PROTEIN INTAKE IN ADULTS AT RISK OF INSULIN RESISTANCE: IMPACT ON GLYCEMIC AND LIPOPROTEIN PROFILES

By

ETSUKO HOSHINO

Bachelor of Science

Marymount College

Tarrytown, New York

1999

Submitted to the Faculty of the
Graduate College of the
Oklahoma State university
In partial fulfillment of
The requirements for
The Degree of
MASTER OF SCIENCE
May, 2003

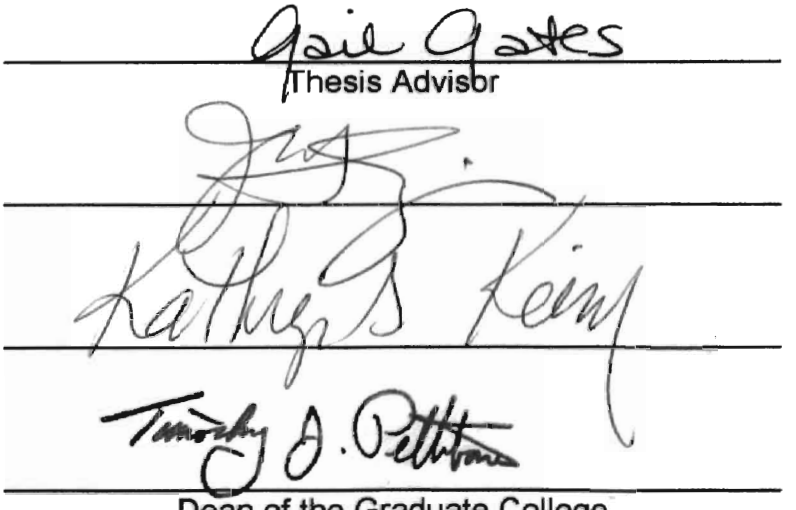
PROTEIN INTAKE IN ADULTS AT

RISK OF INSULIN RESISTANCE:

IMPACT ON GLYCEMIC AND

LIPOPROTEIN PROFILES

Thesis Approved:



Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my advisor, Dr. Gail Gates for all her support, guidance, knowledge, patience, friendship and financial support. I am grateful for all the extra hours you worked to help me complete my thesis and for believing in me, I could not have completed this without such a wonderful and supportive advisor. I would like to extend my gratitude to my committee members Dr. Kathryn Keim and Dr. Maria Spicer for all of their patience, support, friendship, and for providing me with their guidance and knowledge. I am truly indebted to you all. I would like to extend thanks to Chris Taylor for his assistance with the Third National Health and Nutrition Examination Survey (1988-1994) data set. I acknowledge and greatly appreciate National Pork Board for funding this research project.

I would also like to give my special appreciation to my family Yoshihiko and Michiko Hoshino, Kiat and Sooyin Chua, and Kem for your love and supports. I would also like to thank Shinichi Konishi, Teruko Doyle, Elaine Kato, Frances kuba, and Akihiro Hayashi, who supported me spiritually, emotionally, and continuously encouraged me through this process.

Finally, I would like to express thanks to Oklahoma State University and the Department of Nutritional Sciences for this opportunity to further my education.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Evidence suggests that insulin resistance, a notable metabolic disorder, sharply increases the risk of developing chronic disorders such as diabetes and cardiovascular disease, as well as their risk factors such as hypertension, glucose intolerance, and hyperlipidemia (Matsumoto et al., 1999; Taniguchi et al., 2000; Lind et al., 1993). Over 700,000 people died of heart disease in the United States in 1999 (National Vital Statistics Reports, 2001). At an alarming rate, new cases of diabetes are diagnosed each year. Diabetes is the single leading cause of kidney disease and is a risk factor for coronary heart disease (CHD) and stroke (American Diabetes Association, 2002). Diabetes is also a major contributor to blindness, nerve damage, high blood pressure, and amputations. Improving diet quality would not eliminate these serious health risks, but could reduce the current health burden associated with diet-related conditions by delaying and preventing the onset of these diseases (King et al., 1998; Davidson, 1998; Bloomgarden, 2000; Franz et al., 1994).

Background and Significance of the Problem

Adults who consume diets that are high in saturated fat or carbohydrates are at increased risk for development of insulin resistance (Feskens et al., 1995; Riccardi & Rivellese, 2000). Studies show that diets high in saturated fats and simple sugars promote increased insulin responses (Storlien, 2000), but the effect of protein on insulin resistance is not well-studied. Costa et al. (2000) found that protein intake was not associated with blood glucose concentration in a high risk Japanese-Brazilian population. However, another study found that animal protein and fat intake were higher in Japanese-American men who were later diagnosed with diabetes than in nondiabetic men (Tsunehara et al., 1990). Examining data from a large nationally representative sample will help determine if there is an association between protein intake and glycemic profiles in the United States population.

Low calorie diets and weight loss are associated with reduced insulin resistance (American Diabetes Association, 1997). In recent years, dietary guidance for weight loss has focused on encouraging adults to lower their fat intake, however too much emphasis on increasing carbohydrate intake may worsen glycemic control in adults who are insulin sensitive (Riccardi & Rivellese, 2000). Therefore, diets that are lower in fat and carbohydrate but higher in protein may promote weight loss with better glycemic control. In individuals with diabetes, consumption of dietary protein may promote metabolic control by

extending the time substrates are available for hepatic glucose production and result in a lower glycemic response than glucose (Franz et al., 1994).

Studies examining the impact of protein on blood lipid levels are not conclusive. One study found no difference in lipoprotein profiles by protein intake after using analysis of covariance (ANCOVA) adjusted for sex and age effects (Lamon-Fava et al., 1994). However, Smit et al. (1999A) found that subjects in the highest quartile of serum cholesterol consumed more animal protein than subjects in the lowest quartile using age-, sex-, and race-adjusted values. In a Swedish cohort study, adults who ate more protein often consumed more saturated fat and less complex carbohydrate after adjusting for differences in energy intake (Elmstahl et al., 1999). The consumption of very lean meats as part of low fat diets has resulted in reduced blood lipid concentrations (Davidson et al., 1999; Bales 1995; Scott 1994).

Purpose of Study and Hypotheses

Insulin resistance and the development of chronic diseases are probably linked through changes in lipid and glucose metabolism (Cruz et al., 2001; Greco, et al., 2002; American Diabetes Association, 1998; Ozaki et al., 2002; Pyorala M, et al., 2000; Colagiuri et al., 2002; Fujimoto, 2000; Lind et al., 1993). Adults who are at risk of insulin resistance may enhance their glycemic control without elevating lipoproteins if they consume a high protein diet. The purpose of this

study was to examine the relationship of protein intake with lipoprotein and glycemic profiles in US adults who are at risk of insulin resistance. To address this purpose, we examined data from the Third National Health and Nutrition Examination Survey (1988-1994).

Objective of Study

The following questions were identified as being relevant to the problem. These questions were used to develop the specific hypotheses of the study:

1. What is the relation between protein intake and glycemic profiles (glucose, insulin, C-peptide, and hemoglobin A1c) in adults at risk of insulin resistance?
2. What is the relation between protein intake and lipoprotein profiles (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) in adults at risk of insulin resistance?

Research Hypotheses

The following research hypotheses were developed for this study:

1. Glycemic profiles (glucose, insulin, C-peptide, and hemoglobin A1c) will be associated with quartiles of total and animal protein intake in adults at risk of insulin resistance.

1-A. Glycemic profiles will be positively associated with quartiles of total and animal protein intake in adults at risk of insulin resistance.

1-B. Glycemic profiles will be negatively associated with protein intake in adults at risk of insulin resistance.

2. Lipoprotein profiles (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) will be associated with quartiles of total and animal protein intake in adults at risk of insulin resistance.

2-A. Lipoprotein profiles will be positively associated with quartiles of total and animal protein intake in adults at risk of insulin resistance.

Assumptions and Limitations

The study will be based on the following assumptions and limitations:

Assumptions of the Study

1. True information was provided by respondents.
2. All questions were of equal difficulty for each respondent.
3. The nutrient intake reported will accurately represent the usual food intake.

Limitations of the Study

1. Data were self reported.
2. The accuracy of dietary assessment methods depends on the respondent's perception of the portion sizes.
3. The use of memory to record the foods is a limitation because there are many food that are easy to forget.

Definition of Terms

Adults at risk of insulin resistance: adults who are obese (males with a body mass index $\geqq$27.8 kg/m^2; and females with a body mass index $\geqq$27.3 kg/m^2), have a family history of diabetes mellitus, high waist circumference (males >102cm; and females >97cm), impaired fasting glucose (the blood glucose concentrations above 6.1 mmol/L), or mildly elevated triglycerides (150 and 499 mg/dL). (Dickey et al., 1998; Wangerin-Lile et al., 2000; Unwin N et al., 1998; NCEP, 2001)

Diabetes mellitus: a metabolic disease in which carbohydrate utilization is reduced and that of lipid and protein enhanced; it is caused by an absolute or relative deficiency of insulin and is characterized, in more severe cases, by chronic hyperglycemia, glycosuria, water and electrolyte loss, ketoacidosis, and coma; long-term complications include development of neuropathy, retinopathy, nephropathy, generalized degenerative changes in large and small blood vessels, and increased susceptibility to infection (Mahan et al., 1996; Whitney et al., 1994).

Glycemic profile: serum glucose, plasma glucose, serum insulin, serum C-peptide, and glycated hemoglobin.

Insulin resistance: the condition in which a normal amount of insulin produces a subnormal effect; a metabolic consequence of obesity; a common cause of non-insulin-dependent diabetes (Mahan et al., 1996; Whitney et al., 1994).

Lipid profile: total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

CHAPTER II

REVIEW OF RELATED LITERATURE

Insulin Resistance

Insulin is a hormone that has a variety of effects on many types of cells. Anabolic actions of insulin on glucose, lipid, and protein metabolism are essential for life. A lack of insulin will lead to extreme hyperglycemia and hyperlipidemia, protein wasting, and ultimately, keto-acidosis and death. Although insulin is essential for various metabolic systems, its chief control is exerted over glucose metabolism (Ferrannini et al., 1999).

The circulating glucose concentration is a highly homeostatic variable. A rise in plasma glucose concentrations, whether induced by an exogenous (alimentary) or endogenous (hepatic) input, stimulates insulin secretion. Post-prandial surges and inter-prandial declines of insulin level are tightly coupled to glucose availability and prevent both hypoglycemia and hyperglycemia. Thus, the proper response of pancreatic β-cells to glycemic changes by punctually increasing or decreasing insulin release is the conclusive factor to glucose control (Cheatam & Kahn, 1995; Fujimoto, 2000).

Insulin resistance is generally defined as an impaired response to the effect of high insulin concentrations (either exogenous or endogenous) on glucose metabolism. According to Consensus Development Conference on Insulin Resistance, the concept of insulin resistance contains any of the biological actions affected by insulin, such as lipid and protein metabolism, vascular endothelial function, and gene expression (American Diabetes Association, 1998). Insulin resistance is a condition that requires greater than normal insulin levels to bring forth a normal glucose concentration in the whole body, a tissue, or at the cellular level. Insulin resistance results from diminished insulin action or decreased insulin sensitivity (Krentz, 1996; Colagiuri & Miller, 2002).

Insulin resistance can be quantified directly with the euglycemic hyperinsulinemic clamp technique or other methods, such as intravenous glucose tolerance test and fasting insulin concentrations. Intravenous glucose tolerance test and euglycemic hyperinsulinemic clamp technique are not suitable experimental methods for epidemiological studies, and for this reason fasting insulin is commonly used as an estimate of insulin resistance (Ferrannini & Mari, 1998). Also, quantitative comparisons of resistance to the action of insulin are difficult between populations and between individuals because of the need to standardize for physiological (age, gender, race, physical fitness), pathological (obesity, glucose tolerance, blood pressure, hormonal effects) and genetic factors. Thus, an individual with normal glucose tolerance who is in the highest quartile of insulin concentrations of the population may be considered to be

insulin resistant (Colagiuri & Miller, 2002; McAuley et al., 2001). There is no generally acceptable quantitative definition of insulin resistance and therefore of what constitutes normal or abnormal insulin concentration (Ferrannini & Mari, 1998). The homeostasis model assessment (HOMA) provides a simple index for evaluating insulin sensitivity from a single sample, which is closely correlated with insulin resistance index assessed by euglycemic clamp in type 2 diabetic patients, however clinical use is limited (Kanauchi et al., 2002; Fukushima et al., 2000).

Several studies have revealed the link between insulin resistance and various pathophysiological conditions that are coupled with metabolic disturbances such as type 2 diabetes, impaired glucose tolerance, cardiovascular disease, hypertension, dyslipidemia (high triglycerides, low HDL-cholesterol, and smaller, denser LDL particles), and obesity (Ambrosch et al., 1998; Hauner, 2002). According to the Insulin Resistance Atherosclerosis study that examined the relationship between insulin sensitivity and the risk of diabetes and cardiovascular disease by measuring fasting concentrations of insulin and acute insulin response among adults who were nondiabetic, insulin resistance independently predicted development of type 2 diabetes (Hanley et al., 2002). Matsumoto et al. (1999 & 2001) reported that insulin resistance was an independent risk factor for ischemic stroke in Japanese patients with type 2 diabetes along with aging and hypertension.

Taniguchi et al. (2000) compared the cardiovascular disease risk factors in Japanese type 2 diabetes patients with normal insulin sensitivity and insulin resistance and found that patients with normal insulin action have a low cardiovascular disease risk, whereas those with insulin resistance have a significantly increased cardiovascular disease risk. Also, patients with insulin resistance exhibited higher body mass index (BMI) and triglyceride levels than patients with normal insulin sensitivity. According to Lind et al. (1993), insulin resistance, measured by the euglycemic hyperinsulinemia clamp technique, was a better predictor of cardiovascular risk factors including hypertension, glucose intolerance, and indices of hyperlipidemia (elevated free fatty acids, serum triglycerides and low HDL cholesterol) than hyperinsulinemia, but both insulin sensitivity and hyperinsulinemia were significantly related to fasting glucose concentrations.

Obesity is a common cause of insulin resistance and poses a major risk for the development of diabetes (Caro, 1991). Abnormal fat deposition within skeletal muscle has been identified as a mechanism of obesity-associated insulin resistance (Greco et al., 2002). The accumulation of intra-abdominal or visceral fat had the strongest association with insulin resistance (Kissebah & Peiris, 1989; Lean et al., 1995). Ferrannini et al. (1997) reported that the prevalence of insulin hypersecretion was greater than the prevalence of insulin resistance in nondiabetic, normotensive obese subjects, particularly in women with central obesity.

Dietary habits are an important environmental factor associated with the development of glucose intolerance and insulin resistance. High fat diets induced insulin resistance in animal experiments (Storlien et al., 1991; Kusunoki et al, 1995). In humans, high fat diets have been reported to result in decreased insulin sensitivity (Lichtenstein & Schwab, 2000). The results of a follow-up study conducted on Japanese-Americans living in Hawaii and Los Angeles suggested that the westernization of lifestyle such as, conversion to a diet containing markedly more animal fat, simple carbohydrates, and less complex carbohydrates increased the risk of insulin resistance, hyperinsulinemia, and type 2 diabetes among migrant Japanese-Americans (Hara et al., 1996). Similar unfavorable dietary changes were observed with Japanese-Brazilians, but the study did not confirm the association between dietary habits and the risk of type 2 diabetes (Costa et al., 2000). In another longitudinal study, higher plasma insulin was associated with consumption of more total and saturated fat and less carbohydrate and fiber after adjusting for age, gender, ethnicity, physical activity, BMI, waist circumference, and total energy (Marshall et al., 1997).

Other lifestyle choices also influence the development of insulin resistance and associated metabolic conditions and diseases. Increased physical fitness, weight reduction, smoking cessation, and moderate alcohol consumption are reported to enhance insulin sensitivity and improve insulin resistance (Krentz, 1996). Lower physical activity was observed among Japanese-Americans who

exhibited diminished early insulin release to an oral glucose challenge and increased insulin resistance characterized by hyperinsulinemia (Hara et al., 1996).

The identification of insulin resistance at an early stage is beneficial and encouraged, since the metabolic defects associated with some acquired forms of insulin resistance, such as obesity are potentially fully reversible, while others such as type 2 diabetes and cardiovascular disease are only partially reversible (American Diabetes Association, 1998; Martin et al., 1992; Krentz, 1996).

Metabolic Syndrome

Insulin resistance is a common underlying abnormality in a number of chronic conditions, which cluster together and have collectively been referred to as the 'metabolic syndrome.' Originally, Reaven (1988) proposed that insulin resistance was at the center of and pathophysiologicaly link to a syndrome characterized by a clustering of metabolic abnormalities associated with increased cardiovascular risk, impaired glucose tolerance, type 2 diabetes mellitus, dyslipidemia, hypertension and obesity (Everson et al., 1998; Hauner, 2002). The most common feature of metabolic syndrome is insulin resistance, then abdominal fat distribution, high tissue concentrations of triglycerides, and general or central obesity (Anderson, 2001). According to a study conducted in a Chinese population, the most influential factor associated with metabolic syndrome included general and central adiposity, impaired insulin sensitivity, and

glucose intolerance. The second factor included hypertension and general and central obesity. Elevated plasma triglycerides and low HDL-cholesterol loaded very highly on the third factor, and waist circumference was weakly associated. The study suggested that the clustering of variables in metabolic syndrome was the result of multiple factors linked by adiposity and not a single etiology (Anderson et al., 2001).

Everson et al. (1998) reported that hypertension, hyperinsulinemia, and dyslipidemia (low HDL-cholesterol and high serum triglycerides) were identified with insulin resistance. They found that obesity, particularly a weight gain from early aduldhood to middle age was independently associated with metabolic and hemodynamic abnormalities in men. Each 5% weight gain over reported weight at age 20 was associated with 20% increased risk of insulin resistance syndrome by middle age after adjusting for age and height. Their findings were independent from subjects' age, height, physical acitivity, smoking, education, and parental history of diabetes. Fasting hyperinsulinemia in association with the clustering of cardiovascular disease risk factors such as glucose intolerance, central adiposity, hypertension, and elevated triglycerides and lowered HDL cholesterol levels were characteristics of metabolic syndrome in native Hawaiians (Mau et al., 1997).

Several studies pointed to the association between metabolic syndrome and obesity, especially central obesity. As suggested by Barker's (1994) fetal origins hypothesis, Yajnik (2001) found that small size at birth (or poor fetal

growth) and subsequent obesity was associated with increased risk of insulin resistance syndrome (diabetes, hypertension, and coronary heart disease) in later life among an Indian population. Yajnik also reported that adults from India with higher body fat for a given BMI, central adiposity, and small muscle mass were at increased risk of insulin resistance. Similar results were reported for a Japanese population. Takami et al. (2000) examined the association between precise abdominal fat distribution and cardiovascular disease and reported that abdominal fat, regardless of its intraabdominal or subcutaneous localization, was closely associated with atherosclerotic metabolic factors (glucose tolerance, insulin resistance, serum triglyceride, HDL cholesterol, and systolic blood pressure) and predicts carotid atherosclerosis. On the other hand, central adiposity and hypertension were not independently associated with insulin resistance syndrome among native Hawaiian population (Mau et al., 1997).

The results of the Oslo Diet and Exercise Study suggested that an intervention of diet and exercise was the most effective treatment in reversing the development of insulin resistance syndrome. Diet intervention, which included increased intake of fish and reduced total fat intake, was more effective in reducing fasting serum levels of glucose, insulin resistance, BMI, and mean blood pressure, while the exercise intervention was more effective in reducing C-peptides and triglycerides (Torjesen et al., 1997)

It is unlikely that environmental factors such as the intrauterine environment, early life nutrient intake, increasing age, overweight, lack of physical activity, poor diet, starvation, and pregnancy (for gestational diabetes) act independently to determine metabolic syndrome or insulin resistance syndrome (Anderson et al, 2001).

Glycemic Control

In 1997 the Expert Committee of the American Diabetes Association (ADA) and in 1998 the World Health Organization (WHO) proposed a new classification and diagnostic criteria for diabetes mellitus. ADA encourages the use of fasting glucose as the main diagnostic test of diabetes rather than the oral glucose tolerance test (OGTT) as recommended by WHO. Current WHO criteria defined diabetes as ≥ 11.1 mmol/L (≥ 200 mg/dL) and impaired glucose tolerance as 7.8 to 11.0 mmol/L (140 to 198 mg/dL) based on 2 hour post glucose load venous plasma values. Using the new ADA criteria, diabetes was defined as fasting venous plasma ≥ 7.0 mmol/L (≥ 126 mg/dL) and impaired fasting glucose as 6.1 to 6.9 mmol/L (110 to 124 mg/dL). Unwin et al. (1998) examined the relationship between normal, impaired, and diabetic categories using the new ADA fasting and WHO 2 hour post glucose load criteria using population-based data from three ethnic groups (824 European, 375 Chinese, and 680 South Asian) aged 25 to 74 years in UK. The prevalence of diabetes was higher based on the

ADA criteria, using fasting plasma glucose only, than based on WHO criteria in all ethnic groups.

Some researchers reported another effective diagnostic tool for diabetes. Peters et al. (1996) investigated studies reported between 1966 and 1994 in which glycosylated hemoglobin ($HbA1_c$) levels, which accurately reflects the average blood glucose level for three months, were measured concurrently with performance of OGTT and found that measurement of $HbA1_c$ levels may represent a reasonable approach to identifying treatment-requiring diabetes. The study conducted in Japan found high correlations among all three measures of glycemic control: fasting plasma glucose, 2 hour plasma glucose in OGTT, and $HbA1_c$ (Ito et al., 2000).

Rising blood sugar levels may harm the body directly, by damaging blood vessels throughout the body. It is also a signal of metabolic disorders and insulin resistance that sharply increase the risk of developing diabetes, cardiovascular disease, and possibly cancer (Guerrero-Igea et al., 2001; Harvard Heart Letter, 1998; Toeller, et al., 2001). Thus, maintenance of as near-normal blood glucose levels as possible (glycemic control), along with achieving optimal lipid levels is important for prevention and treatment of insulin resistance and its associated metabolic diseases and conditions, such as hyperinsulinemia, hyperglycemia, hypoglycemia, diabetes mellitus, hypertension, cardiovascular disease, and renal disease (Feskens, et al., 1995; Franz, et al., 1994).

Glycemic control may be improved by regular aerobic exercise, weight maintenance and reduction, smoking cessation, and consumption of a healthy diet (ADA, 2002). Since a high intake of fat, especially that of saturated fatty acids, contributes to the risk of glucose intolerance and type 2 diabetes, eating foods such as fish, lean meat, vegetables, and legumes along with reducing caloric intake and spacing of meals may have a protective effect (Franz et al., 1994; Feskens et al., 1995; King et al., 1998).

Control of Dyslipidemia

Dyslipidemia is a disorder characterized by hypercholesterolemia, hyperlipidemia, and hypertriglyceridemia. Dyslipidemia is associated with a high risk of cardiovascular disease. Other risk factors common in patients with dyslipidemia are android obesity, insulin resistance, type 2 diabetes, and hypertension (Haffner, 1998). Alterations in lipid metabolism such as elevated plasma triglycerides, decreased plasma HDL cholesterol, and small dense LDL particle distribution are commonly associated with insulin resistance. An impairment of postprandial lipid metabolism may be an underlying cause linking insulin resistance and the development of cardiovascular disease, since insulin plays a central role in determining triglyceride clearance via activation of lipoprotein lipase, and also triglyceride output, through effects on the synthesis and secretion of very low density lipoprotein (VLDL). A delay in plasma

lipoprotein triglycerides clearance allows for cholesterol esters to be passed on from LDL cholesterol and HDL cholesterol to triglyceride-rich particles, making them potentially atherogenic (Cruz et al., 2001).

Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel or ATP III) recommends primary prevention in persons with multiple risk factors (cigarette smoking, hypertension, low HDL cholesterol (< 40 mg/dL), family history of premature CHD (CHD in male first degree relative < 55 years; CHD in female first degree relative < 65 years), age (male ≥ 45 years and female ≥ 55 years), and diabetes. ATP III defined the optimal values of a fasting lipoprotein profile as follows: total cholesterol <200 mg/dL; LDL cholesterol <100 mg/dL; HDL cholesterol between 40 and 60 mg/dL in males and between 50 and 60 mg/dL in females; and triglycerides <150 mg/dL. According to ATP III, persons with 3 of the metabolic risk factors (metabolic syndrome) are candidates for intensive therapeutic lifestyle changes (NCEP, 2001).

One of important component of intensive therapeutic lifestyle change is diet. Reduction of saturated fats (< 7% of total calories) and cholesterol (<200 mg/day) intakes along with a well balanced diet (total fat 25 to 35% of total calories, carbohydrate 50 to 60%, protein 15%) with increased fiber (20 to 30 g/day) is effective in controlling lipid profiles and reducing the risk of CHD and other metabolic disorders. A balanced energy intake and expenditure to

maintain desirable body weight, prevent weight gain or reduce weight by increased physical activity is also essential (NCEP, 2001).

Serum C-Peptide and Serum Insulin

Pancreatic β-cells secrete C-peptide with insulin. Both fasting insulin and C-peptide levels reflect the degree of insulin production (Laakso, 1993; Chen et al., 1999). Olesky (1981) reported that fasting insulin levels are reliable markers of insulin resistance and have been used as a reasonable method for determining insulin resistance in a population study.

However, several authors reported that C-peptide is a more accurate measure of insulin production than fasting insulin. A high C-peptide concentration indicates elevated β-cell production of insulin (Haban et al, 2002), while an elevated insulin concentration may be caused by both excess β-cell production and reduced hepatic clearance (Giugliano et al., 1993). Serum C-peptide is not extracted by the liver, so it better reflects true pancreatic secretion of insulin. In addition, plasma C-peptide, unlike serum insulin, is unaffected by hemolysis (O'Rahilly et al., 1987). According to Chen et al. (1999), serum C-peptide is a better indicator of metabolic syndrome than serum insulin, because serum C-peptide had greater correlation with the recognized markers of

metabolic syndrome independent of insulin. They also found significant interactions of sex and BMI for serum C-peptide, but not for serum insulin. Haban et al. (2002) also reported that serum C-peptide levels constitute a clinically important marker of the cardiovascular risks associated with clusters of known risk factors of metabolic syndrome.

Harris et al. (2002) examined the racial-ethnic differences in fasting insulin and C-peptide concentrations in adults with no prior history of diabetes. They found that non-Hispanic blacks exhibited lower C-peptide values (640.5±12.7 pmol/L) than whites (696.8±9.3 pmol/L) and Mexican Americans (750.5±11.0 pmol/L). The results also showed that non-Hispanic blacks (68.5±1.6 pmol/L) had higher insulin concentrations than whites (59.6±1.6 pmol/L) but not Mexican Americans (70.8±1.4 pmol/L). The study results implied that African Americans' hyperinsulinemia is explained by impaired β-cell function and impaired insulin clearance (probably due to reduced hepatic extraction) in the basal state, despite their peripheral tissue insulin resistance. Thus, using both serum insulin and serum C-peptide helps us understand the pathophysiologic background (portal versus peripheral) of insulin resistance.

Effects of Dietary Intake

Improving insulin sensitivity and correcting/preventing the associated metabolic and cardiovascular abnormalities (hypertension, type 2 diabetes, and

dyslipidemia: high triglyceride; low HDL-cholesterol and smaller & denser LDL particles) that are linked with insulin resistance are critical for treatment and prevention of metabolic syndrome (Riccardi et al., 2000). Since most of the individuals affected by the metabolic syndrome are overweight, particularly with central obesity (Everson et al., 1998), dietary treatment focused on weight reduction with increased physical activities is beneficial and important (Torjesen et al., 1997).

Dietary patterns of individuals are influenced by various aspects, such as cultural and ethnic background, religious and philosophical belief, psychosocial (depression, substance abuse) and sociodemographic (food availability) factors. Changes in nutritional habits by modifications of food intake (quality and quantity) are a foremost challenge (Dickey, et al., 1998). Major lifestyle changes accompanied with weight reducing strategies, such as low-calorie diet, low-fat diet, and regular aerobic exercise, are challenging and hard to adopt and sustain (Costa et al., 2000; Hara et al., 1996; Krentz, 1996).

An Expert Panel of the National Heart, Lung, and Blood Institute (NHLBI) Obesity Education Initiative reported that lower-fat diets with targeted caloric reduction promote greater weight loss than lower-fats diets alone. Reducing dietary carbohydrates along with dietary fat can further facilitate calorie reduction. Physical activity is also recommended as an integral part of weight loss therapy and weight maintenance (Pi-Sunyer et al., 1998). The American Association of Clinical Endocrinologists/American College of Endocrinology

(AACE/ACE) Obesity Task Force emphasized the importance of weight loss maintenance, because it requires a lifelong commitment to a change in lifestyle, behavioral responses, and dietary practices (Dickey et al., 1998).

Maintaining weight loss seems to be more difficult than losing weight, particularly for patients who are treated with caloric restriction. Simple instructions are not sufficient for behavioral changes. Long-term lifestyle changes are necessary to achieve lasting health improvements (Costa et al., 2000; Hara et al., 1996; Krentz, 1996).

Protein has greater thermogenic and satiating effects than does carbohydrate, which may be relevant for the prevention and treatment of obesity if these effects can be maintained over 24 hours (Mikkelsen et al., 2000). Mikkelsen et al. (2000) conducted a randomized, single-blind, 3-way crossover study lasting four days (with a 1-10 week washout period) on twelve young, healthy, overweight and mildly obese (BMI: 26-32) nonsmoking men. The effects of three isoenergetic intervention diets as follows: pork diet (29% of energy as fat and 29% as protein, mainly from pork meat), soy diet (29% of energy as fat and 28% as protein, mainly from soy) , and carbohydrate diet (28% of energy as fat and 11% as protein) were compared. 24-hour energy expenditure was measured in a respiratory chamber at baseline and on day 4 of each intervention period. Daily energy expenditure was 2% higher with the pork than with the soy or carbohydrate diet. In addition, energy intake was 10-15% lower on pork diet, due to a higher satiating effect. According to Skov et al.

(1999), reduced fat diets with protein substituted for carbohydrate showed greater improvements in weight loss. However, a high dietary protein intake is often accompanied by increased saturated fat and cholesterol intakes. Thus, application of these findings to public dietary advice should be done cautiously (Hu et al., 1999; Marshall et al., 1997; Elmstahl et al., 1999).

As demonstrated in the Insulin Resistance Atherosclerosis Study, the relationship between dietary factors and insulin sensitivity is complex and controversial. In obese subjects with a sedentary lifestyle, a high intake of dietary fats (40% of total energy) was associated with worsened insulin sensitivity, but not in non-obese subjects (Mayer-Davis et al., 1997; Hauner, 2002). Glucose and lipid metabolism were strongly related. A high saturated fat intake (16% of total energy) was linked with glucose intolerance and other metabolic disturbances such as elevated total and LDL-cholesterol. On the other hand, low-fat, high-carbohydrate diets reduce LDL concentrations when saturated or trans fats are replaced with carbohydrates, but these diets are also associated with an elevation of fasting triglycerides and a decrease in HDL-cholesterol in both normal individuals and subjects with type 2 diabetes (Hauner, 2002; Reaven, 1997; Hu et al., 1999; Mensink et al., 1992; Elmstahl et al., 1999).

Several researchers examined the association between fatty acid composition in serum and insulin sensitivity. A high insulin sensitivity in humans was associated with low proportions of palmitic acids (16:0) and palmitoleic acids (16:1 n-7), and high proportion of linoleic acids (18:2 n-6), which are mainly

found in plant foods. The proportions of γ-linolenic acids (18:3 n-6) and dihomo-γ-linolenic acids (20:3 n-6), which are metabolites of linoleic acid in the insulin sensitive subjects, were low. The changes in the fatty acid pattern among insulin resistant or diabetic subjects indicated that they may have had an altered dietary fat composition, compared to healthy people (Hauner, 2002; Vessby, 2000). Increased intake of saturated fat, monounsaturated fat and linolenic acids appeared to be associated with hyperinsulinemia, while polyunsaturated fatty acids and linoleic acid were not (Marshall et al., 1997). Replacing saturated fat with monounsaturated or polyunsaturated fat, or both, has some metabolic benefits along with a fall in LDL-cholesterol. A high monounsaturated fat diet significantly improved insulin sensitivity compared to a high-saturated-fat diet. However this beneficial effect of monounsaturated fat disappeared in individuals whose total fat intake exceeded 38% of total energy (Hauner, 2002; Vessby et al., 1999). A reduction of fat intake (monounsaturated fat) counterbalanced by an increased consumption of starchy foods was also reported to slightly worsen insulin sensitivity (Garg et al., 1988 & 1994; Coulston et al., 1987 & 1989). In short, many features of the metabolic syndrome are worsened by increasing dietary carbohydrate (Riccardi et al., 2000). Hauner (2002) suggests that it is the quality rather than the total amount of fat that really matters.

Based on physiologic, epidemiologic, and clinical evidence, the low-fat, high-carbohydrate diet is not appropriate for the insulin resistant and/or hyperinsulinemic patient. In the insulin resistant patient, a high-carbohydrate

diet produces a greater insulin response to glucose and plasma insulin level (Hollenbeck et al., 1991). A high-carbohydrate diet may not improve insulin sensitivity and has potentially unfavorable effects on lipoprotein metabolism (Garg et al., 1994; Reaven, 1997). For patients with diabetes, high-carbohydrate diets may contribute to deterioration of glycemic control, accentuation of hyperinsulinemia, and increased plasma VLDL-cholesterol and triglycerides levels (Wangerin-Lile et al., 2000; Garg et al., 1994; Reaven, 1997).

Some researchers suggest that the most appropriate diet for insulin resistant/hyperinsulinemic individuals is adequate (not high) protein, moderate complex carbohydrate, minimal amounts of refined carbohydrate, and healthy fat, such as monounsaturated fatty acids. According to Wangerin-Lile et al. (2000), this type of diet could be readily integrated into the lifestyle of patients with insulin resistance or type 2 diabetes with clinically significant improvements in fasting insulin levels, $HbA1_c$, triglycerides, and triglycerides/HDL-cholesterol ratio. A high intake of low glycemic index foods, such as foods containing soluble fibers, was not only associated with an improvement in insulin sensitivity, but also an improvement in other disturbances characteristic of the metabolic syndrome (Hauner, 2002; Jenkins et al., 2000).

Several studies investigated the relationship between dietary protein intake and lipid profiles and the risk of related metabolic disorders. Exchange of animal protein for carbohydrates in human diets significantly reduced LDL-cholesterol and triacylglyceride concentrations and increased HDL-cholesterol

However, the consumption of very lean meats as part of low fat diets has resulted in reduced blood lipid concentrations (Davidson et al., 1999). Scott et al. (1994) examined the comparability of lean beef and chicken in a Step I diet (8 to 10% of energy intake from saturated fatty acids) and concluded that lean beef and chicken were interchangeable in the Step I Diet, since they had similar effects on plasma levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride. Bales et al. (1995) found that very lean red meats such as lean pork, with a fat content 35 to 61% lower than traditional pork, can be used as successfully as chicken in reduced-fat diets which contain substantial amounts of meat. A total of 51 subjects were randomly assigned to either a skinless chicken or lean pork diet, both providing 25% of calories as fat (calorie levels were adjusted to avoid weight loss or gain) for 28 days. Serum lipids were measured at baseline and the end of the study. Both diets reduced total cholesterol and LDL cholesterol. HDL cholesterol was reduced in the skinless chicken group, but not in lean pork group. The incorporation of lean red meats can thus be used to enhance dietary variety and nutritional completeness for individuals seeking to improve their blood lipids.

Therefore, animal protein from lean meat should be recommended for prevention and treatment of insulin resistance and metabolic syndrome. Foods containing animal protein are consumed by most adults in the US population. Eating more protein may enhance the successful compliance to diet therapy for insulin resistance by improving glycemic control without elevating lipid levels.

CHAPTER III

METHODOLOGY

The objective of this study was to examine the relationship of protein intake with lipoprotein profiles (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) and glycemic profiles (fasting and postprandial glucose and insulin, C-peptide, and hemoglobin A1c) in people who are at risk of insulin resistance. This study analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III).

Study Population and Design

The National Center for Health Statistics (NCHS) of the Centers for Disease Control conducted a cross-sectional survey, NHANES III from 1988 through 1994 (NCHS 1994). NHANES III was designed to provide nationally representative reference data and prevalence estimates for numerous nutrition, health status, and health condition measures. Complex, stratified, multistage probability cluster sampling was used to select a representative sample of the civilian noninstitutionalized population of the United States aged 2 months and

older. NHANES III oversampled Mexican-Americans, African-Americans, children aged 2 months to 5 years old, and adults aged 60 years and older to provide representative data from these population subgroups. Socioeconomic, demographic, health behavior, lifestyle, personal and family health, and food frequency data were collected via extensive questionnaires administered at home by health interviewers to 33,994 participants from 19,528 households throughout the United States.

Additional health, 24-hour dietary recall and laboratory data were collected from 30,818 of the participants by health professionals during visits to NHANES mobile examination centers (MEC) at 89 sites. Participants who were unable for health reasons or unwilling to attend the examination centers were offered a limited home examination. The survey procedures were approved by the NCHS Internal Review Board, and all participants signed informed consent forms. Details of the plan and operation of NHANES III and laboratory procedures used for the NHANES III have been published (NCHS 1994).

Study Sample

For this study, we selected participants who met at least one of the identified criteria (Table 1). According to literature adults who are obese, have a family history of diabetes mellitus, high waist circumference, impaired fasting glucose, or mildly elevated triglycerides are at high risk of being insulin resistant.

We selected obese males with a body mass index ≧27.8 kg/m^2 or waist circumference >102cm; and obese females with a body mass index ≧27.3 kg/m^2 or waist circumference >97cm. (Dickey et al., 1998) We used the blood glucose concentrations above 6.1 mmol/L as an indicator of impaired fasting glucose according to the American Diabetes Association criteria (Unwin N et al., 1998). Serum triglycerides concentrations between 150 and 499 mg/dL were selected as mildly elevated according to National Cholesterol Education Program's Adult Treatment Panel III (NCEP, 2001). We excluded adults who reported being told they had diabetes, took insulin or oral hypoglycemic agents or had high serum triglycerides concentrations (≧500mg/dL).

The initial sample for our analyses included 14788 people. From this initial sample, we excluded 240 participants who had no food frequency data. We excluded an additional 754 participants whose 24-hour dietary recalls were not reliable and complete. The final sample size for analysis included 13794 adults (7245 women and 6549 men).

Measurement of Diet

Dietary data were collected using two instruments: a single 24-hour dietary recall and a 1-month qualitative 60-item food frequency questionnaire (FFQ). In NHANES III, 24-hour dietary recalls were used as the principal methodology to obtain quantitative information on food and nutrient intakes of

the US population. Nutrient intakes for each participant were calculated using the gram amounts of the food consumed. The USDA Survey Nutrient Database and The University of Minnesota Nutrition Coordinating Center nutrient database were used for the dietary nutrient intake analysis. Results in this study are reported for nutrient data analyzed using the University of Minnesota database.

The 24-hour dietary recall was administered at the MEC using an automated, interactive interview and coding system that featured a standardized interview format and automated probes to obtain detailed information about all foods and beverages consumed the previous day, including brand names, food preparation methods and ingredients used in food preparation methods (NCHS 1994). Portion sizes were quantified using abstract food models, shape charts and measuring aids such as rulers, cups, and spoons. Seasoning added to prepared foods at the table, nutrients from dietary supplements and medications were not included. Each individual's intake of energy, fat, fiber, protein, vitamins and minerals was estimated from their 24-hour dietary recall. Dietary recalls were collected on every day of the week; weekend days are underrepresented, whereas Fridays are overrepresented. For further details of these procedures see the description by Briefel et al. (1997).

The FFQ was administered during the household interview and asked the average number of times foods were eaten during the 1-month period preceding the respondent's interview date. Frequencies of specific types of foods from the following designated food groups and subgroups were ascertained: milk and milk

products, meat and meat dishes, eggs and egg dishes, fruits and fruit juices (including citrus fruits and fruit juices), vegetables (including dark green leafy vegetables, deep orange and yellow vegetables and white potatoes), grains and legumes (including cereals, breads, legumes and salty snacks), desserts and sweets, beverages (including nonalcoholic and alcoholic beverages) and added fats. The NHANES III FFQ did not include information about portion size and cannot be used to estimate nutrient intakes. However, this method of dietary assessment is appropriate for comparing frequencies of food intakes between groups of individuals (Thompson et al., 1994).

Protein intake was estimated by 24-hour dietary recall data. Participants were divided into quartiles by two classification methods: grams of total dietary protein and grams of animal protein from the 24-hour recall (See Table 2).

Measurement of Lipid and Glycemic Profiles

Blood was collected from participants in the MEC through venipuncture using standard protocols. Several blood components were analyzed for NHANES III. Concentrations of serum cholesterol, serum triglycerides, serum HDL cholesterol, serum glucose, serum C-peptide, serum insulin, plasma glucose, and glycated hemoglobin were measured in this study. All participants were instructed to fast at least 8.5 hours if examined in the morning or at least 6 hours if examined in the afternoon (NCHS 1994). The following assay

methods/instrumentations were used: for serum glucose, Hitachi 737 Analyzer (Thermo Trace Automated Colorimetric Lithium test)/Boehringer-Mannheim Diagnostics; for plasma glucose, Hexokinase System/Roche COBAS MIRA Chem System; for serum cholesterol and triglycerides, Hitachi 704 Analyzer (Thermo Trace Automated Colorimetric Lithium test)/Boehringer-Mannheim Diagnostics; for serum insulin, Insulin Radioimmunoassay Kit/Pharmacia Diagnostics; for C-peptide, Radioimmunoassay/Novo BioLabs; and for Glycated hemoglobin, DIAMAT high-pressure liquid chromatography/Bio-Rad Laboratories. Values for serum LDL cholesterol were calculated by the Friedewald equation (Friedewald et al., 1972).

Blood samples for NHANES III were collected at the MEC and analyzed by the designated laboratories. Detailed information about the procedures and quality control protocols used for the measurement of these serum lipid and glycemic profiles are provided in the NHANES III documentation (NCHS 1994, 1996) and in the Laboratory Procedures used for NHANES III (Gunter et al. 1990).

Measurement of Covariates

Dietary and biochemical data vary by sociodemographic and behavioral characteristics. To determine whether protein intake was independently associated with differences in lipoproteins or indicators of glycemic control, we

adjusted for several potentially confounding variables in our analyses. Self-reported gender and age were collected during the household interview. Other confounding variables included clinically measured BMI (kg/m2); waist circumference; dietary fat and carbohydrate intake; and the number of risk factors (Table 1: inclusion criteria). All body measurements were taken using standard anthropometric protocols (NCHS, 1988).

Behaviors, including alcohol consumption and leisure-time physical activities were determined from questions asked during the household interview or at the MEC. Alcohol consumption was estimated from the 1-month FFQ. We summed the total number of alcohol drinks (beer, wine, and hard liquor) consumed in the past month to estimate alcohol consumption. Respondents were also asked about specific leisure-time physical activities (walking, jogging/running, bicycling, swimming, aerobics/dancing, calisthenics, gardening, and lifting weights) and their frequency and intensity during the past month. We estimated metabolic equivalents (METs) by multiplying frequency by intensity (See Table 3) and summing all measures of energy expenditure reported by each respondent in the past month.

Statistical Analysis

First, we compared histograms of data to the normal curve. Biochemical data were not normally distributed. In order to improve normality of data, we

used SPSS to convert BMI, biochemical, and dietary variables to a log scale. Total protein and animal protein quartiles were calculated.

We used SUDAAN (release 7.5.6, 2000, Research Triangle Institute, Research Triangle Park, NC), a computer program that takes into account the complex, stratified, multistage survey design and sample weights of NHANES III (Shah et al., 1997) to analyze the data. The association between protein intake and lipid and glycemic profiles for crude values and adjusted values were tested with analysis of variance and analysis of covariance followed by the Scheffe's multiple comparisons test to determine differences between quartiles of protein intake. To adjust for their known confounding effects, age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors were entered into the analysis as covariates. Pearson correlation coefficients were calculated to determine bivariate relationships between glycemic and lipid profiles and age, sex, race-ethnicity, BMI, waist circumference, physical activity, dietary intake (protein, total fat, total saturated fatty acids, cholesterol, carbohydrate, and total dietary fiber), family history, and number of risk factors. P values of <0.05 indicated statistical significance.

Research Hypotheses

Hypothesis One

(a) Null Hypothesis – Glycemic profiles (glucose, insulin, C-peptide, and hemoglobin A1c) are not associated with quartiles of total and animal protein intake in adults at risk of insulin resistance

(b) Research Hypothesis – Glycemic profiles are associated with quartiles of total and animal protein intake in adults at risk of insulin resistance

(c) Sub Hypothesis – Glycemic profiles are positively associated with quartiles of total and animal protein intake in adults at risk of insulin resistance

(d). Sub Hypothesis – Glycemic profiles are negatively associated with protein intake in adults at risk of insulin resistance

Statistical analysis Analyses of covariance were used to determine the relationship between glycemic profiles and protein intake in adults at risk of insulin resistance after adjusting for sex, age, BMI, waist circumference, physical activity, fat, carbohydrate, and alcohol intake, and number of risk factors.

Hypothesis Two

(a) Null Hypothesis – Lipoprotein profiles (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) are not associated with quartiles of total and animal protein intake in adults at risk of insulin resistance

(b) Research Hypothesis – Lipoprotein profiles are associated with quartiles of total and animal protein intake in adults at risk of insulin resistance

(c) Sub Hypothesis – Lipoprotein profiles are positively associated with quartiles of total and animal protein intake in adults at risk of insulin resistance

Statistical analysis Analyses of covariance were used to determine the relationship between lipoprotein profiles and protein intake in adults at risk of insulin resistance after adjusting for sex, age, BMI, waist circumference, physical activity, fat, carbohydrate, and alcohol intake, and number of risk factors.

Table 1 Inclusion and Exclusion Criteria

Inclusion Criteria	Value Indicating Risk	Source of Value
Body Mass Index (kg/m^2)	♂:≧27.8; ♀:≧27.3	Dickey RA, Bray GA, Bartuska DG, et al. AACE/ACE position statement on the prevention, diagnosis, and treatment of obesity. *Endocrine Practice.* 1998;4:297-330.
Waist Circumference	♂:>102cm; ♀:>97cm	Dickey RA, Bray GA, Bartuska DG, et al. AACE/ACE position statement on the prevention, diagnosis, and treatment of obesity. *Endocrine Practice.* 1998;4:297-330.
Family History	Type 2 Diabetes Mellitus	Wangerin-Lile D, Goar SL. Insulin resistance and hyperinsulinemia: recognizing the risk and reversing the process. *Physician Assistant.* 2000;24:23-31.
Blood Glucose (mmol/L)	>6.1	Unwin N, Alberti KGMM, Bhopal R, et al. Comparison of the current WHO and new ADA criteria for the diagnosis of diabetes mellitus in three ethnic groups in the UK. *Diabetic Medicine.* 1998;15:554-557.
Serum Triglycerides (mg/dL)	150 to 499mg/dL	Third report of the National Cholesterol Education program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in Adults (Adult Treatment Panel III)

Exclusion Criteria	Value Indicating Risk	Source of Value
Diabetes Mellitus	Reported diagnosis of Diabetes Mellitus, or taking insulin or oral hypoglycemic agents	Unwin N, Alberti KGMM, Bhopal R, et al. Comparison of the current WHO and new ADA criteria for the diagnosis of diabetes mellitus in three ethnic groups in the UK. *Diabetic Medicine.* 1998;15:554-557. Peters AL, Davidson MB, Schriger DL, Hasselblad V. A clinical approach for the diagnosis of diabetes mellitus. *JAMA.* 1996;276:1246-1252. Feskens EJM, Stengard J, Virtanen SM, et al. Dietary factors determining diabetes and impaired glucose tolerance. *Diabetes Care.* 1995;18:1104-1112.
Severely Elevated Serum Triglycerides (mg/dL)	≧500mg/dL	Third report of the National Cholesterol Education program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in Adults (Adult Treatment Panel III)

Table 2 Total protein and animal protein intake by quartiles

	<25		25-50		50-75		>75	
Variables	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Total Protein (gm)	36.01	0.21	60.08	0.13	83.70	0.19	139.10	1.35
Male	38.20	0.58	60.81	0.22	84.69	0.28	143.42	1.59
Female	35.33	0.25	59.65	0.16	82.69	0.28	125.43	1.45
Animal Protein (gm)	19.27	0.14	38.03	0.10	57.02	0.18	103.71	1.00
Male	20.41	0.39	38.68	0.17	57.69	0.28	107.66	1.25
Female	18.83	0.14	37.60	0.14	56.38	0.26	93.03	1.23

Table 3 Metabolic equivalents provided by various phsical activities (adapted from Physical Activity and Health: A Report of the Surgeon General, 1996)

Intensity	Activity	METs[1]
Moderate	Volleyball, noncompetitive	3.0
Moderate	Walking, moderate pace (3 mph, 20 min/mile)	3.5
Moderate	Walking, brisk pace (4 mph, 15 min/mile)	4.0
Moderate	Table tennis	4.0
Moderate	Social dancing	4.5
Moderate	Lawn mowing (powered push mower)	4.5
Hard	Jogging (5 mph, 12 min/mile)	7.0
Hard	Field hockey	8.0
Very hard	Running (6 mph, 10 min/mile)	10.0

[1]METs=Metabolic equivalents

CHAPTER IV

RESULTS

The objective of this study was to examine the relationship of protein intake with lipoprotein and glycemic profiles in adults who were at risk of insulin resistance. Data were obtained from NHANES III (1988-1994) conducted by NCHS as described in chapter III.

Sample Size and Characteristics

NHANES III (1988-1994) data were obtained from 33,994 participants from 19,528 households throughout the United States. This study included 13,794 people (7254 women and 6549 men) who had at least one risk factor for insulin resistance (Table 1) after excluding adults who had been diagnosed with diabetes, reported taking insulin or oral hypoglycemic agents, or had very high triglyceride concentrations. The average number of risk factors per participant was 1.7.

Risk of insulin resistance was assessed by BMI, waist circumference, serum triglycerides, blood glucose or family history of diabetes (Table 4 and 5,

Figures 1 and 2). About half the adults exhibited only one risk factor and about 40% exhibited two risk factors. Few people (2.2%) exhibited more than four risk factors. A high BMI was most common risk factor (71.3%) for both men and women. Family history was the second most common risk factor for women, whereas serum triglycerides was the second risk factor for men.

Table 6 lists the demographic characteristics of the sample population. Participants ages ranged from 17 to 90 years old. The average age of participants was 43 years. The average BMI of participants was 26 kg/m^2 and the average waist circumference was 90 cm. Participants reported average total METs of 16 (with 0 indicating no leisure time physical activity and 10 indicating one very hard intensity activity such as running at 6 mph once during the month) (See Table 3) and an alcohol consumption of 9 drinks per month. The average dietary intake of participants (% of total energy) was: total fat 35.5%; protein 14.9%; animal protein 10.1%; and carbohydrate 49.7%. Average protein intake based on 24 hour recalls in this sample was 79.4 ± 44.7 gm/day (1.3 g/Kg).

When assessed separately by gender, the average age of women was slightly higher than men's age and men had larger waist circumferences than women, though physical activity (total METs) reported by both genders were similar (Table 7). Men consumed about twice as much alcohol per month as women. Men consumed more fat, total protein, animal protein, carbohydrate, and alcohol than women, although intake was similar when expressed as percent of total energy intake: total fat, male 35.9%, female 34.7%; protein, male

15.1%, female, 14.7%; animal protein, male 10.5%, female 9.9%; and carbohydrate, male 48.9%, female 50.6%.

Lipid and Glycemic Profiles

Tables 8 & 8A present the average lipid and glycemic values for adults at risk for insulin resistance. Referring to Adult Treatment Panel III (NCEP 2001) and the American Diabetes Association criteria (Unwin et al., 1998), the average total cholesterol concentrations were borderline high and LDL cholesterol were near optimal/above optimal. Average HDL cholesterol, triglycerides, and glycemic values were within the normal range. Average values were similar for both genders (Table 9 & 9A). Women had slightly higher HDL cholesterol than men. Men exhibited slightly higher triglycerides than women.

Lipid and Glycemic Profiles and Descriptive Variables

Table 10 presents the Pearson's correlation coefficients between lipid and glycemic profiles and descriptive variables. Sex, age, BMI, waist circumference, and number of risk factors were correlated significantly with all lipid and glycemic profiles. Physical activity was correlated only with HDL cholesterol. Sex (1=male, 2=female) was negatively correlated with LDL cholesterol, triglycerides, serum glucose, plasma glucose, and glycated hemoglobin, indicating that females had

lower concentrations for these measures. Age was strongly positively correlated with all lipid and glycemic profiles, except HDL cholesterol which was weakly correlated with age. Other strong positive correlations were observed between BMI, waist circumference, and number of risk factors and lipid and glycemic profiles (except HDL which was negatively correlated with BMI and waist circumference).

Lipid and Glycemic Profiles and Dietary Variables

Table 11 presents the correlations between lipid and glycemic profiles and dietary variables. Macronutrient intakes were weakly correlated with lipid and glycemic laboratory values. Total protein intake was negatively correlated with total cholesterol, HDL cholesterol, and glycated hemoglobin. Animal protein intake was negatively correlated with total cholesterol and HDL cholesterol. Fat intake was negatively correlated with total cholesterol, HDL cholesterol, triglycerides, plasma glucose, and glycated hemoglobin. Saturated fat intake was negatively correlated with total cholesterol, HDL cholesterol, and glycated hemoglobin. Carbohydrate intake was negatively correlated with all lipid and glycemic profiles except serum glucose, plasma glucose, and serum insulin. Dietary fiber was positively correlated with triglycerides and serum glucose, and negatively correlated with HDL cholesterol, serum insulin, and serum C-peptide. HDL cholesterol was negatively correlated with all dietary variables.

Lipid and Glycemic Profiles by Protein Quartiles

Tables 12 & 12A and 13 & 13A present the association between lipid and glycemic profiles and total and animal protein intake by quartiles. When adjusted for age, sex, BMI, waist circumference, life style factors (physical activity and alcohol consumption), dietary factors (fat and carbohydrate intake), and number of risk factors, total cholesterol, HDL cholesterol, serum insulin, and serum C-peptide were significantly different by total protein quartile ($p<0.05$) and total cholesterol, HDL cholesterol, and serum insulin were significantly different by animal protein quartile ($p<0.05$). For both total cholesterol and HDL cholesterol, average values were higher in the lowest protein quartile compared to the highest protein quartile. However, serum insulin was lowest in the second quartile of both total and animal protein and serum c-peptide was lower in the second and highest quartiles of total protein.

Table 14 & 14A presents the association between lipid and glycemic profiles and total protein quartiles by gender. When adjusted for age, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary factors (fat and carbohydrate intake), and number of risk factors, total cholesterol for men and serum C-peptide for women exhibited significant differences by total protein quartiles ($p<0.05$). Males in the second highest total protein quartile (3^{rd} quartile) exhibited slightly higher total cholesterol levels than

other quartiles ($P<0.04$). Although overall statistical significance was exhibited, no quartile differences were observed in serum C-peptide levels of female by total protein quartiles.

Table 15 & 15A presents the association between lipid and glycemic profiles and animal protein quartiles by gender. When adjusted for age, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary factors (fat and carbohydrate intake), and number of risk factors, total cholesterol for both men and women, and serum insulin for men were significantly different ($p<0.05$). As exhibited in total and animal protein quartiles, men in second highest total protein quartile (3^{rd} quartile) had slightly higher total cholesterol levels than men in other quartiles. Women in the lowest quartile (1^{st} quartile) and the highest quartile (4^{th} quartile) as well as the second highest quartile (3^{rd} quartile) exhibited differences in total protein. Also women in the second lowest quartile (2^{nd} quartile) and the highest quartile (4^{th} quartile) exhibited differences. Notably, total cholesterol levels decreased as animal protein intake increased from lowest to highest in women. Men in the second lowest animal protein quartiles (2^{nd} quartile) exhibited slightly lower serum insulin levels than the rest of the quartiles.

Table 4 Number of risk factors for insulin resistance exhibited by people who have valid food intake record

Number of Risk Factor	No.[1]	%
1	6287	47.6
2	5301	38.0
3	1855	12.2
4	335	2.1
5	16	0.1

[1]No.=unweighted sample size

Table 5 Percentage of people who exhibited specific risk factors for insulin resistance

	%
Body Mass Index	71.3
Waist Circumference	28.0
Family History	33.1
Blood Glucose	7.1
Serum Triglycerides	29.6

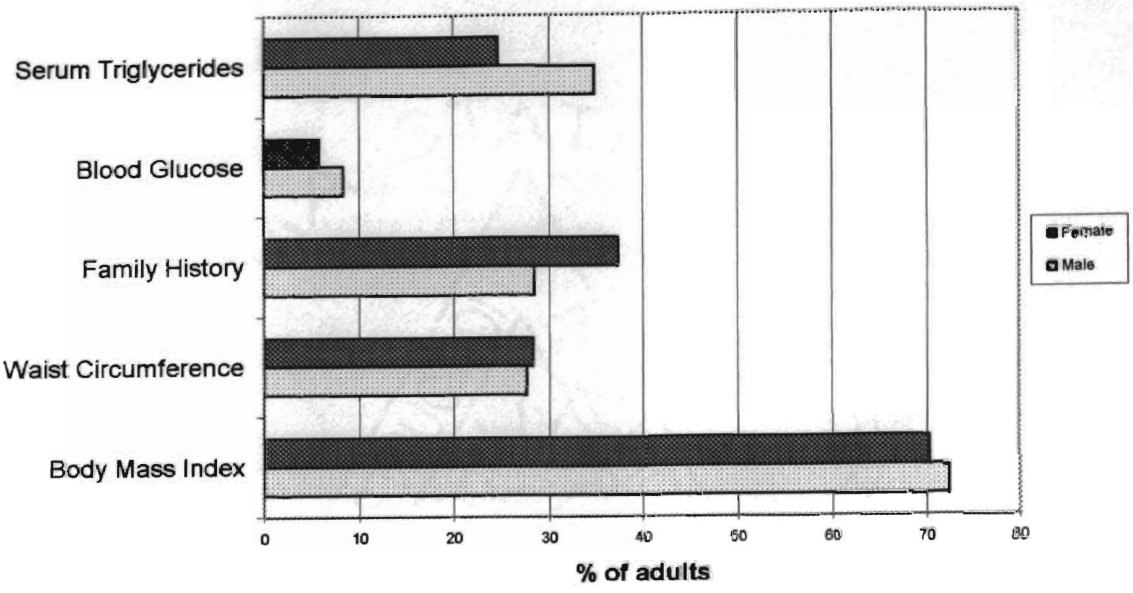


Figure 1. Proportion of males & females who exhibited risk factors for insulin resistance

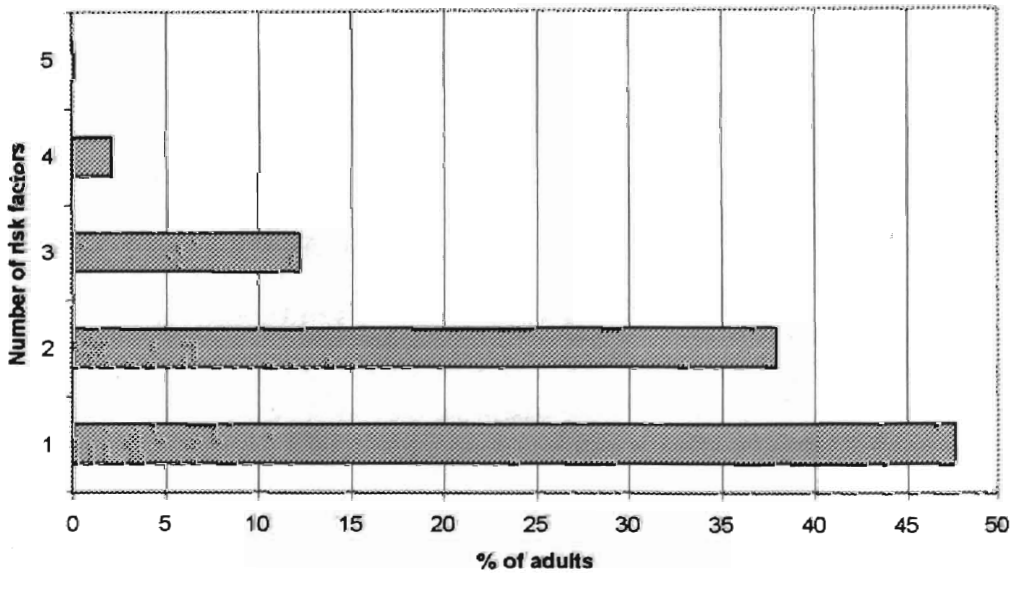


Figure 2. Proportion of subjects who exhibited different numbers of risk factors for insulin resistance

Table 6 Characteristics and Dietary Intake of the Study Population

Characteristics	No.[1]	Mean	S.E.
Age (Years)	13794	42.58	0.11
Body Mass Index (kg/m^2)	13773	25.85	0.13
Waist Circumference (cm)	13269	90.45	0.24
Physical Activity Score (METs[2])	13794	15.85	2.14
Total Fat Intake (gm)	13794	86.88	0.92
Protein Intake (gm)	13794	82.45	0.71
Animal Protein Intake (gm)	13794	56.42	0.60
Carbohydrate Intake (gm)	13794	273.59	2.05
Alcohol (drinks/month)	13790	8.75	0.29
Risk Factor (no.)	13794	1.69	0.01

[1]No.=unweighted sample size
[2]METs=metabolic equivalents

Table 7 Characteristics and Dietary Intake of the Study Population by Gender

	Male			Female		
Characteristics	No.[1]	Mean	S.E.	No.[1]	Mean	S.E.
Age (Years)	6549	41.67	0.15	7245	43.43	0.14
Body Mass Index (kg/m^2)	6543	26.19	0.10	7230	25.85	0.13
Waist Circumference (cm)	6324	93.91	0.25	6945	87.23	0.33
Physical Activity Score (METs[2])	6549	19.87	3.97	7245	12.13	1.11
Total Fat Intake (gm)	6549	105.93	1.59	7245	69.16	0.70
Total Protein Intake (gm)	6549	100.39	1.11	7245	65.77	0.66
Animal Protein Intake (gm)	6549	69.54	0.92	7245	44.23	0.55
Carbohydrate Intake (gm)	6549	324.30	3.24	7245	226.43	1.89
Alcohol (drinks/month)	6548	12.51	0.46	7242	5.26	0.28
Risk Factors (no.)	6549	1.72	0.02	7245	1.66	0.01

[1]No.=unweighted sample size
[2]METs=metabolic equivalents

Table 8 Average lipid and glycemic values for adults at risk for insulin resistance

	No.[1]	Mean	S.E.
Total Cholesterol (mg/dL)	13793	200.99	0.70
LDL Cholesterol (mg/dL)	13764	123.98	0.62
HDL Cholesterol (mg/dL)	13765	51.06	0.25
Triglycerides (mg/dL)	13794	130.08	1.64
Serum Glucose (mg/dL)	13792	92.33	0.28
Plasma Glucose (mg/dL)	12819	94.47	0.24
Serum Insulin (uU/mL)	12784	10.03	0.18
Serum C-peptide (pmol/mL)	12825	0.67	0.01
Glycated Hemoglobin (%)	13728	5.22	0.01

[1]No.=unweighted sample size

Table 8 A Average lipid and glycemic values (expressed as SI units) for adults at risk for insulin resistance

	No.[1]	Mean	S.E.
Total Cholesterol (mmol/L)	13793	5.23	0.02
LDL Cholesterol (mmol/L)	13764	3.22	0.02
HDL Cholesterol (mmol/L)	13765	1.33	0.01
Triglycerides (mmol/L)	13794	1.47	0.02
Serum Glucose (mmol/L)	13792	5.31	0.02
Plasma Glucose (mmol/L)	12819	5.24	0.01
Serum Insulin (pmol/L)	12784	60.17	1.06
Serum C-peptide (nmol/L)	12825	0.67	0.01
Glycated Hemoglobin (%)	13728	5.22	0.01

[1]No.=unweighted sample size

Table 9 Average lipid and glycemic values for adults at risk for insulin resistance by gender

	Male			Female		
Characteristics	No.[1]	Mean	S.E.	No.[1]	Mean	S.E.
Total Cholesterol (mg/dL)	6549	199.10	0.78	7244	202.76	0.85
LDL Cholesterol (mg/dL)	6530	124.97	0.62	7234	123.06	0.78
HDL Cholesterol (mg/dL)	6530	46.18	0.30	7235	55.59	0.33
Triglycerides (mg/dL)	6549	140.36	2.38	7245	120.51	1.63
Serum Glucose (mg/dL)	6548	94.31	0.28	7244	90.49	0.35
Plasma Glucose (mg/dL)	6090	96.51	0.28	6729	92.58	0.27
Serum Insulin (uU/mL)	6068	10.32	0.22	6716	9.76	0.17
Serum C-peptide (pmol/mL)	6092	0.69	0.01	6733	0.65	0.01
Glycated Hemoglobin (%)	6516	5.27	0.01	7212	5.17	0.01

[1]No.=unweighted sample size

Table 9 A Average lipid and glycemic values (expressed as SI units) for adults at risk for insulin resistance by gender

	Male			Female		
Characteristics	No.[1]	Mean	S.E.	No.[1]	Mean	S.E.
Total Cholesterol (mmol/L)	6549	5.18	0.02	7244	5.27	0.02
LDL Cholesterol (mmol/L)	6530	3.25	0.02	7234	3.20	0.02
HDL Cholesterol (mmol/L)	6530	1.20	0.01	7235	1.45	0.01
Triglycerides (mmol/L)	6549	1.59	0.03	7245	1.36	0.02
Serum Glucose (mmol/L)	6548	5.23	0.02	7244	5.02	0.02
Plasma Glucose (mmol/L)	6090	5.36	0.02	6729	5.14	0.02
Serum Insulin (pmol/L)	6068	61.93	1.31	6716	58.54	1.02
Serum C-peptide (nmol/L)	6092	0.69	0.01	6733	0.65	0.01
Glycated Hemoglobin (%)	6516	5.27	0.01	7212	5.17	0.01

[1]No.=unweighted sample size

Table 10 Correlation between lipid and glycemic profiles and descriptive variables[1]

Variables	Sex[2]	Age	Physical Activity[3]	BMI[4]	Waist circumference	Number of Risk Factors
Total Cholesterol	0.039*	0.403*	0.001	0.242*	0.301*	0.265*
LDL Cholesterol	-0.065*	0.315*	-0.053	0.246*	0.313*	0.171*
HDL Cholesterol	0.322*	0.025*	-0.013*	-0.283*	-0.343*	-0.241*
Triglycerides	-0.132*	0.261*	0.013	0.381*	0.299*	0.524*
Serum Glucose	-0.151*	0.326*	-0.003	0.259*	0.325*	0.354*
Plasma Glucose	-0.162*	0.314*	0.001	0.245*	0.320*	0.351*
Serum Insulin	0.038*	0.091*	-0.017	0.578*	0.562*	0.349*
Serum C-peptide	0.038*	0.245*	-0.000	0.559*	0.593*	0.384*
Glycated Hemoglobin	-0.085*	0.390*	-0.007	0.237*	0.291*	0.263*

[1]Values reported as Pearson-product-moment correlation coefficient r, * indicates significance ($p<.05$)
[2]1=Male, 2=Female
[3]METs=metabolic equivalents
[4]BMI=body mass index wt^{kg}/ht^{m2}

Table 11 Correlation between lipid and glycemic profiles and dietary variables[1]

Variables	Total Protein	Animal Protein	Fat	Saturated Fat	Carbohydrate	Dietary fiber
Total Cholesterol	-0.051*	-0.034*	-0.067*	-0.063*	-0.119*	-0.005
LDL Cholesterol	-0.005	-0.012	-0.004	-0.000	-0.046*	-0.002
HDL Cholesterol	-0.093*	-0.073*	-0.086*	-0.088*	-0.157*	-0.026*
Triglycerides	-0.010	-0.007	-0.030*	-0.022	-0.038*	0.051*
Serum Glucose	-0.005	-0.001	-0.022	-0.018	-0.010	0.027*
Plasma Glucose	-0.000	-0.005	-0.029*	-0.017	-0.019	0.012
Serum Insulin	-0.004	-0.019	-0.003	-0.014	-0.009	-0.063*
Serum C-peptide	-0.017	-0.010	-0.005	-0.015	-0.049*	-0.052*
Glycated Hemoglobin	-0.020*	-0.010	-0.040*	-0.038*	-0.045*	-0.009

[1]Values reported as Pearson-product-moment correlation coefficient r, * indicates significance ($p<.05$)

Table 12 Differences in lipid and glycemic profiles by total protein quartiles[1-2]

	<25		25-50		50-75		75-100		
Variables	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	P
Total Cholesterol (mg/dL)	204.85[a]	1.12	201.01[b]	0.87	201.69[ab]	1.18	197.18[c]	1.03	<0.0067
LDL Cholesterol (mg/dL)	126.60	1.01	122.95	1.00	124.70	1.08	122.09	0.80	<0.3286
HDL Cholesterol (mg/dL)	52.31[a]	0.45	52.67[a]	0.41	50.86[b]	0.42	48.79[c]	0.32	<0.0263
Triglycerides (mg/dL)	129.73	2.42	127.37	2.49	130.87	2.01	132.03	2.33	<0.2161
Serum Glucose (mg/dL)	92.41	0.39	92.36	0.39	92.19	0.41	92.37	0.47	<0.9466
Plasma Glucose (mg/dL)	94.74	0.41	94.39	0.29	94.20	0.41	94.59	0.38	<0.5716
Serum Insulin (uU/mL)	10.32[a]	0.30	9.57[b]	0.19	10.01[a]	0.25	10.23[a]	0.25	<0.0117
Serum C-peptide (pmol/mL)	0.70[a]	0.01	0.65[b]	0.01	0.67[a]	0.01	0.66[b]	0.01	<0.0042
Glycated Hemoglobin (%)	5.23	0.02	5.23	0.02	5.22	0.01	5.20	0.02	<0.8569

[1]Means in a row with different superscripts are significantly different ($p<.05$)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

Table 12 A Differences in lipid and glycemic profiles (expressed in SI units) by total protein quartiles[1-2]

	<25		25-50		50-75		75-100		
Variables	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	P
Total Cholesterol (mmol/L)	5.33[a]	0.03	5.23[b]	0.02	5.24[ab]	0.03	5.13[c]	0.03	<0.0067
LDL Cholesterol (mmol/L)	3.29	0.03	3.20	0.03	3.24	0.03	3.17	0.02	<0.3286
HDL Cholesterol (mmol/L)	1.36[a]	0.01	1.37[a]	0.01	1.32[b]	0.01	1.27[c]	0.01	<0.0263
Triglycerides (mmol/L)	1.47	0.03	1.44	0.03	1.48	0.02	1.49	0.03	<0.2161
Serum Glucose (mmol/L)	5.13	0.02	5.13	0.02	5.12	0.02	5.13	0.03	<0.9538
Plasma Glucose (mmol/L)	5.26	0.02	5.24	0.02	5.23	0.02	5.25	0.02	<0.5968
Serum Insulin (pmol/L)	61.91[a]	1.80	57.41[b]	1.14	60.04[a]	1.49	61.39[a]	1.49	<0.0106
Serum C-peptide (nmol/L)	0.70[a]	0.01	0.65[b]	0.01	0.67[a]	0.01	0.66[b]	0.01	<0.0042
Glycated Hemoglobin (%)	5.23	0.02	5.23	0.02	5.22	0.01	5.20	0.02	<0.8569

[1]Means in a row with different superscripts are significantly different ($p<.05$)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

Table 13 Differences in lipid and glycemic profiles by animal protein quartiles[1-2]

	<25		25-50		50-75		75-100		
Variables	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	P
Total Cholesterol (mg/dL)	202.93[a]	1.13	201.30[a]	0.92	202.68[a]	1.18	197.50[b]	1.03	<0.0017
LDL Cholesterol (mg/dL)	124.78	0.99	123.73	0.97	125.18	1.16	122.39	0.79	<0.1998
HDL Cholesterol (mg/dL)	52.01[a]	0.42	52.01[a]	0.50	51.56[a]	0.44	48.93[b]	0.33	<0.0310
Triglycerides (mg/dL)	131.18	2.50	127.85	2.42	129.75	2.12	131.43	2.20	<0.5003
Serum Glucose (mg/dL)	92.51	0.34	92.21	0.43	92.19	0.42	92.41	0.45	<0.6228
Plasma Glucose (mg/dL)	94.53	0.40	94.47	0.30	94.29	0.40	94.59	0.37	<0.4978
Serum Insulin (uU/mL)	10.14[a]	0.27	9.42[b]	0.20	10.16[a]	0.24	10.36[a]	0.23	<0.0084
Serum C-peptide (pmol/mL)	0.68	0.01	0.66	0.01	0.67	0.01	0.67	0.01	<0.7109
Glycated Hemoglobin (%)	5.23	0.02	5.23	0.02	5.22	0.01	5.20	0.02	<0.2209

[1]Means in a row with different superscripts are significantly different ($p<.05$)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

Table 13 A Differences in lipid and glycemic profiles (expressed in SI units) by animal protein quartiles[1-2]

	<25		25-50		50-75		75-100		
Variables	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	P
Total Cholesterol (mmol/L)	5.28[a]	0.03	5.23[a]	0.02	5.27[a]	0.03	5.14[b]	0.03	<0.0017
LDL Cholesterol (mmol/L)	3.24	0.03	3.22	0.03	3.25	0.03	3.18	0.02	<0.1998
HDL Cholesterol (mmol/L)	1.35[a]	0.01	1.35[a]	0.01	1.34[a]	0.01	1.27[b]	0.01	<0.0310
Triglycerides (mmol/L)	1.48	0.03	1.44	0.03	1.47	0.02	1.49	0.02	<0.5003
Serum Glucose (mmol/L)	5.13	0.02	5.12	0.02	5.12	0.02	5.13	0.03	<0.6458
Plasma Glucose (mmol/L)	5.25	0.02	5.24	0.02	5.23	0.02	5.25	0.02	<0.5142
Serum Insulin (pmol/L)	60.86[a]	1.64	56.52[b]	1.19	60.95[a]	1.43	62.15[a]	1.41	<0.0120
Serum C-peptide (nmol/L)	0.68	0.01	0.66	0.01	0.67	0.01	0.67	0.01	<0.7109
Glycated Hemoglobin (%)	5.23	0.02	5.23	0.02	5.22	0.01	5.20	0.02	<0.2209

[1]Means in a row with different superscripts are significantly different ($p<.05$)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

Table 14 Differences in lipid and glycemic profiles by total protein quartiles for men and women[1-2]

Variables	Sex	<25	25-50	50-75	75-100	P
Total Cholesterol (mg/dL)	M	201.84±2.41[a]	197.24±1.43[a]	202.09±1.33[b]	197.32±1.07[a]	<0.0366
	F	205.78±1.14	203.23±1.17	201.29±1.71	196.76±2.34	<0.3902
LDL Cholesterol (mg/dL)	M	127.95±2.02	122.25±1.49	127.53±1.23	123.78±0.93	<0.4095
	F	126.18±1.05	123.36±1.19	121.83±1.46	116.74±1.80	<0.5555
HDL Cholesterol (mg/dL)	M	45.59±0.68	46.89±0.64	45.38±0.45	46.51±0.38	<0.1136
	F	54.38±0.46	56.05±0.54	56.42±0.59	55.97±0.58	<0.1867
Triglycerides (mg/dL)	M	141.32±4.32	141.57±4.48	146.46±4.16	135.72±2.67	<0.2159
	F	126.15±2.66	119.03±2.12	114.95±2.61	120.38±4.10	<0.1330
Serum Glucose (mg/dL)	M	97.03±1.06	94.43±0.42	94.44±0.42	93.49±0.49	<0.4790
	F	90.98±0.40	91.14±0.51	89.89±0.56	88.84±0.70	<0.6126
Plasma Glucose (mg/dL)	M	99.41±1.17	96.38±0.47	96.56±0.46	95.79±0.38	<0.4463
	F	93.29±0.38	93.22±0.35	91.76±0.51	90.81±0.66	<0.3678
Serum Insulin (μU/mL)	M	11.40±0.64	9.79±0.33	10.30±0.36	10.30±0.27	<0.1529
	F	9.982±0.27	9.44±0.02	9.71±0.23	10.02±0.46	<0.0775
Serum C-peptide (pmol/mL)	M	0.77±0.03	0.68±0.02	0.71±0.02	0.65±0.01	<0.2232
	F	0.68±0.01[a]	0.64±0.01[a]	0.64±0.01[a]	0.66±0.03[a]	<0.0147
Glycated Hemoglobin (%)	M	5.37±0.04	5.27±0.02	5.29±0.02	5.22±0.02	<0.3791
	F	5.19±0.02	5.20±0.02	5.14±0.02	5.12±0.02	<0.6790

[1]Means in a row with different superscripts are significantly different (p<.05)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

Table 14 A Differences in lipid and glycemic profiles (expressed as SI units) by total protein quartiles for men and women[1-2]

Variables	Sex	<25	25-50	50-75	75-100	P
Total Cholesterol (mmol/L)	M	5.25±0.06[a]	5.13±0.04[a]	5.25±0.03[b]	5.13±0.03[a]	<0.0336
	F	5.35±0.03	5.28±0.03	5.23±0.04	5.12±0.06	<0.3902
LDL Cholesterol (mmol/L)	M	3.33±0.05	3.18±0.04	3.32±0.03	3.22±0.02	<0.4095
	F	3.28±0.03	3.21±0.03	3.17±0.04	3.04±0.05	<0.5555
HDL Cholesterol (mmol/L)	M	1.19±0.02	1.22±0.02	1.18±0.01	1.21±0.01	<0.1136
	F	1.41±0.01	1.46±0.01	1.47±0.02	1.46±0.02	<0.1867
Triglycerides (mmol/L)	M	1.60±0.05	1.60±0.05	1.65±0.05	1.53±0.03	<0.2159
	F	1.43±0.03	1.35±0.02	1.30±0.03	1.36±0.05	<0.1330
Serum Glucose (mmol/L)	M	5.39±0.06	5.24±0.02	5.24±0.02	5.19±0.03	<0.4855
	F	5.05±0.02	5.06±0.03	4.99±0.03	4.93±0.04	<0.6021
Plasma Glucose (mmol/L)	M	5.52±0.06	5.35±0.03	5.36±0.03	5.32±0.02	<0.4554
	F	5.18±0.02	5.17±0.02	5.09±0.03	5.04±0.04	<0.3747
Serum Insulin (pmol/L)	M	68.38±3.83	58.74±1.98	61.77±2.14	61.79±1.61	<0.1489
	F	59.90±1.61	56.64±1.19	58.26±1.40	60.12±2.78	<0.0670
Serum C-peptide (nmol/L)	M	0.77±0.03	0.68±0.02	0.71±0.02	0.66±0.01	<0.2232
	F	0.68±0.01[a]	0.64±0.01[a]	0.64±0.01[a]	0.66±0.03[a]	<0.0147
Glycated Hemoglobin (%)	M	5.37±0.04	5.27±0.02	5.29±0.02	5.22±0.02	<0.3791
	F	5.19±0.02	5.20±0.02	5.14±0.02	5.12±0.02	<0.6790

[1]Means in a row with different superscripts are significantly different (p<.05)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

Table 15 Differences in lipid and glycemic profiles by animal protein quartiles for men and women[1-2]

Variables	Sex	<25	25-50	50-75	75-100	P
Total Cholesterol (mg/dL)	M	198.35±2.36[a]	198.29±1.41[a]	203.11±1.39[b]	197.23±1.07[a]	<0.0366
	F	204.70±1.20[a]	203.26±1.15[ab]	202.28±1.59[bc]	198.24±2.12[c]	<0.0382
LDL Cholesterol (mg/dL)	M	124.65±1.95	123.64±1.58	127.98±1.48	123.84±0.89	<0.6847
	F	124.83±1.12	123.79±1.11	122.58±1.35	118.46±1.77	<0.0713
HDL Cholesterol (mg/dL)	M	45.04±0.65	46.73±0.73	46.03±0.47	46.37±0.39	<0.0522
	F	54.70±0.46	55.45±0.47	56.70±0.65	55.88±0.65	<0.1540
Triglycerides (mg/dL)	M	144.74±4.47	139.66±4.23	146.01±3.84	135.78±2.79	<0.0704
	F	125.93±2.77	120.16±1.93	114.57±2.45	119.64±3.39	<0.4428
Serum Glucose (mg/dL)	M	96.27±0.74	94.29±0.62	94.53±0.42	93.54±0.47	<0.8653
	F	91.05±0.40	90.85±0.48	90.01±0.61	89.35±0.65	<0.7815
Plasma Glucose (mg/dL)	M	98.45±0.90	96.52±0.59	96.55±0.39	95.83±0.37	<0.8321
	F	92.99±0.42	93.14±0.31	92.14±0.56	91.28±0.61	<0.6103
Serum Insulin (μU/mL)	M	10.96±0.44[a]	9.43±0.38[b]	10.48±0.33[a]	10.45±0.28[a]	<0.0034
	F	9.82±0.27	9.42±0.16	9.85±0.29	10.12±0.39	<0.3707
Serum C-peptide (pmol/mL)	M	0.73±0.02	0.66±0.01	0.71±0.02	0.67±0.01	<0.3264
	F	0.66±0.01	0.65±0.01	0.64±0.02	0.67±0.02	<0.2000
Glycated Hemoglobin (%)	M	5.33±0.02	5.28±0.03	5.28±0.02	5.23±0.02	<0.6533
	F	5.19±0.02	5.19±0.02	5.15±0.02	5.14±0.03	<0.1806

[1]Means in a row with different superscripts are significantly different (p<.05)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

Table 15 A Differences in lipid and glycemic profiles (expressed as SI units) by animal protein quartiles for men and women[1-2]

Variables	Sex	<25	25-50	50-75	75-100	P
Total Cholesterol (mmol/L)	M	5.16±0.06[a]	5.16±0.04[a]	5.28±0.04[b]	5.13±0.03[a]	<0.0366
	F	5.32±0.03[a]	5.28±0.03[ab]	5.26±0.04[bc]	5.15±0.06[c]	<0.0382
LDL Cholesterol (mmol/L)	M	3.24±0.05	3.21±0.04	3.33±0.04	3.22±0.02	<0.6847
	F	3.25±0.03	3.22±0.03	3.19±0.04	3.08±0.05	<0.0713
HDL Cholesterol (mmol/L)	M	1.17±0.02	1.21±0.02	1.20±0.01	1.21±0.01	<0.0522
	F	1.42±0.00	1.44±0.01	1.47±0.02	1.45±0.02	<0.1540
Triglycerides (mmol/L)	M	1.64±0.05	1.58±0.05	1.65±0.04	1.53±0.03	<0.0704
	F	1.42±0.03	1.36±0.02	1.29±0.03	1.35±0.04	<0.4428
Serum Glucose (mmol/L)	M	5.34±0.04	5.23±0.03	5.25±0.02	5.19±0.03	<0.8596
	F	5.05±0.02	5.04±0.03	5.00±0.03	4.96±0.04	<0.7943
Plasma Glucose (mmol/L)	M	5.47±0.05	5.36±0.03	5.36±0.02	5.32±0.02	<0.8309
	F	5.16±0.02	5.17±0.02	5.11±0.03	5.07±0.03	<0.6107
Serum Insulin (pmol/L)	M	65.77±2.66[a]	56.55±2.28[b]	62.88±1.96[a]	62.69±1.67[a]	<0.0036
	F	58.95±1.64	56.49±0.98	59.13±1.72	60.69±2.32	<0.4265
Serum C-peptide (nmol/L)	M	0.73±0.02	0.66±0.01	0.71±0.02	0.67±0.01	<0.3264
	F	0.66±0.01	0.65±0.01	0.64±0.02	0.67±0.02	<0.2000
Glycated Hemoglobin (%)	M	5.33±0.02	5.28±0.03	5.28±0.02	5.23±0.02	<0.6533
	F	5.19±0.02	5.19±0.02	5.15±0.02	5.14±0.03	<0.1806

[1]Means in a row with different superscripts are significantly different ($p<.05$)
[2]Model includes following covariates: age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohydrate), and number of risk factors

CHAPTER V

DISCUSSION

As described earlier, the purpose of the present study was to investigate the relationship of protein intake with lipoprotein profiles (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) and glycemic profiles (fasting and postprandial glucose and insulin, C-peptide, and hemoglobin A1c) in adults who were at risk of insulin resistance. The study used data from a nationally representative sample of the US civilian non-institutionalized population from NHANES III (1988-1994). The results of this study did not demonstrate a consistent association between total and animal dietary protein intakes and glycemic and lipoprotein profiles.

Dietary Intake

The overall dietary pattern for the U.S. population reported by NHANES III and our samples are almost identical: 34%/35.5% energy from fat, 15%/14.9% of energy from protein, 50%/49.7% of energy from carbohydrate, respectively (McDowell et al., 1994). The mean protein intakes were also similar between our samples and NHANES III estimates; both samples showed similar gender differences (men consumed greater amounts of protein than women) and

exceeded the RDA in both gender groups. Smit et al. (1999B) conducted a study describing detailed protein sources using the NHANES III data. They found that the main protein source in the US diet is animal protein, with most of the animal protein coming from the combination of meat, fish, and poultry, followed by dairy protein. With respect to the specific animal protein sources, beef protein contributed the most, followed by milk and yogurt protein. Most of the plant protein came from grains. They also reported that the consumption of animal protein had nearly doubled when compared to data collected by Carroll (1982). In the present study, 67.8% of total protein came from animal sources.

Lipid Profiles

Many studies examined the association between lipid and/or glycemic profiles and health or nutritional status. Very few articles focus directly on specific nutrients, and those that do tend to focus on nutrients that are considered unhealthy if consumed in excess such as saturated fat and cholesterol. There are limited studies that examine the protein intake and its effects on human health. Unlike most other studies, the present study focuses on total protein and animal protein and lipid and glycemic profiles.

Wolfe and Piche (1999) reported that moderate replacement of dietary carbohydrate with low-fat, high-protein foods in a diet significantly improved plasma lipoprotein cardiovascular risk profiles in healthy humans. Similarly, the

results of present study showed that total cholesterol was higher with lower protein intake (both total and animal protein intake) when controlled for lifestyle variables. No differences were found for LDL cholesterol. Interestingly, our findings were different from results of Smit et al. (1999A) that found the positive association between animal protein intake and cholesterol concentrations. However, Coggins et al. (1994) reported that serum total and LDL cholesterol levels tended to decrease with reduced protein intake.

A number of investigations have shown that a vegetable protein diet results in lower plasma levels of cholesterol in humans and animals compared to animal protein diets. (Carroll and Hamilton, 1975; Kritchevsky, 1979; Zulet and Martinez, 1995) Yamada et al. (1987) suggested that a high intake of animal protein would cancel or diminish the hypocholesterolemic effect of vegetable protein diet and physical exercise. However, we found that people who consumed less animal protein had higher total cholesterol.

Besides the difference in types of protein, there are several other concerns to point out the direct relationship between protein intake and lipid profiles, since most foods containing protein also contain other nutrients such as fat. Although our analyses controlled for the amount of fat and carbohydrate in the diet, it is almost impossible to avoid the effects of nutrient and biological interactions since most meals are a mixture of various food items that contain many nutrients.

In the current study, risk of insulin resistance was assessed by BMI, waist circumference, serum triglycerides, blood glucose or family history of diabetes and a high BMI was most common risk factor (71.3%) for both men and women. Trichopoulou et al. (2002) examined the relation between intake of protein and BMI and suggested that a high protein intake was conducive to obesity.

Many studies, both recent and old, have examined the insulin resistance, metabolic syndrome, and their dietary treatment. Insulin resistance and metabolic syndrome is the cause of major health problems in US. Many articles focus on risk factors of insulin resistance, etiology of metabolic syndrome, treatment of insulin resistance. The Oslo Diet and Exercise Study reported that increased protein intake with a reduced fat intake was effective in decreasing both fasting serum glucose and insulin levels in overweight adults with mildly elevated blood pressure and lipids (Torjesen et al, 1997). They also found that C-peptides were reduced more with the exercise intervention than the diet intervention. However, no published study examined the association between protein consumption and glycemic profiles of individuals at risk for insulin resistance. In the current study, we found no significant association between protein intake and insulin or C-peptide concentrations.

Limitations

One of the main limitations of this study was the use of self-reported information, which may not be accurate and may give inconsistent results. Participants in the survey may have answered questions based on what they thought was correct and not on what they actually did.

Dietary risk factors are notoriously difficult to study and the 24-hour recall data are presumably only rough estimators of average intake of individuals. High correlations among intakes of fat, protein, and other nutrients make it difficult to single out the nutrient responsible for affecting lipid and glycemic profiles and associated risk of insulin resistance, diabetes, and cardiovascular diseases. Also the questions asked on the original study were not specifically developed to meet the needs of the current study to investigate the relations between protein intake and lipid and glycemic profiles.

Conclusion

For both total cholesterol and HDL cholesterol, this study showed that average values were higher in the group with the lowest protein intake after controlling for age, sex, BMI, waist circumference, lifestyle factors (physical activity and alcohol consumption), dietary intake (fat and carbohyderate), and number of risk factors. This study also showed that total cholesterol levels

decreased as animal protein intake increased in women. Other lipid values (LDL and triglycerides) did not vary by quartiles of protein intake.

The present study found that the Americans consumed sufficient amount of dietary protein based on RDA and average HDL cholesterol, triglycerides, and glycemic values were within the normal range for both genders. Sex, age, BMI, waist circumference, family history, and number of risk factors were associated with all lipid and glycemic profiles. All lipid (except HDL cholesterol) and glycemic profiles, BMI, waist circumference, and number of risk factors were higher in participants who were older, more obese (higher BMI), had a more andoroid shape (higher waist circumference), and more risk factors for insulin resistance.

Implications

Most people in the US are consuming enough protein, probably too much protein from the animal sources. However, most research findings reported benefits of increased vegetable protein intake, rather than animal protein intake. Such findings are not practical to apply to a population that consumed more animal protein than vegetable protein.

As the evidence mounts for a relationship between protein intake and glycemic and lipid profiles, it may become increasingly important to evaluate the status of these nutrients in patients with insulin resistance, diabetes, and

cardiovascular diseases. At present, it remains unclear whether a high protein intake provides an additional benefit over a lower protein intake to reduce diabetes and cardiovascular risk in individuals who exhibit characteristics that increase their risk of insulin resistance.

Future Research

Further investigations under controlled clinical settings with precise protein intake measurements are needed to determine the effect of protein on glycemic and lipid profiles in individuals with metabolic syndrome, and related chronic diseases such as diabetes and cardiovascular disease. Because we could not find studies that reported strong beneficial effects of protein intake on glycemic control, further studies on this area should be helpful to deepen our understanding. In addition, researchers should consider assessing the effect of nutrition education on protein intake to minimize the risk of further metabolic complications such as diabetes and cardiovascular diseases.

BIBLIOGRAPHY

Ambrosch A, Kope D, Augustin W, Muhlen I, Dierkes J, Konig W, Luley C, Lehnert H. LDL size distribution in relation to insulin sensitivity and lipoprotein patten in young and healthy subjects. *Diabetes Care*. 1998;21:2077-2084.

American Diabetes Association. Consensus development conference on insulin resistance 5-6 November 1997. *Diabetes Care*. 1998;21:310-314.

American Diabetes Association. Standards of medical care for patients with diabetes mellitus. *Diabetes Care*. 2002;25:S33-49.

Anderson PJ, Critchley JAJH, Chan JCN, Cockram CS, Lee ZSK, Thomas GN, Tomlinson B. Factor analysis of the metabolic syndrome: obesity vs insulin resistance as the central abnormality. *J Obes*. 2001;25:1782-1788.

Bakhit RM, Klein BP, Essex-Sorlie D, Ham JO, Erdman JW, Potter SM. Intake of 25g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. *J Nutr*. 1994;124:213-222.

Bales C, Drezner MK, Guyton J, Moreno K, McGee M, Currie K, Yunker P. Both chicken and lean pork can be used effectively in a lipid-lowering regimen. *J Am Coll Nutr*. 1995;14:A.

Barker DJP. *Mothers, Babies and Disease in Later Life*. London: BMJ Publishing Group, 1994.

Bloomgarden ZM. American Diabetes Association 60th scientific sessions, 2000 Nutrition, lipids, and alternative medicine. *Diabetes Care*. 2000;23:1847-1851.

Boyko EJ, DeCourten M, Zimmet PZ, Chitson P, Tuomilehto J, Alberti KG. Features of the metabolic syndrome predict higher risk of diabetes and impaired glucose tolerance. *Diabetes Care*. 2000;23:1242-1248.

Briefel RR, McDowell MA, Alaimo K, Caughman CR, Bischof AL, Carroll MD, Johnson CL. Total energy intake of the US population: the Third National Health and Nutrition Examination Survey, 1988-1991. *Am J Clin Nutr*. 1995;62:1072S-1080S.

Caro JF. Insulin resistance in obese and nonobese man. *J Clin Endocrinol Metab*. 1991;73:691-695.

Carroll KK. Hypercholesterolemia and atherosclerosis: effects of dietary protein. *Fed Proc.* 1982;41:2792-2796.

Caroll KK, Hamilton RMG. Effect of dietary protein and carbohydrate on plasma cholesterol level in relation to atherosclerosis. *J Food Sci.* 1975;40;18-23.

Centers for Disease Control and Prevention. Mortality from coronary heart disease and acute myocardial infarction – United States, 1998. *MMWR.* 2001;50:90-93.

Cheatam B, Kahn CR. Insulin action and the insulin signaling network. *Endocr Rev.* 1995;16:117-142.

Chen CH, Tsai ST, Chou P. Correlation of fasting serum C-peptide and insulin with markers of metabolic syndrome-X in a homogeneous Chinese population with normal glucose tolerance. *Int J Cardiol.* 1999;68:179-186.

Coggins CH, Dwyer JT, Greene T, Petot G, Snetselaar LG, Lente FV. Serum lipid changes associated with modified protein diets: results from the feasibility phase of the modification of diet in renal disease study. *Am J Kid Dise.* 1994;23:514-523.

Colagiuri S, Miller JB. The 'carnivore connection' – evolutionary aspects of insulin resistance. *Eur J Clin Nutr.* 2002;56:S30-35.

Costa MB, Ferreira SR, Franco LJ, Gimeno SG, Iunes M, Japanese-Brazilian Diabetes Study Group. Dietary patterns in a high-risk population for glucose intolerance. *J Epidemiol.* 2000;10:111-117.

Coulston AM, Hollenbeck CB, Swislocki AL, Reaven GM. Effect of source of dietary carbohydrate on plasma glucose and insulin responses to mixed meals in subjects with NIDDM. *Diabetes Care.* 1987;10:395-400.

Coulston AM, Hollenbeck CB, Swislocki AL, Reaven GM. Persistence of hypertriglyceridemic effect of low-fat high carbohydrate diets in NIDDM patients. *Diabetes Care.* 1989;12:94-101.

Cruz ML, Evans K, Frayn KN. Postprandial lipid metabolism and insulin sensitivity in young Northern Europeans, South Asians and Latin Americans in the UK. *Atherosclerosis.* 2001;159:441-449.

Davidson MB. Diabetes research and diabetes care. *Diabetes Care.* 1998;21: 2152- 2160.

Davidson MH, Hunninghake D, Maki KC, Kwiterovich PO, Kafonek S. Comparison of the effects of lean red meat vs lean white meat on serum lipid levels among free-living persons with hypercholesterolemia. *Arch Intern Med.* 1999;159:1331-1338.

Dickey RA, Bray GA, Bartuska DG, Callaway CW, Davidson ET, Feld S, Ferraro RT, Hodgson SF, Jellinger PS, Kennedy FP, Lawrence AM, Palumbo PJ, Seibel JA, Spitz AF. AACE/ACE position statement on the prevention, diagnosis, and treatment of obesity. *Endocr Pract.* 1998;4:297-330.

Elmstahl S, Holmqvist O, Gullberg B, Johansson U, Berglund G. Dietary patterns in high and low consumers of meat in a Swedish cohort study. *Appetite.* 1999;32:191-206.

Everson SA, Goldberg DE, Helmrich SP, Lakka TA, Lynch JW, Kaplan GA, Salonen JT. Weight gain and the risk of developing insulin resistance syndrome. *Diabetes Care.* 1998;21:1637-1643.

Ferrannini E, Galvan AQ, Gastaldelli A, Camastra S, Sironi AM, Toschi E, Baldi S, Frascerra S, Monzani F, Antonelli A, Mannipieri M, Mari A, Seghieri G, Natali A. Insulin: new roles for an ancient hormone. *Eur J Clin Invest.* 1999;29:842-852.

Ferrannini E, Mari A. How to measure insulin sensitivity. *J Hypertens.* 1998;16:895-906.

Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G. Insulin resistance and hypersecretion in obesity. *J Clin Invest.* 1997;100:1166-1173.

Feskens EJM, Virtanen SM, Tuomilehto J, Rasanen L, Stengard J, Pekkanen J, Nissinen A, Kromhout D. Dietary factors determining diabetes and impaired glucose tolerance. *Diabetes Care.* 1995;18:1104-1112.

Franz MJ, Horton ES, Bantle JP, Beebe CA, Brunzell JD, Coulston AM, Henry RR, Hoogwerf BJ, Stacpoole PW. Nutrition principles for the management of diabetes and related complications. *Diabetes Care.* 1994;17:490-518.

Frazao E. The American diet: health and economic consequence. U.S. Department of Agriculture, Economic Research Service, Agriculture Information bulletin. 1995.

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18:499-502.

Fujimoto WY. The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus. *Am J Med.* 2000;108:9S-14S.

Fukushima M, Taniguchi A, Sakai M, Doi K, Nagata I, Nagasaka S, Tokuyama K, Nakai Y. Assessment of insulin sensitivity from a single sample. *Diabetes Care.* 2000;23:1434-1435.

Garg A, Bantle JP, Henry RR, Coulston AM, Griver KA, Raatz SK, Brinkley L, Chen YD, Grundy SM, Huet BA. Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes mellitus. *JAMA.* 1994;271:1421-1428.

Garg A, Grundy SM. Lovastain for lowering cholesterol levels in non-insulin-dependent diabetes mellitus. *N Engl J Med.* 1988;14:81-86.

Giugliano D, Quartaro A, Minei A, De Rosa N, Coppola L, D'Onofrio F. Hyperinsulinemia in hypertension: increased secretion, reduced clearance or both? *J Endocrinol Invest.* 1993;16:315-321.

Greco AV, Mingrone G, Giancaterini A, Manco M, Morroni M, Cinti S, Granzotto M, Vettor R, Camastra S, Ferrannini E. Insulin resistance in morbid obesity reversal with intramyocellular fat depletion. *Diabetes.* 2002;51:144-151.

Guerrero-Igea FJ, Lepe-Jimenez JA, Garrido-Serrano A, Palomo-Gil S. Association among hyperinsulinemia, family history of diabetes, and diminutive stature in normoglycemic premenopausal women. *Diabetes Care.* 2001;24:602-603.

Gunter EW, Bowman BA, Caudill SP. Quality control in planning and operating the laboratory component for the Third National Health and Nutrition Examination Survey. *J Nutr.* 1990; 120:1451-1454.

Haban P, Simoncic R, Zidekova E, Ozdin L. Role of fasting serum C-peptide as a predictor of cardiovascular risk associated with the metabolic X-syndrome. *Med Sci Monit.* 2002;8:CR175-179.

Haffner SM. Management of dyslipidemia in adults with diabetes. *Diabetes Care.* 1998;21:160-178.

Hanley AJ, D'Agostino R, Wagenknecht LE, Saad MF, Savage PJ, Bergman R, Haffner SM. Increased proinsulin levels and decreased acute insulin response independently predict the incidence of type 2 diabetes in the Insulin Resistance Atherosclerosis Study. *Diabetes.* 2002;51:1263-1270.

Hara H, Egusa G, Yamakido M. Incidence of non-insulin-dependent diabetes mellitus and its risk factors in Japanese-Americans living in Hawaii and Los Angeles. *Diab Med.* 1996;13:S133-142.

Harris MI, Cowie CC, Gu K, Francis ME, Flegal K, Eberhardt MS. Higher fasting insulin but lower fasting C-peptide levels in African Americans in the US population. *Diabe Metab Res Rev.* 2002:18:149-155.

Harvard Heart Letter. Insulin resistance and heart disease. *Harvard Heart Letter.* 1998;9:1-3.

Hauner H. Insulin resistance and the metabolic syndrome – a challenge of the new millennium. *J Clin Nutr.* 2002;56:S25-29.

Hollenbeck CB, Coulston AM. Effects of varying carbohydrate and fat intake on glucose and lipoprotein metabolism in individuals with diabetes mellitus. *Diabetes Care.* 1991;14:774-785.

Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary protein and risk of ischemic heart disease in women. *Am J Clin Nutr.* 1999;70:221-227.

Ito C, Maeda R, Ishida S, Sasaki H, Harada H. Correlation among fasting plasma glucose, two-hour plasma glucose levels in OGTT and $HbA1_c$. *Diab Res Clin Prac.* 2000;50:225-230.

Jenkins DJA, Axelsen M, Kendall CWC, Augustin LSA, Vuksan V, Smith U. Dietary fibre, lente carbohydrates and the insulin resistant diseases. *Br J Nutr.* 2000;83:S157-163.

Kanauchi M, Tsujimoto N, Hashimoto T. Validation of simple indices to assess insulin sensitivity based on the oral glucose tolerance test in the Japanse population. *Diab Res Clin Pract.* 2002;55:229-235.

Kagan A, Popper JS, Rhoads GG, Yano K. Dietary and other risk factors for stroke in Hawaiian Japanese men. *Stroke.* 1985;16:390-396.

King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025. *Diabetes Care.* 1998;21:1414-1431.

Kissebah AH, Peiris AN. Biology of regional body fat distribution: relationship to non-insulin dependent diabetes mellitus. *Diabet Metab Rev.* 1989;5:83-109.

Krentz AJ. Insulin resistance. *BMJ.* 1996;313:1385-1389.

Kritchevsky D. Vegetable protein and atherosclerosis. *J Am Oil Chem Soc.* 1979;56:135-140.

Kusunoki M, Cooney GJ, Hara T, Storlien LH. Amelioration of high-fat feeding-induced insulin resistance in skeletal muscle with the antiglucocorticoid RU486. *Diabetologia.* 1995;44:718-720.

Laakso M. How good a marker is insulin level for insulin resistance? *Am J Epidemiol.* 1993;137:959-965.

Lamon-Fava S, Jenner JL, Jacques PF, Schaefer EJ. Effects of dietary intakes on plasma lipids, lipoproteins, and apolipoproteins in free-living elderly men and women. *Am J Clin Nutr.* 1994;59:32-41.

Lean MEJ, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. *Br Med J.* 1995;311:158-161.

Lichtenstein AL, Schwab US. Relationship of dietary fat to glucose metabolism. *Atherosclerosis.* 2000; 150:227-243.

Lind L, Lithell H, Pollare T. Is it hyperinsulinemia or insulin resistance that is related to hypertension and other metabolic cardiovascular risk factors? *J Hypertens.* 1993;11:S11-16.

Mahan LK, Escott-Stump S. Nutritional Care in Diabetes Mellitus and Reactive Hypoglycemia. *Krause's Food, Nutrition, & Diet Therap.* 9th ed. Philadelphia, PA: W.B. Saunders Company; 1996:681-713.

Marshall JA, Bessessen DH, Hamman RF. High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: The San Luis Valley Diabetes Study. *Diabetologia.* 1997;40:430-438.

Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet.* 1992;340:925-929.

Matsumoto K, Kizaki Y, Fukae S, Tomihira M, Sera Y, Ueki Y, Tominaga T, Miyake S. Insulin resistance and coronary risk factors in Japanese type 2 diabetic patients with definite coronary artery disease. *Diab Res Clin Pract.* 2001;51:181-186.

Matsumoto K, Miyake S, Yano M, Ueki Y, Miyazaki A, Hirao K, Tominaga Y. Insulin resistance and classic risk factors in type 2 diabetic patients with different subtypes of ischemic stroke. *Diabetes Care*. 1999;22:1191-1195.

Mau MK, Grandinetti A, Arakaki RF, Chang HK, Kinney EK, Curb JD. The insulin resistance syndrome in native Hawaiians. *Diabetes Care*. 1997;20:1376-1380.

Mayer-Davis EJ, Monaco JH, Hoen HM, Carmichael S, Vitolins MZ, Rewers MJ, Haffner SM, Ayad MF, Bergman RN, Karter AJ. Dietary fat and insulin sensitivity in a triethnic population: the role of obesity. The Insulin Resistance Atherosclerosis Study (IRAS). *Am J Clin Nutr*. 1997;65:79-87.

McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, Duncan AW. Diagnosing insulin resistance in the general population. *Diabetes Care*. 2001;24:460-464.

McDowell MA, Briefel RR, Alaimo K, Bischof AM, Caughman CR, Carroll MD, Loria CM, Johnson CL, Division of Health Examination Statistics. Energy and macronutrient intakes of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988-91. Vital and Health Statistics. Advance Data, No.255. 1994.

Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis of 27 trials. *Arterioscler Thromb*. 1992;12:911-919.

Mikkelsen PB, Toubro S, Astrup A. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. *Am J Clin Nutr*. 2000;72:1135-1141.

National Center for Health Statistics. National Health and Nutrition Examination Survey III. Body Measurements (Amthropometry). Rockville, MD: Westat, Inc. 1988.

National Center for Health Statistics. Plan and Operation of the Third National health and Nutrition Examination Survey, 1988-1994. Vital Health statistics, series 1, No.32. Hyattsville, MD: Public Health Service, 1994.

National Cholesterol Education Program Expert Panel. Detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) Executive Summary. NIH Publication, No. 01-3670. 2001.

National Vital Statistics Reports. Vital Health Statistics 49(11) 2001.

Olefsky JM. Insulin resistance and insulin action. An in vitro and in vivo perspective. *Diabetes*. 1981;30:148-161.

O'Rahilly S, Burnett MA, Smith RF, Darley JH, Turner RC. Haemolysis affects insulin but not C-peptide immunoassay. *Diabetologia*. 1987;30:394-396.

Ozaki K, Okubo M, Mori H, Mito K, Hara H, Kohno N. Decreased insulin secretion and dyslipidemia coexist in subjects with impaired fasting glucose. *Diab Res Clin Pract*. 2002;55:159-164.

Peters AL, Davidson MB, Schriger DL, Hasselblad V. A clinical approach for the diagnosis of diabetes mellitus. *JAMA*. 1996;276:1246-1252.

Pi-Sunyer FX, Becker DM, Bouchard C, Carleton RA, Colditz GA, Dietz WH, Foreyt JP, Garrison RJ, Grundy SM, Hansen BC, Higgins M. Clinical guidelines on the identifications, evaluation, and treatment of overseight and obesity in adults. The evidence report. NIH publication No.98-4083. 1998.

Potter SM, Bakhit RM, Exxex-Sorlie DL, Weingartner KE, Chapman KM, Nelson RA, Prabhudesai M, Savage WD, Nelson AI, Winter LW. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. *Am J Clin Nutr*. 1993;58:501-506.

Pyorala M, Miettinen H, Halonen P, Laakso M, Pyorala K. Insulin resistance syndrome predicts the risk of coronary heart disease and stroke in healthy middle-aged men. *Arterioscler Thromb Vasc Biol*. 2000;20:538-544.

Reaven GM. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1595-1607.

Reaven GM. Do high carbohydrate diets prevent the development or attenuate the manifestations (or both) of syndrome X? A viewpoint strongly against. *Curr Opin Lipidol*. 1997;8:23-27.

Riccardi G, Rivellese AA. Dietary treatment of the metabolic syndrome – the optimal diet. *Br J Nutr*. 2000;83:S143-148.

Scott LW, Dunn JK, Pownall HJ, Brauchi DJ, McMann MC, Herd JA, Harris KB, Savell JW, Cross HR, Gotto AM. Effects of beef and chicken consumption on plasma lipid levels in hypercholesterolemic men. *Arch Intern Med*. 1994;154:1261-1267.

Shah BV, Barnwell BG, Hunt PN. *SUDAAN User's Manual, Release 7.5*. Research Triangle Park, NC: Research Triangle Institute; 1997.

Shaw S, Worner TM, Lieber CS. Comparison of animal and vegetable protein sources in the dietary management of hepatic encephalopathy. *Am J Clin Nutr.* 1983;38:59-63.

Skov AR, Toubro S, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab Disord.* 1999;23:528-536.

Smit E, Nieto FJ, Crespo CJ. Blood cholesterol and apolipoprotein B levels in relation to intakes of animal and plant proteins in US adults. *Br J Nutr.* 1999A;82:193-201.

Smit E, Nieto FJ, Crespo CJ, Mitchell P. Estimates of animal and plant protein intake in US adults: results from the Third National Health and Nutrition examination Survey, 1988-1991. *J Am Diet Assoc.* 1999B;99:813-820.

SPSS Inc. *SPSS 6.1 Syntax Reference Guide.* Chicago,IL: SPSS Inc. 1994.

Storlien LH, Higgins JA, Thomas TC, Brown MA, Wang HQ, Huang XF, Else PL. Diet composition and insulin action in animal models. *Br J Nutr.* 2000;83:S85-90.

Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes.* 1991;40:280-289.

Takami R, Takeda N, Hayashi M, Takami K, Kawachi S, Sasaki A, Yoshino K, Hattori J, Yamakita N, Yasuda K. Abdominal fat as a predictor of insulin resistance syndrome and early carotid atherosclerosis. *Diabetes.* 2000;49:A149.

Taniguchi A, Fukushima M, Sakai M, Kataoka K, Nagata I, Doi K, Arakawa H, Nagasaka S, Tokuyama K, Nakai Y. The role of the body mass index and triglyceride levels in identifying insulin-sensitive and insulin-resistant variants in Japanese non-insulin-dependent diabetic patients. *Metabolism.* 2000;49:1001-1005.

Thompson FE, Byers T. Dietary assessment resource manual. *J Nutr.* 1994;124:2245S-2317S.

Toeller M, Buyken AE, Heitkamp G, Cathelineau G, Ferriss B, Michel G, EURODIAB IDDM Complications Study Group. Nutrient intakes as predictors of body weight in European people with type 1 diabetes. *J Obes.* 2001;25:1815-1822.

Torjesen PA, Birkeland KI, Anderssen SA, Hjermann I, Holme I, Urdal P. Lifestyle changes may reverse development of the insulin resistance syndrome. *Diabetes Care.* 1997;20:26-31.

Trichopoulou A, Gnardellis C, Benetou V, Lagiou P, Bamia C, Trichopoulos D. Lipid, protein and carbohydrate intake in relation to body mass index. *Eur J Clin Nutr.* 2002;56:37-43.

Tsunehara CH, Leonetti DL, Fujimoto WY. Diet of second-generation Japanese-American men with and without non-insulin-dependent diabetes. *Am J Clin Nutr.* 1990;52:731-738.

Unwin N, Alberti KGMM, Bhopal R, Harland J, Watson W, White M. Comparison of the current WHO and new ADA criteria for the diagnosis of diabetes mellitus in three ethnic groups in the UK. *Diabet Med.* 1998;15:554-557.

U.S. Department of Health and Human Services. Physical Activity and Health: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Center for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, 1996.

Vessby B. Dietary fat and insulin action in humans. *Br J Nutr.* 2000;83:S91-96.

Wangerin-Lile D, Goar SL. Insulin resistance and hyperinsulinemia: Recognizing the risk and reversing the process. *Physician Asst.* 2000;24:23-39.

Whitney EN, Cataldo CB, Rolfes SR. Nutrition, Diabetes, and Hypoglycemia I : *Understanding Normal and Clinical Nutrition.* 4th ed. Minneapolis/St. Paul, MN: West Publishing Company; 1994:842-874.

Widhalm K, Brazda G, Schneider B, Kohl S. Effect of soy protein diet versus standard low fat, low cholesterol diet on lipid and lipoprotein levels in children with familial or polygenic hypercholesterolemia. *Pediatrics.* 1993;123:30-34.

Wolfe BM. Potential role of raising dietary protein intake for reducing risk of atherosclerosis. *Can J Cardiol.* 1995;11:127G-131G.

Wolfe BM, Piche LA. Replacement of carbohydrate by protein in a conventional-fat diet reduces cholesterol and triglyceride concentrations in healthy normolipidemic subjects. *Clin Invest Med.* 1999;22:140-148.

Wong WW, Smith EO, Stuff JE, Hachey DL, Heird WC, Pownell HJ. Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *Am J Clin Nutr.* 1998;68:S1386-1389.

Yajnik CS. The insulin resistance epidemic in India: Fetal origins, later lifestyle, or both? *Nutr Rev.* 2001;1:1-9.

Yamada T, Tohori M, Ashida T, Kajiwara N, Yoshimura H. Comparisons of effects of vegetable protein diet and animal protein diet on the initiation of anemia during vigorous physical training (sports anemia) in dogs and rats. *J Nutr Sci Vitaminol.* 1987;33:129-149.

Zulet MA, Martinez JA. Corrective role of chickpea intake on a dietary-induced model of hypercholesterolemia. *Plant Foods Hum Nutr.* 1995;48:269-277.

VITA #2

Etsuko Hoshino

Candidate for the Degree of

Master of Science

Thesis: PROTEIN INTAKE IN ADULTS AT RISK OF INSULIN RESISTANCE: IMPACT ON GLYCEMIC AND LIPOPROTEIN PROFILES

Major Field: Nutritional Sciences

Biographical:

Personal Data: Born in Tokyo, Japan on April 2, 1964, the daughter of Yoshihiko Hoshino and Michiko Hoshino.

Education: Graduated from Tamagawa Seigakuin High School, Tokyo, Japan in March 1983; received a Bachelor of Science degree in Foods and Nutrition from the Marymount College, Tarrytown, New York in May 1999. Completed the requirements for the Master of Science degree with a major in Nutritional Sciences at Oklahoma State University in December 2002.

Experience: Employed by Oklahoma State University, Department of Nutritional Sciences as a graduate research assistant, 2001 to 2002.

Professional Memberships: American Dietetic Association, Oklahoma Dietetic Association, Diabetes Care and Education Dietetic Practice Group, Gerontological Nutritionists Dietetic Practice Group, and Kappa Omicron Nu Honor Society.