202.

119. Ransohoff DF, Feinstein AR. Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. N Engl J Med. 1978;299:926–930

120. Mulherin SA, Miller WC. Spectrum bias or spectrum effect? Subgroup variation in diagnostic test evaluation. Ann Intern Med. 2002;137:598–602

121. Scobbo RR. Subgroup variation in diagnostic test evaluation [letter]. Ann Intern Med. 2003;138:686; author reply 686–687.

122. Swarr D and Keren R. Comparison of Alternative Diagnostic Approaches for Managing Appendicitis in Children: The Effect of Disease Prevalence and Spectrum. Pediatrics 2004;114;513-514.

123. Weng X, Liu Y, Ma J, Wang W, Yang G, Caballero B. Use of body mass index to identify obesity-related metabolic disorders in the Chinese population. Eur J Clin Nutr. 2006;60:931–7.

124. Fischer S, Hanefeld M, Haffner SM, et al. Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. Acta Diabetol 2002;39:105–110.

124. Yvold WBO , Nilsen TIL , ger OKRU , Holmen TL , Krokstad S, Midthjell K and Holmen J. Change in height, weight and body mass index: Longitudinal data from the HUNT Study in Norway. International Journal of Obesity. 2006; 30: 935–939.

125. Canatan H, Bakan I, Akbulut M, Baydas G, Halifeoglu I, Gursu MF. Comparative analysis of plasma leptin levels in both genders of patients with essential hypertension and healthy subjects. Endocr Res. 2004; 30: 95–105.

126. Salopuro T, Pulkkinen L, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Tuomilehto J, Laakso M, Uusitupa M. Finnish Diabetes Prevention Study Group. Genetic variation in leptin receptor gene is associated with type 2 diabetes and body weight: The Finnish Diabetes Prevention Study. Int J Obes (Lond). 2005; 29(10): 1245-51.

127. Thompson DB, Ravussin E, Bennett PH, Bogardus C. Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians. Hum Mol Genet. 1997; 6: 675–9.

128. Francke S, Clement K, Dina C, et al. Genetic studies of the leptin receptor gene in morbidly obese French Caucasian families. Hum Genet. 1997; 100: 491– 6.

129. Morris DL and Rui L. Recent advances in understanding leptin signaling and leptin resistance. Am J Physiol Endocrinol Metab. 2009; 297(6): E1247-59.

130. Reed AS, Unger EK, Olofsson LE, Piper ML, Myers MG Jr, Xu AW. Functional role of Socs3 up-regulation in hypothalamic leptin resistance and long-term energy homeostasis. Diabetes. 2010 Jan 12. [Epub ahead of print]

131. Kumagai S, Kishimoto H, Masatakasuwa, Zou B, Harukasasaki. The leptin to adiponectin ratio is a good biomarker for the prevalence of metabolic syndrome, dependent on visceral fat accumulation and endurance fitness in obese patients with diabetes mellitus. Metab Syndr Relat Disord. 2005; 3(2): 85-94.

132. Zhuo Q, Wang Z, Fu P, Piao J, Tian Y, Xu J, Yang X. Comparison of adiponectin, leptin and leptin to adiponectin ratio as diagnostic marker for metabolic syndrome in older adults of Chinese major cities. Diabetes Res Clin Pract. 2009; 84(1): 27-33.

133. Sánchez-García S, García-Peña C, Duque-López MX, Juárez-Cedillo T, Cortés-Núñez AR, Reyes-Beaman S. Anthropometric measures and nutritional status in a healthy elderly population. BMC Public Health. 2007; 7: 2.

134. Gottlieb MG, Bodanese LC, Leite LE, Schwanke CH, Piccoli Jda C, da Rocha MI, da Cruz IB. Association between the Gln223Arg polymorphism of the leptin receptor and metabolic syndrome in free-living community elderly. Metab Syndr Relat Disord. 2009; 7(4): 341-8.

135. Samaras K, Botelho NK, Chisholm DJ, Lord RV. Subcutaneous and Visceral Adipose Tissue Gene Expression of Serum Adipokines That Predict Type 2 Diabetes. Obesity (Silver Spring). 2009; Dec 17. [Epub ahead of print]

136. Mohiti J, Talebi F, Afkhami-Ardekani M. Circulation free leptin in diabetic patients and its correlation to insulin level. Pak J Biol Sci. 2009;12(4):397-400.

137. Lee CY, Lee CH, Tsai S, Huang CT, Wu MT, Tai SY, Lin FF, Chao NC, Chang CJ. Association between serum leptin and adiponectin levels with risk of insulin resistance and impaired glucose tolerance in non-diabetic women. Kaohsiung J Med Sci. 2009; 25(3):116-25.

138. Esteghamati A, Khalilzadeh O, Anvari M, Rashidi A, Mokhtari M, Nakhjavani M. Association of serum leptin levels with homeostasis model assessment-estimated insulin resistance and metabolic syndrome: the key role of central obesity. Metab Syndr Relat Disord. 2009; 7(5): 447-52.

139. Park JS, Cho MH, Nam JS, Ahn CW, Cha BS, Lee EJ, Lim SK, Kim KR, Lee HC. Visceral adiposity and leptin are independently associated with C-reactive protein in Korean type 2 diabetic patients. Acta Diabetol. 2009 May 5. [Epub ahead of print]

140. Ganji V, Kafai MR, McCarthy E. Serum leptin concentrations are not related to dietary patterns but are related to sex, age, body mass index, serumtriacylglycerol, serum insulin, and plasma glucose in the US population. NutrMetab (Lond). 2009; 6:3.

141. Potenza MV, Mechanick JI. The metabolic syndrome: definition, global impact, and pathophysiology. Nutr Clin Pract. 2009; 24(5):560-577.

142. Mulligan K, Khatami H, Schwarz JM, Sakkas GK, DePaoli AM, Tai VW, Wen MJ, Lee GA, Grunfeld C, Schambelan M. The effects of recombinant human leptin on visceral fat, dyslipidemia, and insulin resistance in patients with human immunodeficiency virus-associated lipoatrophy and hypoleptinemia. J Clin Endocrinol Metab. 2009; 94(4): 1137-1144

143. Lago, F., Dieguez, C. and Gómez-Reino, J. Adipokines as emerging mediators of immune response and inflammation. Nat. Clin. Pract. Rheumatol. 2007; 3: 716–724

144. Fan AZ. Etiology of the Metabolic Syndrome. Current Cardiology Reviews. 2007; 3: 232-239.

145. Antuna-Puente B, Feve B, Fellahi S, Bastard JP. Adipokines: the missing link between insulin resistance and obesity. Diabetes Metab. 2008; 34(1): 2-11.

146. Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Mol Med. 2008; 14(11-12): 741-751.

147. Iuliano AD, Feingold E, Wahed AS, Kleiner DE, Belle SH, Conjeevaram HS, Zmuda J, Liang TJ, Yee LJ; Virahep-C Study. Host genetics, steatosis and insulin resistance among African Americans and Caucasian Americans with hepatitis C virus genotype-1 infection. Intervirology. 2009; 52(1): 49-56.

148. Ross J. mRNA stability in mammalian cells. Microbiol Rev. 1995; 59: 423 - 450.

149. Ragin CC, Dallal C, Okobia M, Modugno F, Chen J, Garte S, Taioli E. Leptin levels and leptin receptor polymorphism frequency in healthy populations. Infect Agent Cancer. 2009 Feb 10;4 Suppl 1:S13.

150. Sinha M, Larkin EK, Elston RC, Redline S. Self-reported race and genetic admixture. N Engl J Med. 2006; 354(4): 421-2.

151. Wauters M, Mertens I, Renkinen T, Chagnon M, Bouchard C and Van Gaal L. Leptin receptor gene polymorphisms are associated with insulin in obese women with impaired glucose tolerance. J Clin Endocrinol Metab. 2002; 86: 3227–3232