ABSTRACT

Title of Thesis: THE EFFECTS OF OBESITY ON PLASMA LEVELS OF OMENTIN, A DEPOT-SPECIFIC ADIPOCYTOKINE

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Obesity is a chronic pathological condition and a risk factor for diabetes and cardiovascular disease. It has been demonstrated that adipose tissue functions not only as a fat storage depot but also as an endocrine organ. Omentin is a protein expressed and secreted in adipose tissue that increases insulin-stimulated glucose transport. To further elucidate omentin's physiological function, its levels were measured by quantitative western blotting in plasma from 44 healthy nondiabetic volunteers (22 women, 22 men). Participants were organized into sibling pairs based on discordant BMI (3-12 Kg/m^2). Lean subjects had significantly higher omentin levels than obese/overweight subjects (independent of sex), and significantly higher omentin levels were detected in women compared to men. Omentin levels were inversely correlated with BMI and positively correlated with HDL levels. These data suggest that omentin may play a physiological role in the pathogenesis of obesity-dependent insulin resistance.

THE EFFECTS OF OBESITY ON PLASMA LEVELS OF OMENTIN, A DEPOT-SPECIFIC ADIPOCYTOKINE

By

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DEDICATION

To Luis Eduardo, for his love, encouragement, and support, making it possible.

To my parents, Raul (in memoriam) and Catharina, for giving me a model to follow.

To my brother and sister, Jose Carlos and Carmem Lucia, for showing me the way.

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LIST OF ABBREVIATIONS

AFDS	Amish Family Diabetes Study
BMI	Body mass index
BSA	Bovine serum albumin
CHO	Total cholesterol
CNS	Central nervous system
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DTT	Dithiothreitol
FFA	Fatty acids
HDL	High-density lipoprotein
HOMA	Homeostasis model assessment of insulin resistance
LDL	Low-density lipoprotein
mRNA	Messenger ribonucleic acid
NEFA	Non-esterified fatty acid
NHANES II	The Second National Health and Nutrition Examination Survey
NHANES III	The Third National Health and Nutrition Examination Survey
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PVDF	Polyvinylidene fluoride
SBP	Systolic blood pressure
SDS-PAGE	Sodium dodecyl sulphate - polyacrylamide gel electrophoresis
SE	Standard error of the means
TG	Triglyceride
TNF-$\propto$	Tumor necrosis factor $\propto$
TTBS	Tween-Tris Buffered Saline
TZD	Thiazolidinedione
VLDL	Very-low density lipoprotein
WHO	World Health Organization

CHAPTER 1: BACKGROUND INFORMATION

1 – Obesity

1.1 - Definitions and assessments

According to the World Health Organization (WHO), obesity is the accumulation of excessive body fat negatively affecting health. It was declared one of the top ten health risk conditions in the world, affecting about 300 million people worldwide (60 million adults in the United States alone; 1-5).

As simple as the definition of obesity appears, establishing the best technique to measure obesity has been a matter of dispute. The most accurate techniques to assess body fat, such as hydrostatic weighing, absorptiometry, bioelectrical impedance and computed tomography are also the most technically challenging, demanding specialized training and equipment and, therefore, the most expensive. For these reasons, skinfold thicknesses and weight-and-height indexes have been adopted as substitute measurement methods in clinical and public health works (6).

Skinfold thickness is a measurement of the thickness of the double layer of skin and subcutaneous tissue obtained with specialized calipers. This method can be used to estimate fatness and characterize its distribution. The main advantage of this measurement is the possibility to determine adiposity levels. However, this assessment has important limitations such as standardization and reproducibility of measurements. Because of these limitations, weight-and-height indexes are most commonly used worldwide as an assessment of obesity (6).

The body mass index (BMI, Kg/m^2), a weight-and-height index, is the international measure of obesity. Subjects are categorized as healthy (BMI < 18 Kg/m^2), lean (BMI < 25 Kg/m^2), obese (BMI ≥ 30.0 Kg/m^2), or overweight (25 Kg/m^2 ≤ BMI < 30 Kg/m^2). Increasing BMI values are correlated with an increased risk of morbidity and mortality. BMI information has the great advantage of being easy to obtain. However, as its main disadvantage, BMI is not always a measure of adiposity, if the excess weight is caused by muscularity. When used for obesity assessment in children and adolescents, it is necessary to correct BMI for sex and age, because growth and sex hormones affect weight/height and body fat distribution respectively (2, 6, 7, 8).

Waist circumference and the ratio of waist circumference to hip circumference (waist-to-hip ratios) have also been used as an assessment of body fat distribution. Independent of body mass, increased localization of fat in the abdominal region is a risk factor for cardiovascular disease (CVD) and diabetes. Values of waist circumference higher than 102 cm (40.2") for men and 88 cm (34.6") for women are considered an increased risk for morbidity. Waist-to-hip ratios higher than 1 for men and 0.85 for women are also indicative of increased risk for the development of obesity associated risk factors (6).

1.2 – Epidemiology of Obesity

Worldwide, more than one billion adults are overweight and about 300 million are obese. Obesity and overweight have increased dramatically in several countries. For example, in less than 10 years in China (1989 – 1997) the prevalence of overweight individuals doubled in women and tripled in men (1, 9).

Based on BMI values, according to data from the National Health and Nutrition Examination Survey (NHANES III, 1999-2000), almost 65% of the American adult population is overweight. These data represent an increase from the already high incidence rate of 56% found in the previous assessment (NHANES III, 1988-1994). The incidence of obesity in adults paralleled this tendency and increased from 23% to 31% during the same period of time (1, 6, 7, 9).

Based on the BMI distributions from the NHANES II and III studies, Hill et al (2003) projected obesity and overweight incidence to 2008 (figure 1). They estimated that obesity would increase to 39% of the adult American population. The ratio between lean and obese individuals is represented in the axis y, and BMI distributions are shown in the axis x. The cut-off points for overweight (BMI = 25 Kg/m^2) and obesity (BMI = 30 Kg/m^2) are also shown (1).

Interestingly, according to the NHANES III (1988-1994), the prevalence of obesity in American adults was higher in women than in men. A higher prevalence of overweight women was found in African-American (37%) and Mexican-American (33%) populations than in the Caucasian population (22.7%). This pattern was not observed for men (6, table 1). This difference in obesity prevalence between genders has been tentatively explained by the regulation of body fat distribution by sex

hormones. Usually, women have higher proportion of fat relative to total body weight than men. This characteristic is thought to be evolutionarily advantageous because of the metabolic burden of birth and care of children associated with women. However, the molecular and physiological role played by sex hormones in fat distribution is far from understood (10, 11).

Race or ethnicity	Male (% obese)	Female (% obese)
All U.S. adults	19.5	25.0
Non-Hispanic white	19.9	22.7
Non-Hispanic black	20.7	36.7
Mexican American	20.6	33.3

Table 1. Obesity prevalence stratified by sex and race. Data from NHANES III (1988-1994); see figure 1 for details. Adapted from Galuska and Khan, 2001 (6).

It has been observed that obesity is most prevalent for middle-age adults in developed countries. Surprisingly, in the United States, the highest incidence of weight gain has occurred for adults between 24 to 34 years. However, it is important to notice that the prevalence of obesity among children and adolescents in the United States from 1988 to 1994 increased in a 2-fold ratio compared with the previous decade. These data indicate that the number of overweight and obese individuals is increasing, but also, the age that an individual reaches overweight/obesity levels is decreasing. Combined, these data indicate that the deleterious health consequences of excessive body fat will affect an ever younger population (1, 6, 7, 9).

Due to the serious implications of obesity, its direct health care costs in the United States were approximately $70 billion (7% of the total health costs) in 2001. These figures are in agreement with the cost of obesity in other developed nations (2-7% of the national health care costs in Australia, France and the Netherlands). However, this value did not account for the indirect costs related to loss of productivity and quality of life. In 2003, it was estimated that obesity accounted for 5.5 to 7.8% of all health care expenditures, representing about 39.2 million lost work days per year in the United States (1,6).

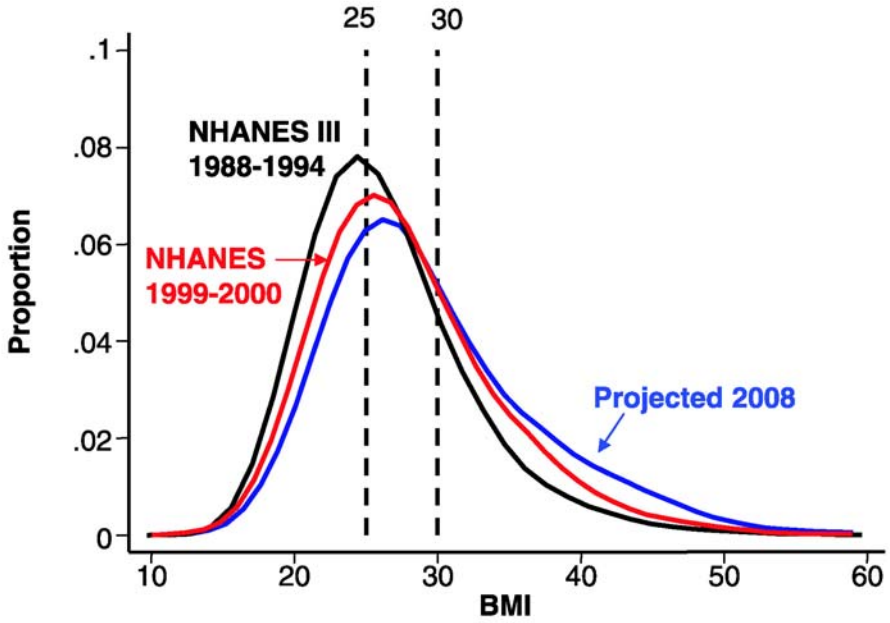

Figure 1. Obesity prevalence continuously increases in American society. From 1988-1994 to 1999-2000, a larger percentage of the population had higher BMI index, indicating increased body weight. Values for obesity were estimated to reach 39% of the total population in only 4 more years. Adapted from Hill et al, 2003.

1.3 – Causes of Obesity

Obesity is the result of an imbalance between energy intake and expenditure. As in most human pathological conditions, genetic and environmental conditions play a role in its pathogenesis. In order to maintain a stable and healthy body weight, according to the equation of energy balance (energy intake = energy output + stored energy), the intake and expenditure of energy should be equivalent. However, due to increasingly sedentary life styles and diet changes, a positive energy balance has been created in the majority of developed countries (12, 13).

Energy intake is directly influenced by environmental conditions. Due to the development of food technologies like processing, storage and distribution, the food industry can largely influence the type and amount of food consumed. Both package and portion sizes of foods have increased. It has been shown that the amount of food provided can affect energy intake. Moreover, food availability in developed countries has increased in the past 20 years. The combined effect of more and higher caloric food with less exercise have fueled the increase in body weight observed in developed societies (13, 14).

Habits and beliefs play a role in food choices, such as meal times, food preferences and portion sizes. In industrialized countries, it has become easier to choose fast food instead of home prepared foods. Social context also influences the selection and consumption of food based on the daily rhythm of life. Consequently, it has been seen an increased propensity to eat without physiological needs (13).

Food sensory properties influence pleasure while eating and, therefore, food selection. It is known that fat is a main component responsible for taste in food.

Consequently, fat combined with sugar and salt improves sensory properties and contributes to selection of food with high fat content (13).

Energy output can be divided into basal metabolic rate, thermic effect of food, and energy expended by physical activity. These three classes represent respectively about 60-70%, 10%, and 20-30% of daily energy expenditure. The most modifiable of these factors is physical activity. However, several factors affect physical activity frequency, such as age, gender, socioeconomic status, and education. Unfortunately, a sedentary life style has become usual in developed countries due to technological advances (13).

Like environmental factors, genetic factors contribute to obesity. During human evolution, energy storage as fat was an important survival adaptation. However, with increased food availability and decreased physical activity, these "thrifty" genes will influence the expression of obesity, a survival disadvantage. Several population studies estimated that the heritability of body mass or body fat can be derived from genetic information in the range of 24-70%. This effect is considered polygenic and may also depend on sex and age (2, 13, 15, 16, figure 2).

Obesity can also be a component of diseases which mostly affect the endocrine system. For example, Cushing’s disease, polycystic ovary syndrome, hypothyroidism, hypogonadism, and growth hormone deficiencies are diseases associated with obesity. Medications, especially antidepressants, glucocorticoids, progestagens, and sulfonylureas can also contribute to weight gain (6, 13).

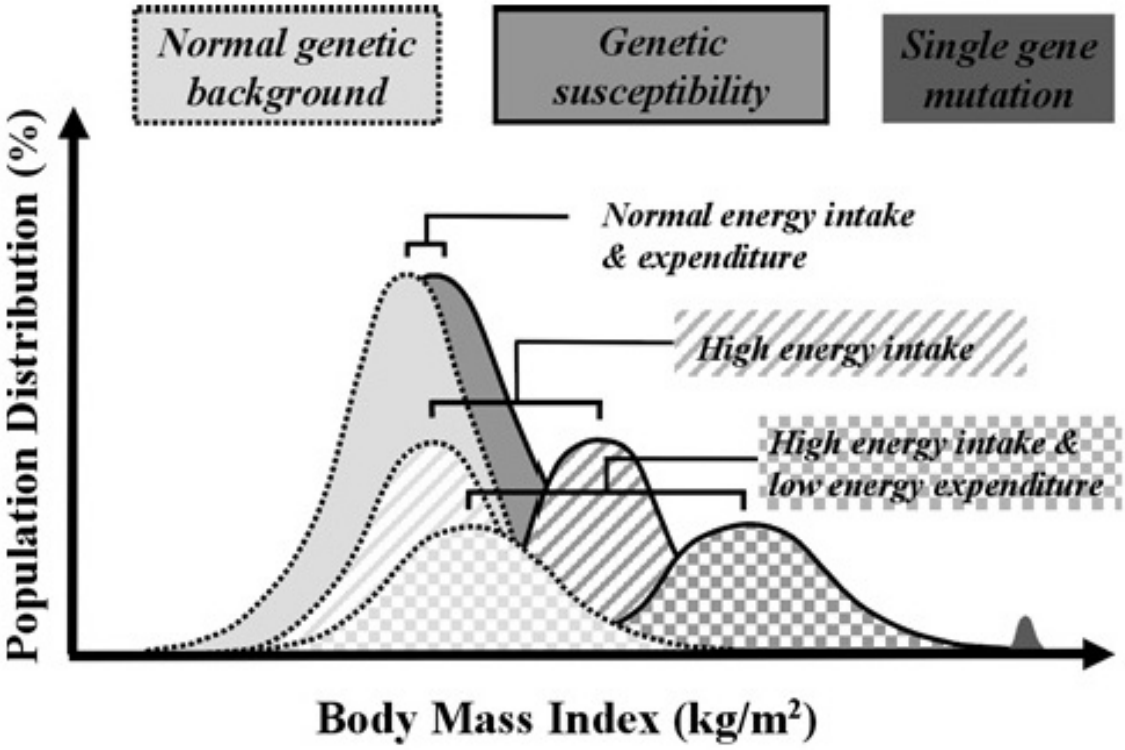

Figure 2. Genetic background modulates obesity, but the effect can be overcome by controlling energy balance. Normal individuals have small variations on BMI upon changes in energy intake or expenditure. The changes in BMI are much more intense for genetically susceptible individuals. Observe the widening and "shift to right" for increase in energy intake and/or decreased expenditure in the graph. Also, rare single gene mutations can condition for extremely high BMI values. Adapted from Hofbauer, 2002.

1.4 – Health Effects of Obesity

Obesity is a chronic pathological condition and a risk factor for type 2 diabetes and cardiovascular disease (CVD). Overweight or obese people have a greater probability than normal-weight people of developing metabolic syndrome, a condition characterized by high blood pressure, insulin resistance, and dyslipidemias (high levels of total cholesterol, triglycerides and LDL and low levels of HDL). It is estimated that 20-25% of adults in the United States have acquired metabolic syndrome (17 – 24).

Obesity during pregnancy may cause congenital malformations, such as neural tube effects, hypertension and gestational diabetes. In addition to these conditions, obesity also increases the risk of polycystic ovary disease, ischemic stroke, sleep apnea, gallbladder disease, gastroesophageal reflux, osteoarthritis, colon cancer, postmenopausal breast cancer, and psychological disorders, such as depression (6).

According to the Rand Institute, obesity is a higher risk for chronic diseases than living in poverty, smoking or drinking. Approximately 300,000 people die every year in the United States of obesity-associated diseases (1).

1.5 – Treatment and Prevention of Obesity

Any intervention aimed at treating obesity should focus on how to restore balance to energy intake and energy expenditure. In a simplified form, these interventions could be divided into three classes: diet modifications, physical activity improvement, and clinical interventions (6, 12, 13).

The main objective of diet modification is to reduce energy intake. It has been shown that overweight subjects on a low-calorie diet (800-1500 Kcal / day) reduced body weight by an average of 8% over 3-12 months. According to several studies, this intervention also works for children and adolescents. A great variety of weight loss diets have been offered commercially. Although some of these popular diets work very well in a short term, they do not offer a nutritionally balanced diet. Therefore, the potential to lose weight but to develop other health problems secondary to poor diet remains (6, 12, 13).

Increased physical activity can be used to balance energy expenditure by increasing the amount of energy the body uses. It is very efficacious because it increases body energy use not only during physical exercise, but physical activity also increases the basal metabolic rate, increasing energy use throughout the day. Physical exercise can be used for weight loss in adults and children, although, in both cases medical supervision is always indicated. Physical activity also offers several other benefits (mental and physical) than weight loss. It is well documented that it increases self-confidence, improves mood, decreases cardio-vascular disease (independent of weight loss) and the risk of drug use. But physical activity alone without diet changes

has a smaller effect on weight loss than that obtained with both interventions (6, 12, 13).

Clinical interventions include pharmacological and surgical therapies. Pharmacological therapies can be divided in three groups: to reduce food intake by increasing the feeling of satiety; to affect metabolism by inhibiting intestinal digestion and absorption of fat; and to increase energy expenditure by causing weight loss. According to recent randomized controlled trials, pharmacological treatments used along with life style changes for 6-12 months reduced body weight in a range of 2-10 Kg in obese people. However, due to the side effects of those drugs, they are only recommended for people who have a higher health risk secondary to obesity. On the other hand, surgical interventions are used to reduce food intake by changing the gastrointestinal capacity. Although gastric bypass results in considerable weight loss, this intervention is indicated only for people who were unsuccessful in using the interventions described above (6, 12, 13).

In addition to the strategies focused on balancing energy intake and expenditure, there is a need of a stronger nutritional knowledge for medical practitioners. Currently, the majority of medical schools do not offer this kind of training. Consequently, the practitioners do not have the skills to advise and guide their patients though life-style changes. On a more broad scale, health and nutritional education of the general public should be a powerful way of changing population life-style. A good example is the nonsmoking campaign. In the past two decades, the number of smokers has decreased significantly; from 50% of the adult population in the 50's to 25% in the beginning of the 90's and with some studies indicating a

reduction to 16% of adult smokers in some population cohorts. In summary, obesity is an epidemic health problem that needs to be combated vigorously without loss of time in order to counteract the already present health consequences (13, 25).

2. Adipose Tissue as an Endocrine Organ

Fat tissue has been traditionally viewed as an energy storage depot composed of fat storing cells (adipocytes) embedded in a simple matrix of connective tissue with stromovascular and immune cells (4, 11).

Recently, this traditional view of fat cells as simply energy storage has been challenged. In 1987, it was found that sex steroids have their major site of production in adipose tissue. Soon thereafter, in 1994, the identification of leptin contributed for the establishment of the adipose tissue as an endocrine organ. Currently, with the identification of a number of pro-hormones, hormones, enzymes and cytokines expressed and released from adipose tissue, it is clear that this tissue functions not only as a passive fat storage depot but also as an endocrine organ (26 – 31, table 2).

Cytokine is a general name for small secreted proteins that are involved in a plethora of processes like immunity, inflammation, and hematopoiesis. Generally, they act over short time at very low concentration. Their mechanism of action involves modulation of gene expression and consequent metabolic alterations. Their actions are mediated by binding with specific membrane receptors which use intracellular second messenger cascades. Interestingly, the production and release of cytokines also obey a cascading order, with one cytokine stimulating its target cells to make additional cytokines. These compounds can also act synergistically (two or

more cytokines acting together) or antagonistically (cytokines causing opposing activities). Therefore, cytokines form an extremely interdependent signaling network with the final metabolic effect being the combined interplay of many different actions. Cytokines expressed and secreted in adipose tissue receive the particular name of adipocytokines. They may act on the cells that secrete them (autocrine action), on proximal cells (paracrine action), or in a systemic level (endocrine action). Therefore, through this integration of other organs, adipose tissue plays a role in the regulation of energy balance, neuroendocrine function, and immunological responses (26, 27).

Adipose tissue can be divided in two types: white and brown. Brown adipose tissue is generally found in small amounts in adults and contributes to resting metabolic rate with its thermogenic capacity. On the other hand, white adipose tissue can be broadly classified as visceral (organ fat) and subcutaneous (under the skin). Omental visceral fat is more metabolically active than subcutaneous adipose tissue. It also contains large insulin-resistant adipocytes, when compared with the smaller more insulin-sensitive adipocytes found in subcutaneous fat (4, 32, 33).

It is known that increased visceral adiposity promotes increased levels of circulating non-esterified fatty acid (NEFA). The resulting increased NEFA levels inhibit insulin clearance by the liver causing increased plasma levels of insulin (hyperinsulinemia), and inhibition of insulin action on insulin-sensitive tissues (insulin resistance) such as liver and muscles. Some studies have also shown that increased release of fatty acids from visceral fat cells to the portal venous system results in increased release of both glucose and insulin. Taken together, these results

suggest that increased NEFA circulating levels cause a significant inhibition of glucose transport activity. Consequently, obesity, especially in the visceral region, is strongly associated with insulin resistance, hyperglycemia, dyslipidemia and hypertension, leading to cardiovascular disease (4, 5, 26).

The Honolulu Heart Study showed that the risk of coronary artery disease was equivalent in both non-obese group with predominant visceral fat distribution and the obese group with equivalent visceral fat distribution. On the contrary, the obese group with subcutaneous obesity had only a slightly higher risk than the non-obese group with predominantly subcutaneous fat distribution. The presence of predominantly visceral fat, even in the absence of body obesity, is sufficient to increase the risk of cardiovascular disease. Interestingly, obesity and its associated morbidities (metabolic syndrome) are also induced by adipose tissue deficit. Therefore, both excess and deficit of adipose tissue have negative impacts on health (4).

Cancers of colon, breast, endometrium, kidney, and esophagus have also been associated with obesity. An imbalance between cell proliferation, differentiation, and apoptosis can be caused by alterations in the metabolism of hormones, such as estrogen, insulin, and insulin-like growth factor. Therefore, the growth of adipose tissue observed in obesity and consequently the increased release of growth factors may facilitate cancer development (26).

Among the several endocrine hormones expressed and secreted by adipose tissue are, in chronological order of discovery, leptin, adiponectin, resistin, and omentin.

Function	Protein secreted by adipocytes
Cytokines and cytokine-related protein	Leptin
	TNF∝
	IL-6
Other immune-related proteins	MCP-1
Proteins involved in the fibrinolytic system	PAI-1
	Tissue factor
Complement and complement-related proteins	Adipsin
	Complement factor B
	ASP
	Adiponectin
Lipids and proteins for lipid metabolism or transport	Lipoprotein lipase (LPL)
	CETP
	Apolipoprotein E
	NEFAs
Enzymes involved in steroid metabolism	Cytochrome P450
	17βHSD
	11βHSD1
Proteins of the RAS	AGT
Other proteins	Resistin

Table 2. Partial list of proteins secreted by adipocytes with predominantly endocrine functions. Not all of these proteins are adipocytokines because they were first characterized in other body organs and/or have their main function in other body systems. However, they evidence the metabolically active state of the adipose tissue. Adapted from Kershaw and Flier, 2004.

2.1 – Leptin

Leptin, the product of the obesity gene ob/ob, was discovered in 1994 by Friedman and colleagues. Leptin, also called OB protein, is a 16-kDa glycosylated protein of 167 amino acid residues produced predominantly by white adipose tissue. Higher levels of leptin mRNA have been detected in subcutaneous than in visceral adipose tissue, a finding that correlates with higher leptin release from subcutaneous adipose tissue. Leptin expression in low levels was also found in the hypothalamus, pituitary placenta, skeletal muscle, and gastric and mammary epithelia (27, 31, 34, 35).

Leptin has been characterized as an important regulator of food intake by exchanging information from the periphery to the central nervous system (CNS). Fat mass is directly correlated with leptin circulating levels, consequently, increased amount of energy stored in adipose tissue increases leptin circulating levels. As a result, decreased hunger, increased sympathetic activity, and increased thermogenesis are observed. Therefore, leptin is involved in the regulation of appetite, food intake and energy expenditure. In addition to that, it has been demonstrated that leptin is also correlated with regulation of sexual maturation, fertility, hematopoiesis, and bone development (4, 26, 29, 34, figure 3).

Initial studies showed that leptin knockout mice became hyperphagic and obese when allowed to eat ad libidum. This phenotype was rescued by administration of leptin, which promoted reduced food intake and increased metabolic rate. These studies suggested that leptin could be used as a pharmacological intervention to treat obesity. However, administration of leptin to obese individuals failed to reduce

adiposity; since they already had high circulating leptin levels, suggesting a state of leptin resistance. The mechanism for leptin resistance is not clear yet, but restricted transport of leptin to the brain, and/or a reduced hypothalamic leptin signaling (impaired intracellular signal transduction) has been proposed to play a role (15, 34). Interestingly, it has also been indicated that women have higher leptin levels than men (36, 37, 38, 40). Additionally, other studies also indicated that leptin correlates positively with BMI (body mass index), and HOMA (homeostasis model assessment of insulin resistance). HOMA (defined as the product of fasting plasma insulin and glucose divided by 22.5) is a tool to estimate insulin sensitivity from a single sample (36, 38, 39, 40).

Several hormones, such as, insulin, glucocorticoids, estrogens, and tumor necrosis factor α (TNF-α) stimulate leptin production. The opposite effect is observed with noradrenaline, adrenaline, androgens, and free fatty acids (26, 31, 41).

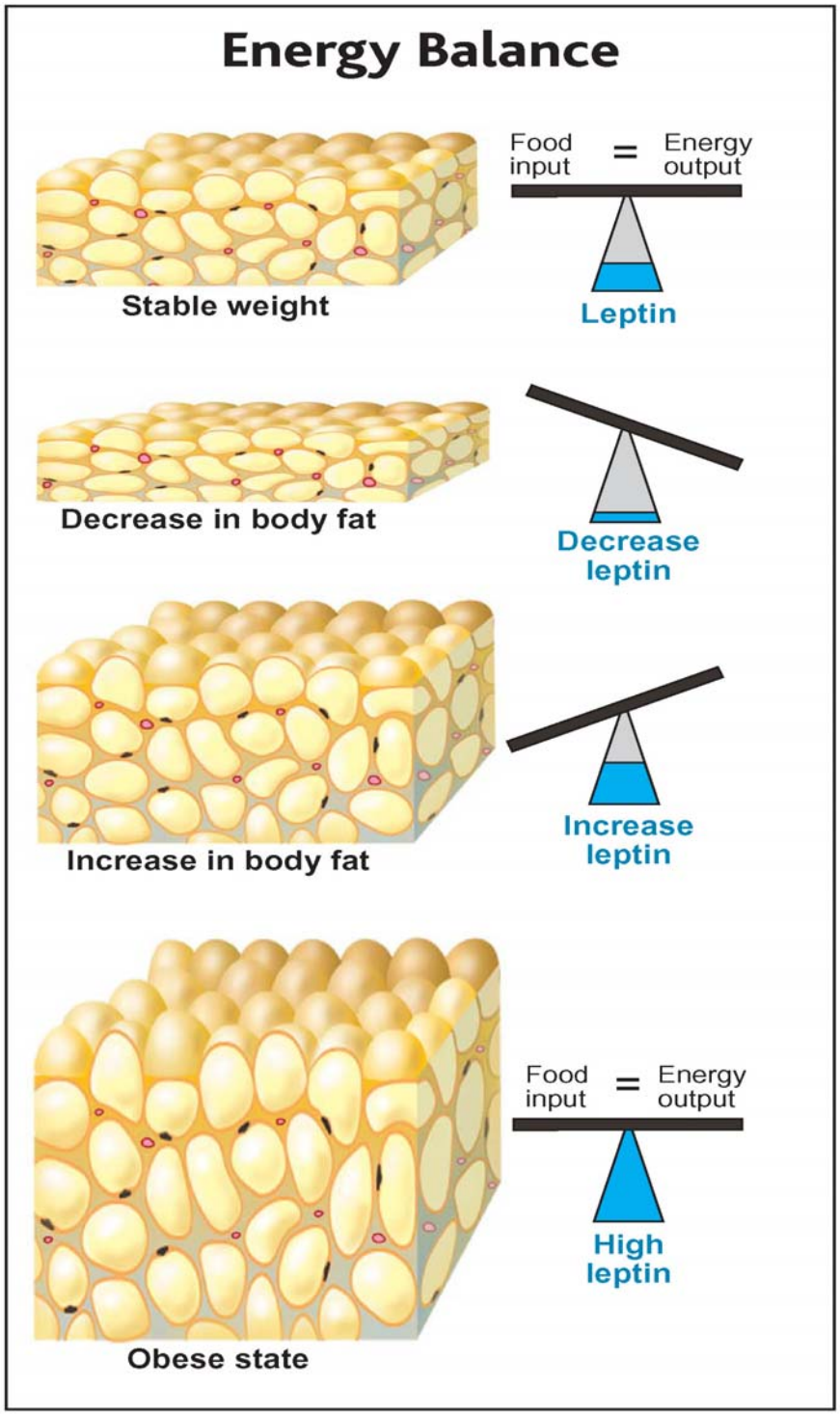

Figure 3. Leptin levels are directly proportional to body fat. It acts as an appetite and metabolism regulator balancing body's fat content. Adapted from Marx, 2003.

2.2 – Adiponectin

Adiponectin, also called apM1, Acrp30, adipoQ, and GBP28, was simultaneously described by four different groups in 1995 and 1996 (26). This 30-kDa polypeptide with 244 amino acids is expressed and secreted exclusively in white adipose tissue (26, 34). Many of its characteristics are the opposite of leptin, i.e., it has been shown that adiponectin expression and secretion are higher in visceral than in subcutaneous adipose tissue. Also its gene expression is decreased by obesity, glucocorticoids, beta-adrenergic agonists, and TNF-α and increased by leanness, activation of the nuclear receptor PPAR-γ (peroxisome proliferator-activated receptor gamma), and cold exposure (26, 28, 42, 43).

Plasma adiponectin circulates at high levels in human bloodstream (range 0.5 to 30 μg/mL). Compared with leptin and insulin, adiponectin is found in about 1000-fold higher than those hormones. Adiponectin is considered the most abundant adipose tissue protein, accounting for about 0.01% of total human plasma protein (26, 29, 42).

It has been established that low adiponectin levels are associated with obesity and several components of the metabolic syndrome, such as, hyperlipidemia, low HDL levels, and insulin resistance/type 2 diabetes (26, 34, 42).

Several studies have indicated that adiponectin acts as an insulin-sensitizing hormone (26, 29, 34, 42, 44). For example, adiponectin administration to rodents showed an increase in glucose uptake and fat oxidation in muscle, a reduction in fatty acid uptake and hepatic glucose production in liver, improving insulin-sensitivity. In

addition, thiazolidinediones (TZD drugs), used to enhance insulin sensitivity, increased plasma adiponectin and mRNA levels in mice (34, 44, figure 4).

In support of those findings, a negative correlation between human plasma adiponectin levels and body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA), body fat mass, insulin resistance, and insulin levels was described (34, 39, 42, 45, 46, 47).

Adiponectin is also considered to protect against cardiovascular disease. It has been hypothesized that low adiponectin levels correlate with the development of atherosclerosis and cardiovascular disease, a hypothesis corroborated by a study showing that adiponectin acts as an anti-atherosclerotic protein on endothelial cells. Moreover, adiponectin is positively correlated with HDL concentrations, and negatively correlated with LDL, and triglyceride levels (45, 46, 48).

It has been reported that adiponectin levels are higher in lean than in obese subjects (36, 42, 46, 49). Adiponectin circulating concentrations are also higher in females than in males, possibly explaining in part the lower risk of cardiovascular disease found in women (29, 36, 37, 42, 46, 49, 50). Moreover, in contrast to decreased adiponectin levels in obese subjects, adiponectin concentrations were increased after weight loss interventions (42).

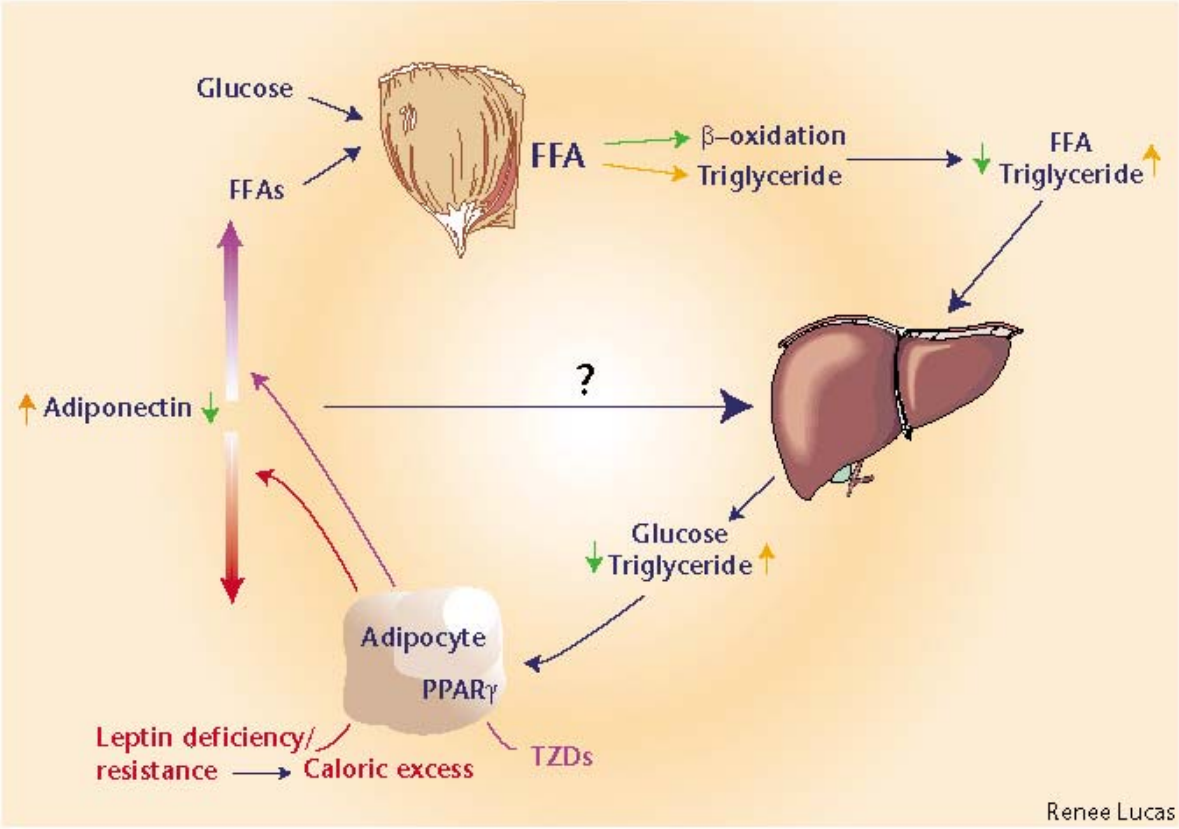

Figure 4. Proposed model for adiponectin secretion and action. In adipocytes, leptin deficiency/resistance and/or caloric excess reduce adiponectin expression and secretion. Conversely, activation of nuclear receptor PPAR-γ increases adiponectin synthesis, which increases fatty acids (FFAs) transport and oxidation in skeletal muscle, as well as, glucose utilization. The combined effect of adiponectin is a reduction in circulating lipids, improved glucose tolerance and insulin sensitivity. Adapted from Saltiel, 2001.

2.3 – Resistin

Resistin, named after “resistance to insulin” and also called “adipose-tissue-specific secretory factor” and “FIZZ3”, is a 12-kDa polypeptide with 114 amino acids, which was identified in 2001 by Steppan and colleagues (26, 34).

Initial studies indicated that resistin is expressed and secreted 15 times higher in visceral than in subcutaneous adipose tissue in rodents (26).

It has been shown that resistin is correlated with obesity-related insulin resistance (26, 51, 54). Resistin administration in rodents induced insulin resistance, impaired glucose tolerance and insulin action. Interestingly, neutralization of resistin by anti-resistin antibodies reduced glucose levels and improved insulin sensitivity (26, 29, 31, 34).

Moreover, some studies have indicated that resistin is down regulated by the antidiabetic drug rosaglitazone (a TZD compound used to enhance insulin sensitivity, 26, 29, 34, 52, figure 5).

In support of those findings, it has been found that plasma resistin levels are higher in obese than in lean subjects (36, 53). In an analysis of subgroups, it was shown higher levels of resistin in obese versus lean females, but without difference between obese and lean males (53). In addition, higher resistin levels were found in women than in men (36, 37, 53). Moreover, resistin levels were also correlated positively with body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA), and insulin levels (36, 53).

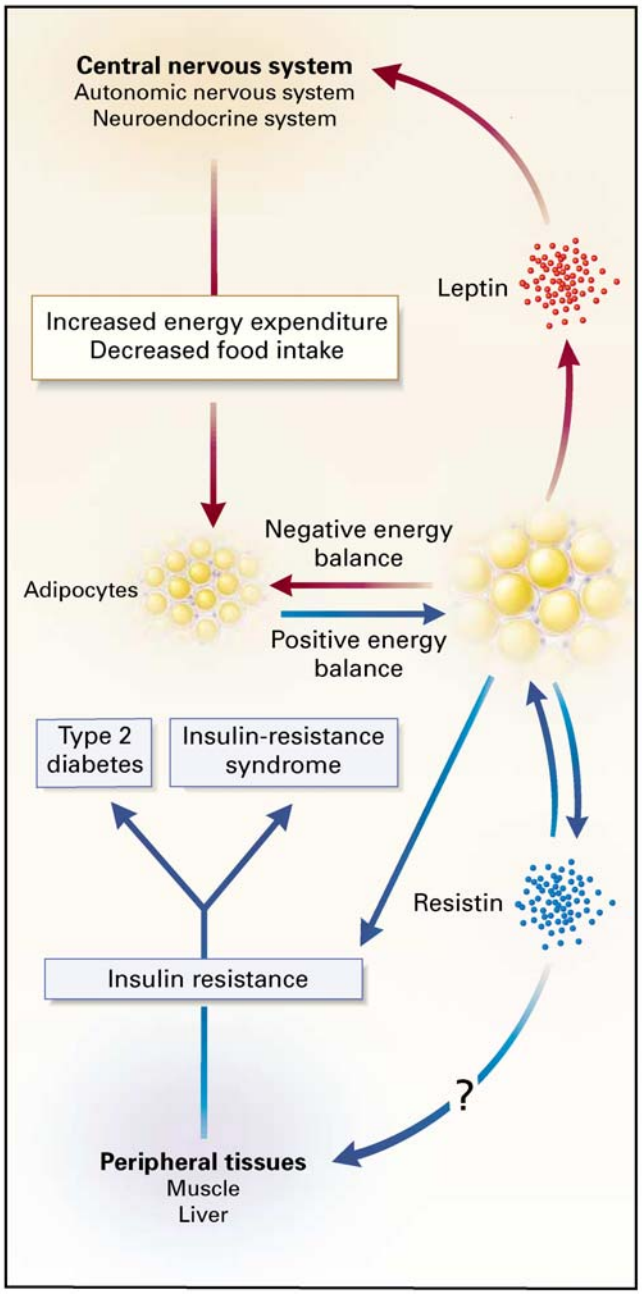

Figure 5. Proposed model for resistin secretion and actions. Higher leptin levels decrease food intake and increase energy expenditure, resulting in negative energy balance and lower resistin levels. This decreases insulin-resistance, benefiting diabetes control. Adapted from Shuldiner et al, 2001.

TABLE 3 – Comparison of metabolic characteristics between adiponectin, leptin, and resistin.

Characteristics	Adiponectin	Leptin	Resistin
Lean x Obese	Higher levels in lean subjects	Higher levels in obese subjects	Higher levels in obese subjects
Lean x Obese (Women)	Higher levels in lean subjects	Higher levels in lean subjects	Higher levels in lean subjects
Lean x Obese (Men)	Higher levels in lean subjects	Higher levels in lean subjects	No difference
Females x Males	Higher levels in females	Higher levels in females	Higher levels in females
Correlation w/ BMI	–	+	+
Correlation w/ HOMA	–	+	+
Correlation w/ TG	–	+	+
Correlation w/ CHO	–	+	+
Correlation w/ LDL	–	+	+
Correlation w/ HDL	+	–	–
Correlation w/ Fasting Insulin	–	+	+
Correlation w/ Fasting Glucose	–	+	+
Correlation w/ SBP	–	+	+
Correlation w/ DBP	–	+	+

2.4 – Omentin

Omentin is a 35-kDa protein with 313 amino acids, named after its preferred expression in omental (visceral) rather than subcutaneous fat tissue. It was cloned and sequenced in 2002 at the Division of Endocrinology, Diabetes and Nutrition (University of Maryland Baltimore).

Preliminary data indicated that omentin can be detected in human serum, and is positively correlated with insulin signal transduction and insulin-stimulated glucose transport in 3T3-L1 adipocytes and isolated human adipocytes. In addition, omentin secretion was positively regulated by dexamethasone and thiazolidininedione (insulin sensitizers).

These preliminary data suggest that omentin may play a positive role in maintaining insulin sensitivity; consequently omentin levels may be decreased in obesity-related insulin resistance.

In consequence, these preliminary findings raise the following hypotheses:

(a) omentin levels are higher in lean than obese individuals;

(b) omentin levels are higher in female lean than female obese individuals;

(c) omentin levels are higher in male lean than male obese individuals;

(d) omentin levels are negatively correlated with BMI;

(e) omentin levels are negatively correlated with HOMA;

(f) omentin levels are negatively correlated with triglyceride levels;

(g) omentin levels are negatively correlated with total cholesterol levels;

(h) omentin levels are negatively correlated with LDL levels;

(i) omentin levels are positively correlated with HDL;

(j) omentin levels are negatively correlated with fasting insulin levels;

(k) omentin levels are negatively correlated with fasting glucose levels;

(l) omentin levels are negatively correlated with blood pressure.

In order to test these hypotheses, this study was designed to (i) compare plasma omentin levels in lean and obese/overweight subjects; (ii) and to investigate the relationships between plasma omentin levels and several metabolic indices such as body mass index (BMI), insulin resistance index (HOMA), lipid profile (triglycerides, total cholesterol, HDL, and LDL levels), fasting insulin and glucose levels, and blood pressure.

CHAPTER 2: RESEARCH DESIGN AND METHODS

2.1 – Subjects

Subjects included 44 healthy nondiabetic volunteers (22 women and 22 men) who participated in the Amish Family Diabetes Study (AFDS) conducted by the Division of Endocrinology, Diabetes and Nutrition, University of Maryland at Baltimore. The study protocol was approved by the Institutional Review Boards for Human Subject Research at University of Maryland (both from College Park and Baltimore) and written informed consent was obtained from all subjects. Participants were organized in sibling pairs based on sex (same gender), age (1-5 years of interval) and BMI (discordant pairs; 3-12 Kg/m^2) (table 4). Subjects of the same family were paired, i.e., sisters with sisters, and brothers with brothers. The sibling pairs were divided in two groups: (a) lean ($BMI<25Kg/m^2$); or (b) overweight ($25Kg/m^2 \leq BMI<30Kg/m^2$) and obese ($BMI \geq 30.0Kg/m^2$).

Table 4. Participants were organized in sibling pairs (relatives). The division was based on sex (same gender), age (1-5 years of interval) and BMI (discordant pairs; 3-12 Kg/m^2).

Pair	Subject ID	Gender	BMI	Age	Subject ID	Gender	BMI	Age
1	577	F	21.5	46	1472	F	26.3	45
2	7280	F	20.3	30	6601	F	33.1	29
3	4317	F	24	30	4316	F	33.5	33
4	7243	F	23.1	25	7241	F	28.9	26
5	1491	F	21.8	27	1493	F	25.5	24
6	7062	F	21.2	20	7063	F	27.6	21
7	7122	F	19.8	70	7043	F	25.2	71
8	7297	F	21.5	25	7296	F	26.8	27
9	593	F	22.9	59	4314	F	28.7	56
10	7049	F	17.5	39	7077	F	25.8	42
11	503	F	22.9	50	478	F	33.6	47
12	1414	M	17.0	43	8027	M	27.8	48
13	7279	M	23.0	57	7271	M	31.0	56
14	7254	M	21.8	58	1295	M	26.6	57
15	520	M	19.8	42	526	M	27.9	44
16	7099	M	21.5	34	7098	M	29.4	39
17	611	M	22.9	44	627	M	30.2	39
18	6554	M	24.8	38	621	M	30.55	35
19	1333	M	23.6	30	1329	M	31.5	33
20	7086	M	24.9	65	7078	M	30.1	61
21	1314	M	23.4	32	1349	M	27.6	38
22	7039	M	21.2	64	7034	M	29.7	65

2.2 – Clinical Characteristics

The clinical characteristics of the participants of the study were contributed by Dr. Alan Shuldiner (Professor & Head, Division of Endocrinology, Diabetes and Nutrition – UMB).

Blood was drawn after an overnight fast, and plasma was separated by centrifugation using standard procedures. Aliquots were prepared for determination of glucose, insulin, triglycerides, total cholesterol and high density lipoprotein (HDL) levels; and frozen at – 80°C for additional assays (omentin). The researchers also measured the height, weight, and blood pressure of the subjects.

Blood glucose was measured using a glucose analyzer (Beckman®). Insulin levels were determined by enzyme-linked immunosorbent assay RIA (Linco Research, Inc.®). Homeostasis model of assessment (HOMA) score was calculated as fasting insulin (μU/mL) x fasting glucose (mM) divided by 22.5 (55).

Total cholesterol, HDL cholesterol and triglycerides were determined enzymatically using commercial kits on a Hitachi 747 auto analyzer (Roche®). Low density lipoprotein (LDL) levels were calculated using the Friedewald Equation, where all concentrations are given in mmol/L and the quotient ([TG]/2.2) is used as an estimate of VLDL-cholesterol concentration (56).

$$[\text{LDL-cholesterol}] = [\text{Total cholesterol}] - \{[\text{HDL-cholesterol}] - ([\text{TG}]/2.2)\}$$

2.3 – Quantification of Plasma Omentin Levels

2.3.1 – Quantification of Purified Omentin Levels (Step 1)

Purified omentin, obtained from Dr. Gong (Division of Endocrinology, Diabetes and Nutrition – UMB), was used as a standard for the quantification of plasma omentin levels in the samples.

The purified omentin concentration was quantified by SDS-PAGE (sodium dodecyl sulphate - polyacrylamide gel electrophoresis) and Sypro Ruby fluorescent protein stain (Bio-Rad®) using purified bovine serum albumin (BSA) as a standard. Five BSA purified solutions (6.25; 12.5; 18.75; 25.0; and 37.5 μg/mL) and a solution prepared from purified omentin (6.6 times more concentrated than the original purified omentin) were denatured with 0.002% DTT (dithiothreitol) in Laemnli Sample Buffer for 3 minutes at 95°C (Bio-Rad®). After this treatment, the standards and purified omentin were electrophoresed in duplicate on 10% SDS-PAGE gels (Criterion Pre- Cast Bio-Rad®) for 30 and 60 minutes respectively at 125 and 150 Volts. The gel was placed into a foil-covered with 50 mL of Sypro Ruby Stain solution (Bio-Rad®), and agitated in a shaker at room temperature for 12 hours. After that, the gel was washed with a 10% methanol/ 7% acetic acid solution twice for 10 and 20 minutes respectively. Fluorescent band intensities were visualized by 305 nm UV light and quantified on a Fluorchem 8000 chemilumenescent/fluorescent imager (Alpha Innotech®) (figure 6).

A standard curve was constructed using the log transformed BSA concentrations (x-axis) versus the band intensities (y-axis) (figure 7, 57).

2.3.2 – Quantification of Standard Plasma Samples (Step 2)

Due to the difficulty of over expressing and purifying omentin, some plasma samples were quantified using purified omentin as a standard. These quantified plasma samples were used as "standard plasma" to quantify the samples from the subjects.

Standard plasma omentin levels were measured by an immunoblotting analysis (quantitative western blotting) using purified omentin as a control. Plasma samples and three purified omentin solutions (0.1; 0.15; and 0.2 μg/mL) were denatured with 0.002% DTT in Laemnli Sample Buffer for 3 minutes at 95°C (Bio-Rad®). Then, the "standard plasma" samples and purified omentin were electrophoresed in triplicate on 10% SDS-PAGE gels (Criterion Pre- Cast Bio-Rad®) for 30 and 60 minutes respectively at 125 and 150 Volts. After that, the proteins were transferred to Immobilon-PVDF membrane (Millipore®) by using the Bio-Rad® transfer system for 50 minutes at 100 Volts. The membranes were removed from the transfer apparatus and the nonspecific binding sites were blocked for 30 minutes at room temperature with starting block solution (Pierce®) plus 0.1% Tween 20 (also called Polysorbate 20, Pierce®). In sequence, the membranes were rinsed twice for 5 minutes with TTBS (Tween-Tris Buffered Saline: 0.1% Tween-20 in 100 mM Tris-CL [pH 7.5], 0.9% NaCl). Incubation in primary antibodies 3G1B3, a human omentin specific monoclonal antibody obtained from Dr. Gong (Professor, Division of Endocrinology, Diabetes and Nutrition – UMB), was carried out for 1.5 h at room temperature in a shaker. Antibodies were diluted 1:24 in starting block solution plus 0.1% Tween 20 (Pierce®). Horseradish peroxidase-conjugated anti-mouse secondary

antibodies (KPL®) were diluted 1:30,000 in starting block solution plus 0.1% Tween 20 (Pierce®) and incubated with the blots for 40 minutes at room temperature in a shaker. After incubation with primary and secondary antibodies, membranes were washed three times for 10 minutes with TTBS. Immunoreactive bands were visualized and quantified by chemiluminescent detection with the Femto-West kit (Pierce®) on a Fluorchem 8000 chemilumenescent/fluorescent imager (Alpha Innotech®) (figure 8).

A standard curve was constructed using the log transformed purified omentin concentrations (x-axis) versus the optical densities (y-axis) (figure 9).

2.3.3 – Quantification of Plasma Samples (Step 3)

Plasma omentin levels were measured by quantitative western blotting using "plasma standards" as a control. Plasma samples from the subjects and previously quantified "plasma standard" solutions (1.11, 1.66, and 2.21 μg/mL) were denatured in 0.002% DTT in Laemnli Sample Buffer for 3 minutes at 95°C (Bio-Rad®). In sequence, the "plasma standard" solutions and plasma samples were electrophoresed in triplicate on 10% SDS-PAGE gels (Criterion Pre- Cast Bio-Rad®) for 30 and 60 minutes respectively at 125 and 150 Volts. After that, the same procedure used for the quantification of the "plasma standards" was followed for the subjects' samples (figure 10).

A standard curve was constructed using the log transformed "standard plasma" concentrations (x-axis) versus the optical densities (y-axis) (figure 11).

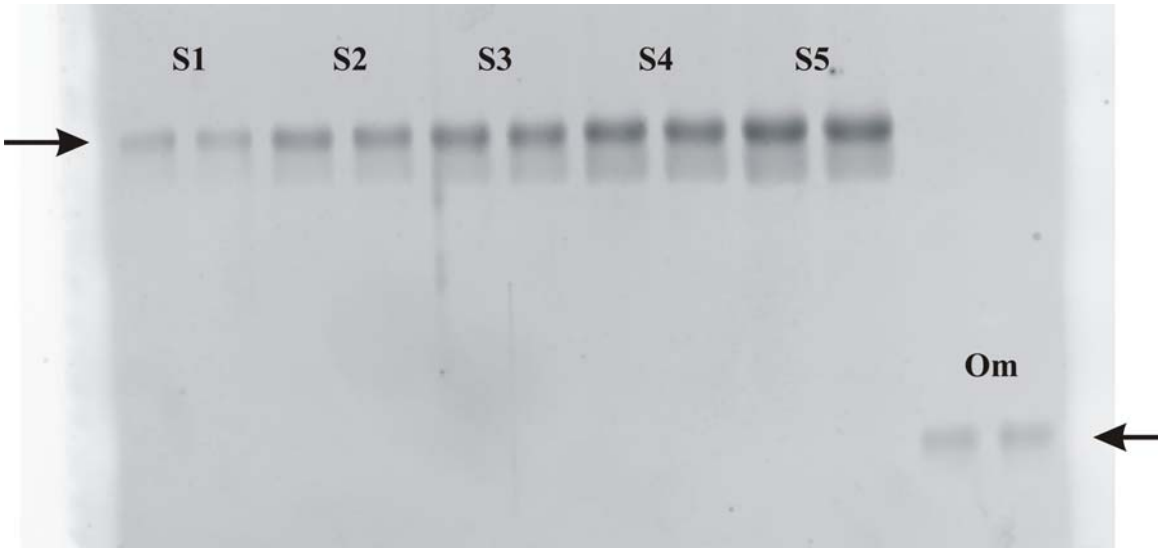

Figure 6. Quantification of purified omentin levels using BSA standards. Five concentrations of BSA (62.5; 125; 187.5; 250; and 375 μg/lane, marked S1 through S5, respectively) and omentin (marked Om) were loaded in duplicate on 10% SDS-PAGE gel. Optical densities were measured on Fluorochem 8000 imager. The arrows indicate BSA and omentin bands.

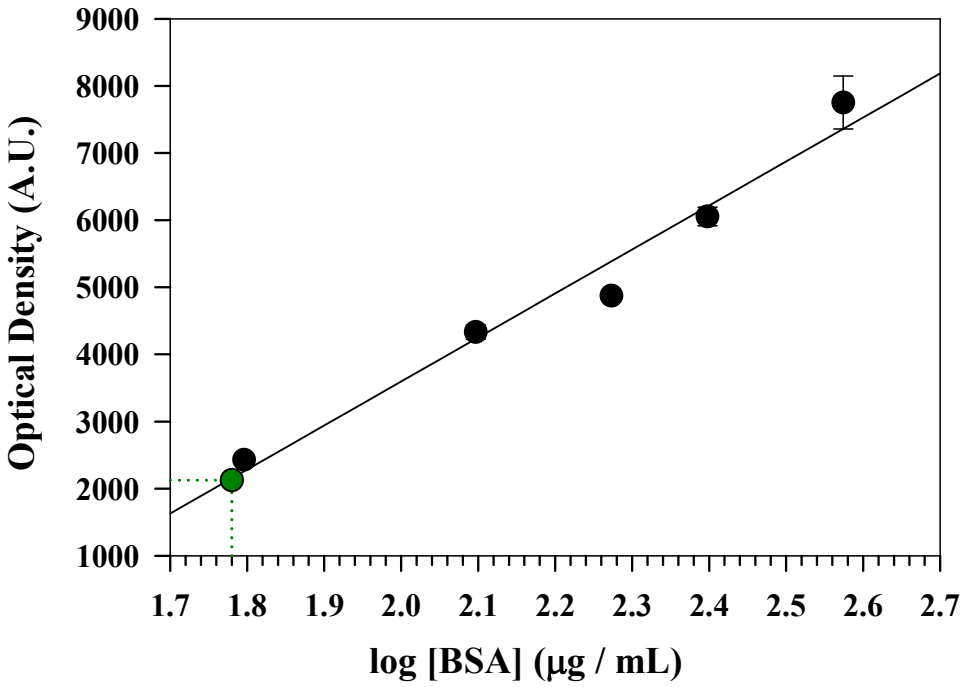

Figure 7. Standard curve obtained for the quantification of purified omentin levels using BSA standards. The standard curve was obtained by plotting the log-transform of five BSA concentrations (6.25; 12.5; 18.75; 25.0; and 37.5 µg/mL) versus optical density (•). The purified omentin concentration (9 µg/mL) was obtained from this curve (•).

Figure 8. Quantification of plasma omentin levels ("standard plasmas") using purified omentin. Plasma samples from human subjects (marked I through III) were run in triplicate on SDS-PAGE gel. On the same gel, three concentrations (1.25, 2.5, 5 μg/lane, marked O1 through O3, respectively) of purified omentin were run in triplicate. Optical densities were measured on Fluorochem 8000 imager.

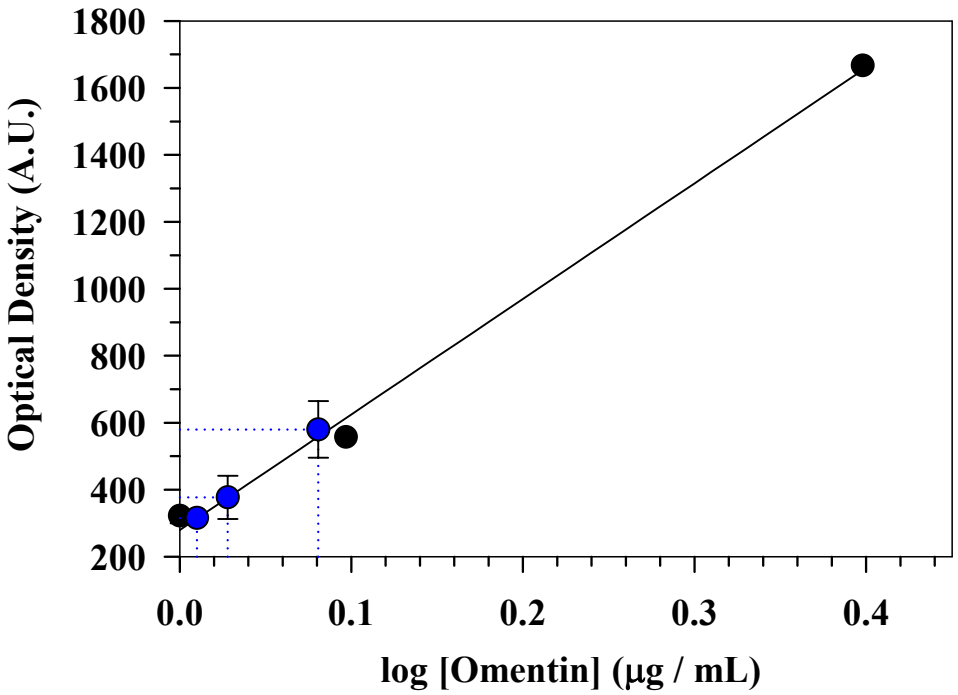

Figure 9. Standard curve constructed for the quantification of "standard plasmas" using purified omentin as a control. The standard curve was obtained by plotting the log-transform of purified omentin concentrations versus their optical densities (•). Omentin concentrations in plasma were extrapolated from this standard curve. Results indicated values of 1.02, 1.07, and 1.22 µg/mL for standard plasmas I, II, and III, respectively (•).

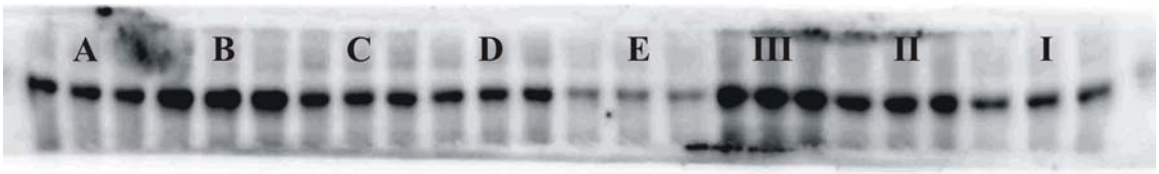

Figure 10. Quantification of plasma omentin levels using plasma samples of known concentrations ("standard plasmas"). Plasma samples from human subjects (marked A through E) were run in triplicate on SDS-PAGE gel. On the same gel, triplicates of "standard plasmas" previously quantified in relation to purified omentin (marked I through III) were also included. Optical densities were measured on Fluorochem 8000 imager.

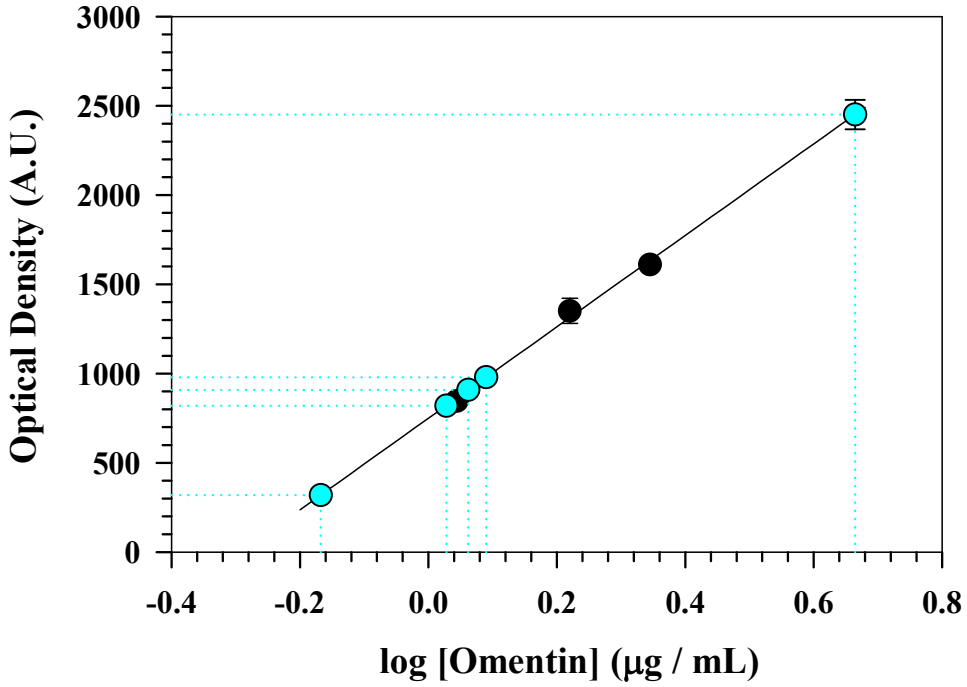

Figure 11. Standard curve obtained for the quantification of plasma omentin levels using "standard plasmas" as a control. The standard curve was constructed by plotting the log-transform of known omentin concentrations ("standard plasmas") versus their optical densities (•). The concentrations of omentin in subjects' plasma were extrapolated to the levels of omentin in plasma standards. The values indicated omentin concentrations of 0.77 ± 0.02, 3.08 ± 0.10, 0.82 ± 0.01, 0.71 ± 0.02, 0.45 ± 0.02 µg/mL for subjects A to E, respectively (•).

2.4 – Statistical Analysis

Data are expressed as means ± S.E. Statistical analysis was performed using either paired Student t-test to investigate the differences in plasma omentin, insulin, glucose, triglycerides, total cholesterol, HDL, and LDL levels between lean and overweight/obese sibling pairs, or unpaired student t-test to verify the differences in omentin concentrations between males and females. Data were tested for normality of distribution by the Shapiro-Wilk test. In consequence, HOMA, plasma omentin, triglyceride, and insulin levels were log transformed to obtain a normal distribution. Pearson correlation coefficients were calculated to evaluate the relationship of plasma omentin concentrations with demographic and metabolic characteristics (age, BMI, HOMA, HDL, LDL, total cholesterol, triglycerides, fasting insulin, fasting glucose and blood pressure).

CHAPTER 3: EXPERIMENTAL RESULTS

Metabolic and demographic characteristics of the subjects are presented in Table 5.

The mean fasting glucose levels were higher in overweight/obese subjects than in lean subjects. However, those values were in the normal non-diabetic range (<120 mg/dL) (table 5).

Fasting insulin levels and HOMA score (assessment of insulin resistance) were significantly lower in lean subjects (8.8 ± 0.4 μU/mL; 1.9 ± 0.1, n = 22) compared with overweight/obese subjects (13.4 ± 1.1 μU/mL; 3.0 ± 0.3; n = 22; P = 0.001 for both variables, paired Student t-test, table 5).

HDL levels were significantly lower in overweight/obese subjects (41.9 ± 1.7 mmol/L, n = 21) than in lean subjects (55.3 ± 2.6 mmol/L, n = 21, P < 0.0001, paired Student t-test, table 5).

Systolic and diastolic blood pressure were significantly higher in overweight/obese subjects (125.5 ± 3.1 mmHg; 82.1 ± 2.1 mmHg, n = 22) than in lean subjects (111.9 ± 3.7 mmHg; 74.8 ± 1.8 mmHg; n = 22; P < 0.05 for both variables, paired Student t-test, table 5).

Plasma omentin levels were significantly higher in lean subjects (1.1 ± 0.1 μg/mL, n = 22) than in overweight/obese subjects (1.04 ± 0.02 μg/mL, n = 22, P = 0.02, paired Student t-test) (figure 12 A, and B).

The same pattern was found among women. Plasma omentin levels were found significantly higher in the lean group (1.25 ± 0.09 μg/mL, n = 11) than in the

overweight/obese group (1.08 ± 0.03 μg/mL, n = 11, P = 0.03, paired Student t-test, figure 13 A, and B).

Interestingly, plasma omentin concentrations were not significantly different between lean males and overweight/obese males (1.03 ± 0.03 μg/mL, 1.01 ± 0.03 μg/mL, respectively, n = 11, P = 0.39, paired Student t-test, figure 14, A and B).

Like adiponectin, resistin and leptin, omentin levels were significantly higher in women compared with men (1.16 ± 0.05 μg/mL vs. 1.02 ± 0.02 μg/mL, n = 44, P = 0.01, unpaired Student t-test, figure 15).

Although age, total cholesterol, triglycerides, LDL, fasting glucose, fasting insulin, and HOMA were not significantly correlated with plasma omentin levels (Table 5), plasma omentin levels were significant negatively correlated with BMI (Pearson correlation coefficient, r = –0.39, r^2 = 0.15, P = 0.0088, n = 44, 22 women, 22 men, figure 16 A) .

These data also indicated a significant positive correlation between omentin and HDL levels (Pearson correlation coefficient, r = 0.41, r^2 = 0.16, P = 0.0078, n = 42, 20 women, 22 men, figure 16 B).

TABLE 5 – Demographic and clinical characteristics of the subjects divided in lean and obese/overweight groups. Data are means ± S.E. P values are for the differences between the two groups by paired Student t-test.

Characteristics	**Lean**	**Obese/Overweight**	**P**
n	22	22	
Sex (males/females)	11/11	11/11	
Age (years)	42 ± 3	43 ± 3	NS
BMI (Kg/m^2)	21.8 ± 0.4	29.0 ± 0.5	<0.0001
Fasting Insulin (μU/mL)	8.8 ± 0.4	13.4 ± 1.1	0.001
Fasting Glucose (mM)	4.9 ± 0.1	5.0 ± 0.1	NS
HOMA	1.9 ± 0.1	3.0 ± 0.3	0.001
Triglycerides (mM)	71.9 ± 8.4	88.4 ± 7.7	NS
Total cholesterol (mM)	217.7 ± 10.5	207.0 ± 8.0	NS
HDL (mM)	55.3 ± 2.6	41.9 ± 1.7	<0.0001
LDL (mM)	148.1 ± 9.4	147.3 ± 6.7	NS
SBP (mmHg)	111.9 ± 3.7	125.5 ± 3.1	0.01
DBP (mmHg)	74.8 ± 1.8	82.1 ± 2.1	0.02

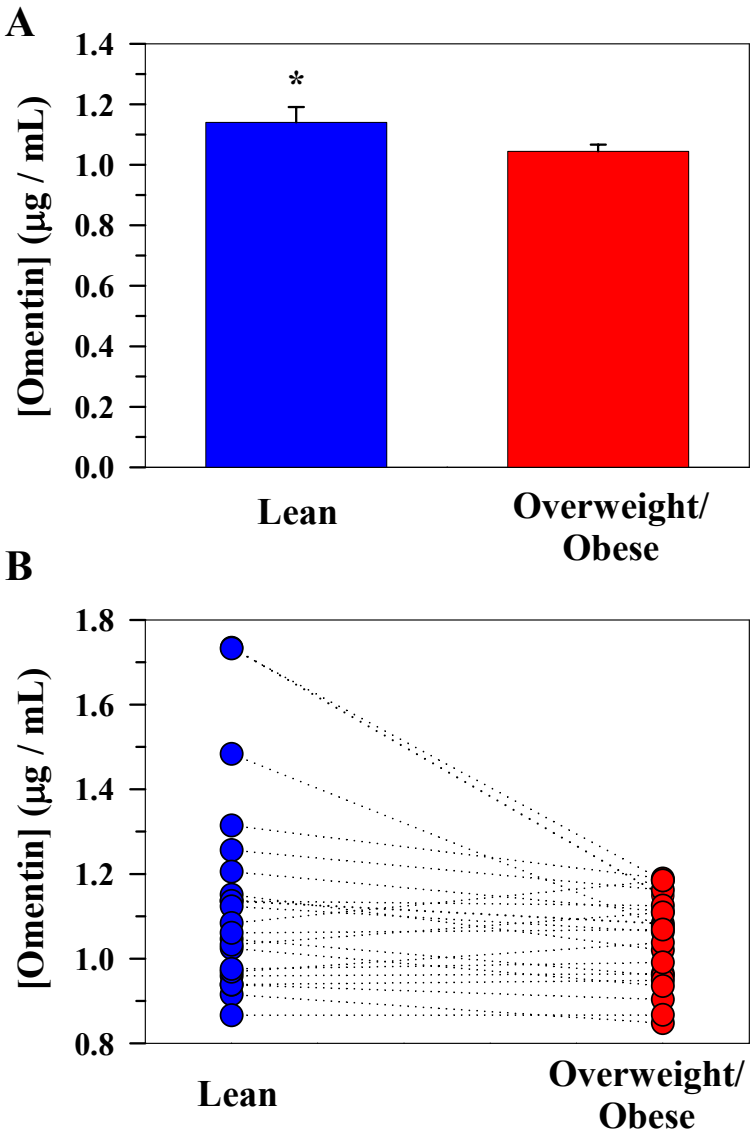

Figure 12. Omentin levels in lean and overweight/obese individuals. Subjects were paired in sib pairs. **(A)** Lean subjects had higher omentin levels (* $P < 0.05$) than overweight/obese subjects. The latter group had a predominance of overweight over obese individuals (14 versus 8 individuals, respectively). **(B)** Omentin concentrations for each subject pair. Note that for most pairs, lean individuals had higher omentin concentration (up to 50% more, data not shown), than the overweight/obese relative.

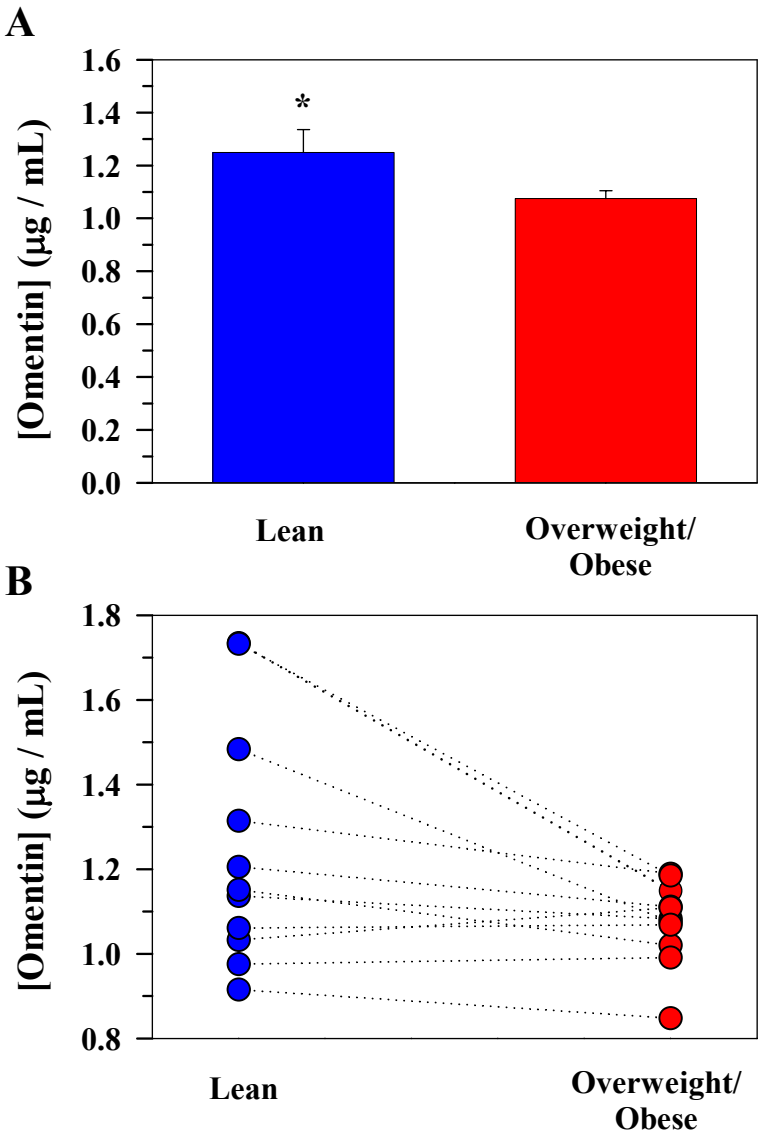

Figure 13. Plasma omentin levels in lean and overweight/obese female individuals. Subjects were paired in sib pairs. **(A)** Female lean subjects had higher plasma omentin levels (1.25 ± 0.09 µg/mL, n = 11) than in the overweight/obese group (1.08 ± 0.03 µg/mL, n = 11, P = 0.03, paired Student t-test). **(B)** Plasma omentin concentrations for each subject pair. Note that for most pairs, lean individuals had higher omentin levels than the overweight/obese relative.

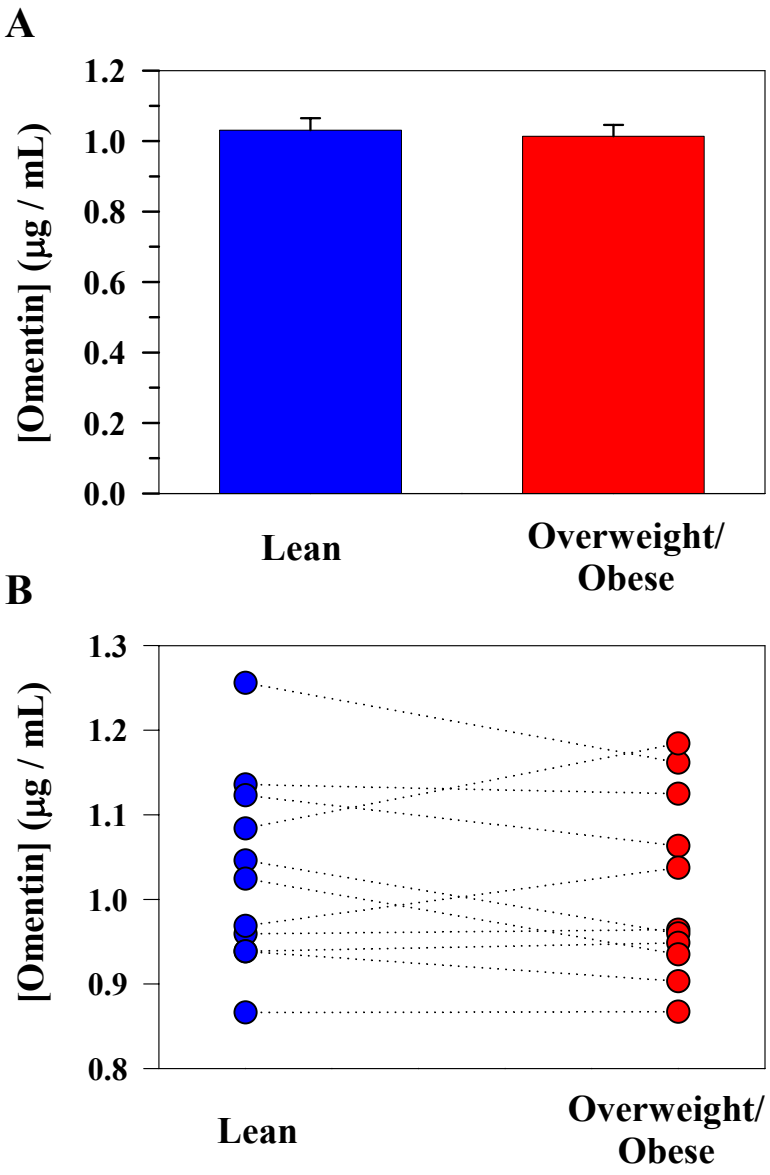

Figure 14. Plasma omentin concentrations in lean and overweight/obese male individuals. Subjects were paired in sib pairs. **(A)** Plasma omentin levels were not significantly different between male lean subjects (1.03 ± 0.03 µg/mL) and overweight/obese subjects (1.01 ± 0.03 µg/mL, n = 11, P = 0.39, paired Student t-test). **(B)** Omentin concentrations for each subject pair.

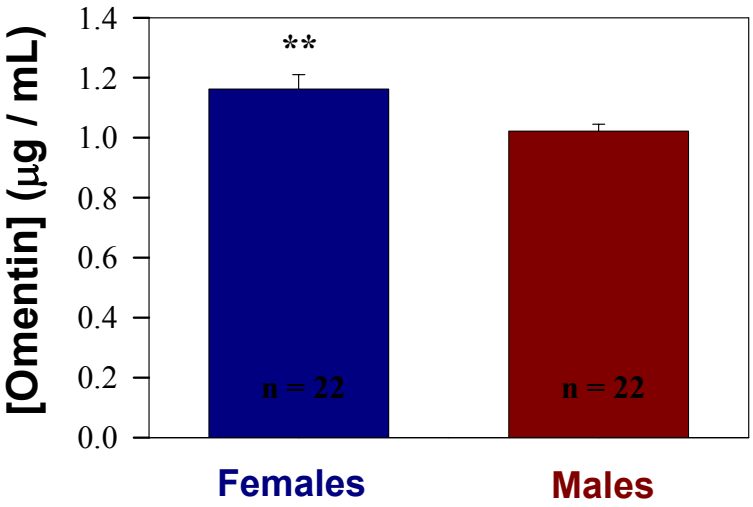

Figure 15. Plasma omentin levels in female and male subjects. Plasma omentin levels were significantly higher in women (1.16 ± 0.05 µg/mL, n = 22) compared with men (1.02 ± 0.02 µg/mL, n = 22, P = 0.01, unpaired Student t-test).

TABLE 6 – Correlations between log-transformed plasma omentin levels and subjects' clinical and demographic characteristics. The Pearson correlation coefficient (r) describes the strength of association between the variables. Prior to regression analysis, data were tested for normality of distribution by the Shapiro-Wilk test. In consequence, HOMA, triglyceride, and insulin levels were log transformed to obtain a normal distribution.

Characteristics	**r**	**P**	**n**
Age	0.08713	NS	44
Total cholesterol (mM)	0.01083	NS	42
Triglycerides (mM)	- 0.2161	NS	42
LDL (mM)	- 0.06577	NS	42
Fasting Insulin (μU/mL)	- 0.1806	NS	44
Fasting Glucose (mM)	0.1630	NS	42
HOMA	- 0.1507	NS	42

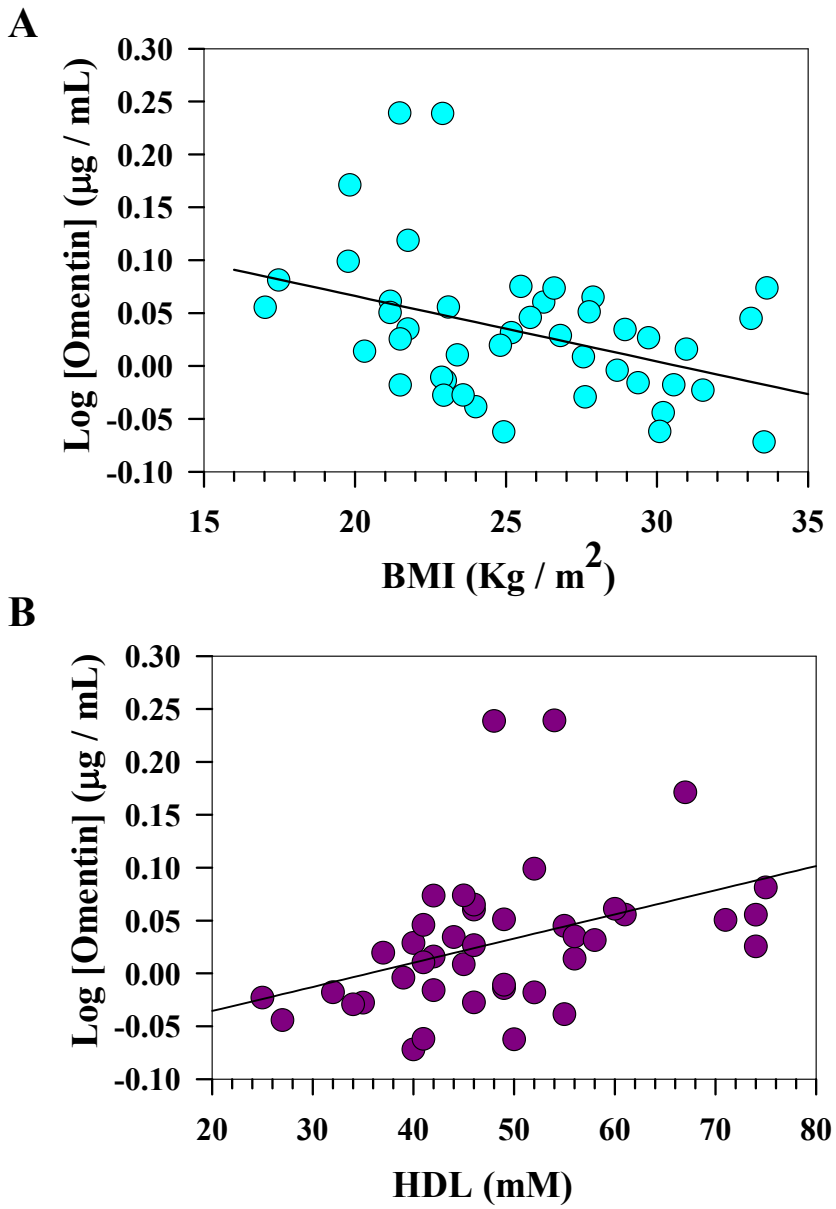

Figure 16. Correlation between log-transformed plasma omentin with BMI and HDL levels. The lines of best fit are indicated in each graph. **(A)** Plasma omentin levels were significant negatively correlated with BMI (Pearson correlation coefficient, r = –0.39, P = 0.0088, n = 44, 22 women, 22 men). **(B)** There was observed a significant positive correlation between plasma omentin and HDL levels (Pearson correlation coefficient, r = 0.41, P = 0.0078, n = 42, 20 women, 22 men).

CHAPTER 4: DISCUSSION AND FUTURE DIRECTIONS

The discovery of adipocytokines has led to the realization that adipose tissue does not only serve as a depot for energy storage, but in fact, also functions as an endocrine organ. This represented a fundamental paradigm shift in human nutrition and physiology (26-31).

Adipocytokines, such as adiponectin, leptin and resistin have been previously correlated with insulin resistance and obesity (26, 29, 31, 34, 42, 44, 51, 52). It has also been shown that omentin increases insulin signal transduction and insulin-stimulated glucose transport in 3T3-L1 adipocytes and isolated human adipocytes. In addition to that, omentin secretion is regulated positively by dexamethasone and thiazolidininedione (TZD) compounds (used to enhance insulin sensitivity) in human adipose explant cultures. These preliminary data suggest that omentin may play a positive role in maintaining insulin sensitivity, possibly improving obesity-related insulin resistance.

As a first step in investigating the role played by omentin in human physiology, plasma omentin levels in lean and obese/overweight subjects were measured; and the relationships between plasma omentin levels and several metabolic indices were investigated.

These preliminary data showed higher plasma omentin levels in lean versus obese/overweight subjects (1.14 ± 0.05 μg/mL vs. 1.04 ± 0.02 μg/mL, $P < 0.05$, paired Student's t-test). This finding correlates with previous reports of higher plasma adiponectin levels in lean compared with obese subjects (36, 42, 46, 49). On the other hand, it is well established that plasma leptin and resistin levels are higher in obese

than in lean subjects (34, 36, 40, 53). Based on several studies, the approximate difference between lean and obese subjects in percentage for adiponectin, leptin, and resistin are respectively 35%, 78%, and 25%. Although the difference found for omentin was only 10% (figure 12), there are important aspects to consider: (i) the different methodologies to measure each adipocytokine used in each study; (ii) the group of subjects used in the study.

First, in this present study, quantitative western blotting was used to measure plasma omentin levels, as opposed to standardized, commercially available immunoassays kits used to measure adiponectin, leptin and resistin in the other investigations. It is well known that quantitative western blotting can be a technically challenging assay. Initial experiments (data not shown) evaluated 3 different types of anti-omentin antibodies: 2 monoclonal mouse anti-omentin (3G1B3 and 4G2E9) and 1 polyclonal rabbit anti-omentin (16070) (all human-omentin specific and developed at the Division of Endocrinology, Diabetes and Nutrition, UMB). These results indicated that anti-omentin antibody 3G1B3 gave the best signal-to-noise ratio and was selected for use in the rest of the experiments (data not shown). Also, initial experiments indicated that this antibody did not detect human adiponectin in serum samples (data not shown). On the other hand, commercially available immunoassays are recognizably more accurate and sensitive than quantitative western blotting analysis, resulting in less variability among different samples. However, in spite of these limitations the differences between obese/overweight and lean subjects were significant.

A second potential confounding factor was the limitation in obtaining plasma samples from a higher number of obese volunteers. Because of the reduced number of obese individuals (8 versus 14 overweight), overweight and obese subjects were combined in a single group and paired against lean relatives. The main advantage of recruiting volunteers from the Amish population of Lancaster County is the ability to obtain subjects with a homogeneous life style. However, most of them are more physically active than their non-Amish urban counter parts (see introduction for details). This suggests the possibility that a comparison between lean and obese subjects (with a standard sedentary lifestyle), such as the subjects investigated in other adipocytokine studies, may indicate greater variation in omentin levels than observed in the Amish.

Surprisingly, the difference in plasma omentin levels was significant only between lean and overweight/obese women (1.25 ± 0.09 μg/mL vs. 1.08 ± 0.03 μg/mL, $P < 0.02$, paired Student's t-test, figure 13). Moreover, plasma omentin levels were significantly higher in women than men (1.16 ± 0.05 μg/mL vs. 1.02 ± 0.02 μg/mL, $P < 0.01$, Student's t-test, figure 15). Adiponectin, leptin and resistin levels are also about 50% higher in women than in men (29, 36, 37, 38, 40, 42, 46, 49, 50, 53). The difference for omentin levels between women and men was about 14%. The smaller relative difference between omentin and the other adipocytokines in women versus men probably derives from the same causes discussed above for the absolute plasma levels of omentin.

On the other hand, there was not difference between lean and overweight/obese individuals in the male population (1.03 ± 0.03 μg/mL vs. 1.01 ±

0.03 μg/mL, figure 14). These data are consistent with the lack of a significant difference in plasma resistin levels between obese and lean males as described by Degawa-Yamauchi et al (2003), (53).

Taken together, these results suggest an effect of sex hormones (estrogen, progesterone, and testosterone) on plasma omentin levels. Epidemiological studies established that women have a lower risk of cardiovascular diseases than men (see introduction for details). If higher omentin levels are associated with this lower risk, or are a consequence of some other, more fundamental cause, this will require further investigation. Further studies with adipose tissue cultures should clarify this situation.

Plasma omentin levels showed a significant negative correlation with BMI ($r = -0.39$, $P = 0.0088$, figure 16A). According to other studies, leptin and resistin are positively correlated with BMI, while adiponectin is negatively correlated with BMI (34, 36, 38, 39, 40, 42, 45, 46, 47, 53). This result corroborates the hypothesis that low omentin levels may be associated with higher risk of metabolic syndrome, indicated by the BMI index. Moreover, to further validate the correlation between omentin and BMI, a study measuring plasma omentin levels before and after weight loss can be conducted.

In agreement with other studies for adiponectin, leptin and resistin, a significant correlation between plasma omentin levels and HOMA (homeostasis model assessment of insulin resistance) was not found in this study (34, 36, 40). Although HOMA is considered by several authors an imprecise method of assessing insulin resistance compared with the hyperinsulinemic euglycemic clamp technique, these data suggest that omentin is more tightly linked with degree of obesity than

insulin resistance levels. In order to verify more carefully the correlation between omentin levels and insulin resistance, a study using hyperinsulinemic euglycemic clamp technique could be designed. In this case, the insulin resistance index would be more precise, not leaving doubts about the possible correlation between omentin and insulin sensitivity.

In addition, plasma omentin levels were significant positively correlated with HDL levels ($r = 0.41$, $P = 0.0078$, figure 16B). Negative correlations without statistical significance were found between omentin and triglycerides, and LDL (table 5). The same pattern has been previously found for adiponectin, while the opposite was found for leptin and resitin (45, 46, 48). These data show that higher omentin levels are associated with a healthy lipid profile, suggesting that omentin may play a role in the same anti-atherogenic behavior as adiponectin.

Interestingly, plasma omentin levels did not have a statistically significant correlation with age, total cholesterol, fasting glucose and blood pressure, probably due to the absence of significant differences in these factors between the lean and overweight/obese subjects, as well as the small sample size. With a higher number of female volunteers, it would be interesting to search for a correlation between age and plasma omentin levels, because of the natural hormonal variation experienced by woman according to age. This might shed light on the hormonal regulation of omentin expression.

Future directions can be drawn from these data. First, omentin's correlation with certain metabolic indices is similar to adiponectin, indicating that a higher omentin level is seen as a positive factor that may affect obesity and its associated

pathologies. Also, *in vitro* experiments can be designed to investigate synergistic effects between the two adipocytokines. In addition, it would be interesting to verify the correlations between plasma omentin levels, and adiponectin, leptin, and resistin levels. Second, sex hormones influence omentin expression/or secretion as evidenced by the higher omentin levels in women than men. Future studies could address this question using hormone-stimulated tissue culture models, as other investigators have conducted with adiponectin.

Finally, it is still necessary to study omentin gene expression and secretion in lean, overweight, and obese subjects. Since it is not possible to investigate mRNA levels in plasma, this study could be developed using surgical biopsies of omental fat from lean, overweight, and obese subjects to gain a greater understanding of the molecular control of omentin expression and secretion. Consequently, using these preliminary data, it should be possible to determine the necessary sample size associated with a satisfactory power of analysis for future studies involving omentin.

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