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DIETARY PATTERNS ASSOCIATED WITH RISK FACTORS FOR CARDIOVASCULAR DISEASE IN U.S. ADULTS

By

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ABSTRACT

DIETARY PATTERNS ASSOCIATED WITH RISK FACTORS FOR CARDIOVASCULAR DISEASE IN U.S. ADULTS

By

Jean Marie Kerver

Dietary intake of specific nutrients, foods, and food groups are well-established dietary risk factors for cardiovascular disease (CVD). As dietary patterns encompass the dietary intake of individuals within the context of lifestyles, we tested the hypotheses that complex dietary behaviors can be systematically classified into dietary patterns that are associated with nutrient intakes and risk factors for CVD. This study utilized data from the third National Health and Nutrition Examination Survey (NHANES III) and all statistical analyses accounted for the survey design and sample weights and controlled for confounding variables (age, gender, ethnicity, income, smoking status, alcohol intake, vitamin/mineral supplement use, BMI, and physical activity level) in regression analyses.

Using the food-frequency questionnaire and collapsing the 64 food groups available into 35, dietary patterns of healthy U.S. adults (≥20y; n=13,130) were identified by factor analysis. Of six dietary patterns identified, two patterns emerged most prominently and were characterized by high intakes of 1) processed meats, eggs, red meats, and high-fat dairy products ("Western"); and 2) green, leafy vegetables, salad dressings, tomatoes, other vegetables, cruciferous vegetables, and tea ("American-healthy"). The Western pattern was associated ($p<0.05$) positively with serum C-peptide, serum insulin, and glycosylated hemoglobin and inversely with RBC folate

concentrations after adjusting for confounding variables. The American-healthy pattern had no linear relationships with any of the biomarkers examined.

Using the 24-h dietary recall, meal and snack patterns of U.S. adults (≥20 y; n=15,978) were described in relation to nutrient intakes. Daily eating frequency was associated ($p<0.05$) positively with carbohydrate (% energy), folic acid, vitamin C, calcium, magnesium, iron, potassium, and fiber intakes and inversely related to protein (% energy), total fat (% energy), cholesterol, and sodium intakes. In additional analyses (n=10,427), daily eating frequency was associated positively with serum C-peptide concentrations ($p<0.05$). Daily eating frequency was associated positively with an "American-healthy" dietary pattern but not with a "Western" dietary pattern. After further controlling for the American-healthy dietary pattern, the relationship between daily eating frequency and serum C-peptide concentrations was no longer statistically significant ($p=0.09$).

We identified common dietary patterns among free-living persons that characterize high-risk groups at the U.S. population level. These dietary patterns are remarkably similar to those reported in other subgroups and are correspondingly related to health risks. Our findings confirm the hypothesis that dietary pattern analysis is a reliable method for assessing dietary intake that predicts CVD risk, and that meal and snack patterns are predictive of nutrient intake and selected biomarkers. The concept of the "total diet" inclusive of both food intakes and dietary behaviors should be considered in investigations of dietary intake and health outcomes.

Dedicated to my Mother who instilled the value of an education in all of her children and truly believed we could do whatever we set our minds to.

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WORKING DEFINITIONS

Daily Eating Frequency. Number of eating occasions reported in a 24-hour time period.

Dietary Index. A summary measure of dietary intake designed to assess overall diet quality, which is typically constructed on the basis of dietary recommendations. An example is the Healthy Eating Index (HEI), which is a summary measure of the degree to which an individual's diet conforms to recommendations in the U.S. Dietary Guidelines for Americans.

Dietary Intake. Foods, nutrients, and other food components ingested by an individual.

Dietary Patterns. Summary measure of foods, nutrients, and dietary behaviors within the total diet.

Dietary Standards. Standards by which dietary intake is compared (e.g., Dietary Reference Intakes).

Factor. A cluster of related variables that are a mathematically distinguishable component of a larger group of variables in factor analysis.

Factor Analysis. A method of analysis used to reduce a large number of variables to a smaller number of variables, or factors; a factor is a set of variables, such as items on a survey, that can be conceptually and statistically related or grouped together. Factor analysis is done by finding patterns among the variations in the values of several variables; a cluster of highly intercorrelated variables is a factor.

Factor Loadings. The correlations between each variable and each factor in a factor analysis. They are analogous to regression coefficients. The higher the loading, the closer the association of the item with the group of items that make up the factor.

Factor Score. A measure of an individual's association with a factor. The higher the factor score of an individual, the closer the association of the individual with the factor.

Meal Frequency. The number of meals (i.e. breakfast/brunch, lunch, dinner) reported in a 24-hour time period.

Observational Study. A research design in which the investigator observes subjects but does not impose experimental protocols.

Quintile. Division of the total cases in a study into five groups of equal size.

Total Diet. An overall pattern of foods eaten, rather than any one food or meal.

Chapter 1

INTRODUCTION

Cardiovascular disease (CVD) is the number one cause of death in the United Sates (US) and is one of the most serious public health problems we face today. The risk varies with age, gender, ethnicity, and socioeconomic status. Modifiable risk factors include smoking, physical inactivity, hypertension, dyslipidemia, diabetes, and overweight (Krauss *et al.*, 2000). The latter four risk factors can potentially be improved by changes in dietary intake. Specific dietary components (e.g. excess saturated fat, inadequate dietary fiber), however, explain only a portion of CVD risk.

Dietary intake consists of consumption of individual foods or food mixtures that are in turn made up of a complex set of macronutrients, micronutrients, and other food components. Nutrition research has largely focused on the study of specific nutrients and other food components in the prevention of chronic disease. In many instances, however, it is not accurate to presume that nutrient intake can represent food intake. A clear example of this concept was evidenced in the attempt to prevent coronary heart disease (CHD) with beta-carotene supplements (ATBC Study Group, 1994; Hennekens *et al.*, 1996; Omenn *et al.*, 1996). Based on animal studies and prospective human cohort studies, beta-carotene was thought to play a role in the prevention of CHD through its antioxidant properties. However, in randomized controlled primary prevention studies beta-carotene supplements either had no effect or increased the incidence of coronary events and cancer death (ATBC Study Group, 1994; Hennekens *et al.*, 1996; Omenn *et al.*, 1996). Partially in response to this occurrence, there has been a surge of interest in

the study of dietary patterns, which is an attempt to summarize the synergy of foods and nutrients within the total diet in relation to human health and disease. Dietary patterns are inclusive of the roles of bioactive compounds in specific foods or food groups (phytochemicals, non-nutritive and nutritive compounds) and their interactions and are consumed within the context of available food supplies and individual lifestyles.

Since dietary intake is such a complex exposure variable, it is now recognized that we must develop new methods and refine existing methods of assessing dietary intake that focus on the total diet and not just individual dietary components (e.g. nutrients, phytochemicals) or behaviors (e.g. adding salt at the table). The improvement of methods for evaluating dietary intake will lead to a better understanding of the risk for CVD that is attributable to diet and may ultimately lead to decreases in CVD incidence through public education and other interventional efforts to promote optimal dietary intakes.

While there are several methods of summarizing certain aspects of dietary intake (e.g. nutrient adequacy ratios, number of servings of specific food groups), it is a challenge to find a method that accurately describes the complete dietary intake of an individual in all of its complexity. Increasingly, multivariate data reduction techniques, such as factor analysis and cluster analysis, are being used to translate food or food group intake into identifiable dietary patterns. The underlying assumption of statistical data reduction regarding food intake is that foods eaten together (i.e. within the total diet) can be characterized as part of a dietary pattern that is more epidemiologically meaningful than individual foods (Slattery *et al.*, 1998). It is important to note, however, that data reduction by any method can occur only after a complete understanding of the individual

components. Thus, dietary pattern analysis is an extension of the many years of research involving specific dietary components. Additionally, while we now have the luxury of studying dietary patterns because we have sufficient information about the individual components of diet, it will remain critically important to continue investigating singular dietary components for many reasons, including the correct interpretation of dietary pattern research.

By using multivariate data reduction techniques, dietary patterns have been identified and associated with various sociodemographic characteristics and chronic disease risks in age- gender- and race-specific populations. In a large case-control study (n=1,993 cases and n=2,410 controls), Slattery, et al. (1998) identified dietary patterns in both men and women that were associated with risk of colon cancer in a multiethnic population. Study participants were from the Kaiser Permanente Medical Care Program of Northern California and the majority were over 60 years old and thus represents an age-specific population. In two large prospective studies including men (Health Professionals Follow-up Study) (Hu *et al.*, 2000) and women (Nurses' Health Study) (Fung *et al.*, 2001b), dietary patterns were associated with increased risk for CHD. Further analyses of the Health Professionals Follow-up Study revealed associations between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk in men (Fung *et al.*, 2001a). Although these were important findings, the participants in both the Health Professionals Follow-up Study and the Nurses' Health Study were mostly white, over 40 years of age, and of higher socioeconomic status, and thus the findings must be reproduced in other populations before any general conclusions can be drawn.

Interestingly, the two major dietary patterns identified in all three studies mentioned above were qualitatively similar and labeled by the authors as 1)"Western," which was characterized by a high intake of red meat, processed meat, high-fat dairy products, eggs, and refined grains, and 2)"prudent," which was characterized by a high intake of vegetables, fruit, legumes, and fish. Broadly speaking, these studies have identified similar major dietary patterns, with the Western pattern consistently associated with increased disease risk, and the prudent pattern consistently associated with decreased disease risk. The similarity of dietary patterns across these diverse study populations lends credibility to the pursuit of identification of dietary patterns related to health and disease that can be used to guide public health recommendations. While the study of dietary patterns appears to have merit based on current literature in specific population groups, general dietary patterns need to be identified in nationally representative populations in order to better understand the impact of diet on CVD risk at the population level.

In addition, dietary patterns based on food intake still do not account for the full range of dietary behaviors. Besides the *composition* of dietary intake, dietary behaviors such as the daily number of meals or snacks may potentially also effect risk for CVD. Increased daily eating frequency has been shown to improve lipid profiles and markers for glucose metabolism in men with diabetes and healthy men, which may mediate the risk for CVD (Jenkins *et al.*, 1989; McGrath & Gibney, 1994).

Early epidemiological observations revealed a higher incidence of obesity and ischemic heart disease with fewer daily eating occasions in men (Fabry & Tepperman, 1970) (Fabry *et al.*, 1968). There have now been numerous observational and

experimental studies regarding daily eating frequency and CVD risk factors, but they have provided inconsistent results. Large observational studies have consistently shown more favorable lipid profiles with increasing daily eating frequency (Titan *et al.*, 2001), (Redondo *et al.*, 1997), (Edelstein *et al.*, 1992). On the other hand, while short-term clinical experiments tend to show that increased frequency of eating leads to favorable blood lipid and glucose concentrations under controlled feeding conditions, longer experiments do not (Gibney & Wolever, 1997). These findings may be explained in part by the observation that increased meal frequency is associated with a decrease in human cholesterol biosynthesis (Jones, 1997), but perhaps metabolic adaptations occur over time.

In one study subjects were identified as men whose usual eating habits included either three or six meals per day (McGrath & Gibney, 1994). The subjects were then instructed to convert to the alternate eating pattern for 3 weeks duration and increasing meal frequency was accompanied by a significant reduction in total and LDL-cholesterol concentrations. Although subjects were instructed not to change the nutritional composition of their diet, significant changes in nutrient intake (% energy from protein, fat, saturated fat, and alcohol) occurred in the group who decreased their meal frequency. This highlights the fact that changing eating frequency may precipitate changes in nutrient composition and may also explain the changes in lipid concentrations seen in this study. This implies that the discrepancy between epidemiological observations and experimental trials may actually be because meal frequency is a surrogate measure that is a strong covariate of some other variable (e.g. nutrient composition) that is affecting lipid profiles and/or glucose metabolism.

In free-living populations, nutrient intakes may vary depending on meal or snacking habits. In fact, increased eating frequency was associated with higher daily intakes of energy, fat, carbohydrate, and protein in the Norfolk population of the European prospective investigation into cancer (EPIC-Norfolk), however, more favorable lipid concentrations were still associated with increasing meal frequency even after adjusting for energy, macronutrient intake, and other confounding variables in multivariate analyses (Titan *et al.*, 2001). In the EPIC-Norfolk study, frequency of eating was assessed using the question, "How many times a day do you eat, including meals, snacks, biscuits with coffee breaks, etc.?" and nutrient intake was assessed with a quantitative 160 item food frequency questionnaire. The assumption was thus made that people habitually eat the same number of meals or snacks each day, although there is no published research to support this assumption. Despite this limitation, the EPIC-Norfolk study provides important information regarding eating frequency, macronutrient intake, and serum lipid concentrations in the Norfolk population and should be verified in other populations.

More research is needed to determine if eating frequency is related to nutrient intake within a given day. In other words, within a 24-hr period, are diets that consist of more frequent eating occasions related to more favorable nutrient intakes in free-living populations? Moreover, since the nutritional composition of eating occasions is likely to be as important as eating frequency, it is important to examine the nutrient intakes among individuals with different daily eating frequencies in free-living populations. Furthermore, the daily eating frequency may not only be related to nutrient intakes, but to overall dietary patterns and both may be part of a larger pattern of health-related

behaviors. Therefore, this study describes the mean nutrient intakes and biomarker concentrations, among groups with different daily eating frequencies and different dietary pattern scores (i.e. factor scores) in an effort to determine associations between meal and snack patterns, nutrient intake, dietary patterns, and risk factors for CVD.

To summarize, CVD imposes a huge burden of illness in the U.S. and both dietary patterns and daily eating frequency have been associated with risk factors for CVD in specific epidemiological cohorts or small clinical trials. However, no similar work has been done to explore the associations between dietary patterns or daily eating frequency and risk factors for CVD using nationally representative populations. Hence, this study examined the associations between measures of dietary intake (i.e. dietary patterns and daily eating frequency) and risk factors for CVD (i.e. sociodemographic and lifestyle characteristics, physical measurements, and biomarkers) in the healthy U.S. adult population. To test the hypotheses that in U.S. adults 1) individual dietary intake data can be systematically classified into major dietary patterns based on food group intake that are related to risk factors for CVD; 2) meal and snack patterns (including daily eating frequency) are associated with nutrient intakes; and 3) meal and snack patterns are associated with both risk factors for CVD and dietary patterns based on food group intake, the specific objectives of this study were to use the most recent national dietary and health data available (NHANES III) to:

1. a) Identify major dietary patterns with a factor analysis of the food frequency questionnaire items; and b) determine the association between major dietary patterns and CVD risk factors (Chapter Three).

2. a) Describe the number of meals and snacks eaten per day (based on 24-hr dietary recall and self-reported definitions of meal and snack occasions); and
   b) determine the association between the number of eating occasions and daily macronutrient (% energy) and micronutrient intakes (Chapter Four).
3. a) Determine the association between daily eating frequency (using aggregate data) and CVD risk factors; and b) determine the association between daily eating frequency (using aggregate data) and the dietary patterns (using the factor score) (Chapter Five).

Chapter 2

REVIEW OF LITERATURE

A. Cardiovascular Disease

1. Pathophysiology

The term "cardiovascular disease (CVD)" encompasses a broad range of diseases that can include any part of the cardiovascular system. Coronary heart disease (CHD) is the most common type of CVD and also the final common pathway of most cardiovascular diseases. The primary pathogenesis of the major cardiovascular diseases is the obstruction of blood vessels by atherosclerosis or thrombosis, either singly or in combination. Atherosclerosis is the principal pathologic process that underlies CHD and other cardiovascular diseases such as peripheral artery disease and some kinds of stroke (Cohn *et al.*, 1997).

The process of atherosclerosis consists of both the accumulation of lipids within the artery wall and a series of cellular and molecular inflammatory responses (Ross, 1999). The initiating event of atherosclerosis is the formation of lesions that obstruct the flow of blood somewhere in the cardiovascular system. These lesions get progressively worse and are commonly classified as fatty streaks, fibrous plaques, and complicated lesions, in that order of severity. The fatty streaks contain atheromas, or hard masses of fat, and foam cells. As the disease progresses, fibrous plaques form, which are areas of thickening of the arterial wall that contain the fatty streaks in the center that are surrounded by smooth muscle cells, macrophages and collagen. Finally, the complicated lesion is formed, which is made up of calcified tissue containing various degrees of

necrosis, thrombosis and ulceration. When the blood vessel is approximately 70% occluded from these lesions, the blood flow is cut down sufficiently that the surrounding tissue is often injured (Phibbs, 1997).

2. Prevalence

CVD is the leading cause of death in the United States (U.S.) in both men and women. In 1998 in the U.S., 949,619 people died from CVD, which is 40.6 percent of all deaths or 1 of every 2.5 deaths. CVD was about 70 percent of "total mention mortality," which means that of the more than 2,000,000 deaths from all causes, CVD was listed as a primary or contributing cause on about 1,400,000 death certificates (AHA, 2000). CHD is the cause of approximately half of all CVD deaths, or about 500,000 deaths annually (NCHS, 2001). Mortality rates from CHD have been declining in the U.S. for the past 30 years, but morbidity rates are less clear and the burden of illness is still enormous.

Women and both black and Mexican-American men appear to have a lower risk for CHD than white men when the data is adjusted for other risk factors including hypertension, age, and income, but mortality rates are declining faster in white men than in women or blacks (Keil *et al.*, 1995), (Ho *et al.*, 1993), (Mitchell *et al.*, 1991). Mortality rates are increasing in poorer persons and decreasing in the wealthy. In the 1950's, heart disease mortality rates were greater among persons with higher socioeconomic status (SES), but now mortality rates are higher among persons with lower SES (NCHS, 2001). The shift is presumably because higher SES groups more quickly adopt practices to reduce the risk of chronic disease. This includes lifestyle changes, but also reflects the greater access to health care and medication that people of higher SES enjoy.

3. Risk Factors

Risk for CVD is multi-factorial and includes non-modifiable risk factors such as genetic predisposition, male gender, and advanced age, but also modifiable risk factors including smoking, physical inactivity, hypertension, dyslipidemia, type 2 diabetes, and overweight (Krauss *et al.*, 2000). Especially notable is the recent increase in overweight and obese individuals in the US, which is contributing substantially to the burden of chronic health conditions. Results from the 1999-2000 NHANES estimate that 64% of U.S. adults are either overweight (body mass index 25.0-29.9) or obese (body mass index $\geq$ 30.0) which represents an 8% higher prevalence than that reported from NHANES III, 1988-94 (Flegal *et al.*, 2002). Additionally, data from the 1999-2000 NHANES estimate that 15 percent of adolescents aged 12-19 years are overweight, which represents a 4% higher prevalence than estimates from NHANES III (Ogden *et al.*, 2002) and will likely lead to continued increases in adult obesity.

Much attention has been focused on the relationship between diet and CVD as mediated by the effect of diet on blood cholesterol and lipoprotein profiles. This is in part because large epidemiological observations have shown that higher concentrations of serum total cholesterol predict CVD mortality (Rosenman *et al.*, 1976) and large clinical prevention trials such as the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) have shown that lowering serum total cholesterol and low density lipoprotein cholesterol (LDL-C) will reduce risk for CVD mortality (LRC-CPPT, 1984a). Regardless of the cause of elevated total serum cholesterol, the LRC-CPPT showed that a 1% decline in serum cholesterol correlates with a nearly 2% decrease in cardiovascular events in a multicenter, prospective, randomized, double-blind study (LRC-CPPT,

1984b). According to the classic "diet—heart" hypothesis, excessive intakes of saturated fats and dietary cholesterol increase the concentration of serum cholesterol, which then leads to the development of coronary artery disease (Gordon, 1988). Furthermore, a large body of research has shown that various other dietary practices can impact blood cholesterol and lipoprotein profiles (Carleton *et al.*, 1991) (NHLBI, 2001).

Plasma homocysteine (Hcy) is also considered to be a biomarker for CVD risk that is affected by specific nutrient consumption (i.e., vitamins B-6 and folate). Data from approximately 80 clinical and epidemiological studies including more than 10,000 patients has indicated that elevated total plasma Hcy is a strong risk factor for atherosclerotic vascular disease in the coronary, cerebral, and peripheral vessels and for arterial and venous thromboembolism (Refsum *et al.*, 1998). Hyperhomocysteinemia is caused by common genetic factors as well as deficiencies of folate and vitamin B6. Other biomarkers for CVD include altered vascular and endothelial factors, Apolipoprotein A-I, lipoprotein(a), fibrinogen, insulin, C-peptide, glycosylated hemoglobin, and C-reactive protein (Fung *et al.*, 2001a).

Creating an accurate risk factor profile for an individual, however, is difficult because of the interactions between risk factors and the possible multiplicative effects of certain risk factors in combination. For example, the effects of smoking on atherosclerosis are thought to be precipitated by changes in vascular tone and increased platelet aggregation, which may be aggravated by hyperhomocysteinemia. Additionally, the benefits of physical activity may be from its effects on maintaining a healthier body weight, lowering total serum cholesterol, increasing high density lipoprotein cholesterol (HDL-C), and improving muscle tone (Schroder *et al.*, 2002).

4. Dietary Effects

Specific dietary factors have been associated with risk of CVD morbidity and mortality and biomarkers for CVD risk. In fact, there are many dietary factors that have been shown to be associated with increased risk of CVD including: total energy, macronutrient intake and distribution, specific fatty acid classes (e.g., saturated, monounsaturated, polyunsaturated, n-3 fatty acids, and trans fatty acids), dietary cholesterol, dietary fiber, antioxidant vitamins and minerals, pro-oxidants (e.g., iron), and minerals affecting blood pressure (e.g., sodium, potassium, calcium, and magnesium). Additionally, specific foods and food groups have been associated with altered CVD risk including alcohol, fruits and vegetables, grains, nuts, fish, soy protein, red meat, and dairy products (both whole fat and non fat dairy products have been implicated in increased and decreased risk, respectively). Although the spectrum of research regarding diet and CVD risk is vast indeed, current population recommendations address only those issues that are known with relative certainty to decrease risk of CVD.

Recommendations from the third Adult Treatment Panel (ATPIII) of the National Cholesterol Education Program (NCEP) include weight management, physical activity, and restriction of calories from saturated fat (< 7% of calories), and dietary cholesterol (< 200 mg/day), and increasing intake of soluble fiber (10-25 g/day) (NHLBI, 2001). The American Heart Association released new guidelines in the year 2000 stating that the major emphasis for weight management should be on avoidance of excess total energy intake and a regular pattern of physical activity. Fat intake of ≤30% of total energy is recommended to assist in limiting consumption of total energy as well as saturated fat. The guidelines continue to advocate a population-wide limitation of saturated fat to <10%

of energy and dietary cholesterol to <300 mg/d. Because of increased evidence for the cardiovascular benefits of fish (particularly fatty fish), consumption of at least 2 fish servings per week is now recommended. Finally, recent studies support a major benefit on blood pressure of consuming vegetables, fruits, and low-fat dairy products, as well as limiting salt intake (<6 grams per day) and alcohol (no more than 2 drinks per day for men and 1 for women) and maintaining a healthy body weight (Krauss *et al.*, 2000).

B. Dietary Patterns

1. Description

As evidenced in the dietary recommendations above, the traditional focus of nutrition science on the amount and distribution of nutrients is being replaced by a combination focus on both nutrients/food components (i.e. fat, saturated fat, cholesterol, fiber) and foods (i.e. fish, vegetables, fruit, low-fat dairy products). The possibility of many undiscovered compounds in foods, and the enormity of interactions among food components are now recognized. As a result, the study of patterns of nutrients, foods, and food-group intakes has begun to emerge in nutrition research in an attempt to capture a snapshot of the entire diet.

2. Measurement

One method of describing the overall diet is to assess the agreement of an individual's diet with pre-defined dietary standards. This approach builds on previous knowledge concerning the health or disease promoting effects of specific dietary components, and is thus referred to as *a priori*. It involves the use of a graded score to assess an individual's diet against our best estimation of an ideal diet, which is usually based on scientific evidence regarding diet-disease relationships and current dietary

recommendations (Hu, 2002). Examples include a scoring system derived from studies of food groups, individual food items, and nutrients in relation to common diseases in the population (Nube *et al.*, 1987), and the healthy eating index (Kennedy *et al.*, 1995), which is a summary measure describing how well an individual's diet conforms to the U.S. dietary guidelines. Necessarily, *a priori* approaches are limited by current scientific knowledge of diet-disease relationships.

On the other hand, factor analysis and cluster analysis detect patterns from existing data with no prior assumptions of health or disease relationships and are referred to as *a posteriori.* Factor analysis is a statistical technique used both to reduce the number of variables required to explain the *relationships* among a set of variables, and to classify the variables into a smaller number of variables called *factors* (Lawley, 1963). Factor analysis does not assess the relation between independent and dependent variables. Instead, it is used to assess the *patterns* of relationship among many dependent variables, with the goal of discovering something about the nature of the independent variables that affect them, even though those independent variables were not measured directly (Lawley, 1963). Therefore, conclusions reached by factor analysis are by definition more hypothetical than when independent variables are directly observed. The inferred independent variables are called *factors*.

Principal component analysis is the method of factor analysis that has been used to define dietary patterns because the principal components of a correlation matrix are mathematical functions of the observed variables. The central concept of principal components analysis is summarization of a large set of variables that can be replaced by a smaller set, which best represents the larger set (Kim, 1978). Results of a principal

components analysis include a factor loading matrix, which comprises the dietary patterns (i.e. factors) for the entire sample, and a factor score for each individual, which is derived by summing the individual intakes of the food items weighted by standardized scoring coefficients for each factor. Thus each subject has a factor score for each factor that emerges from the data reduction.

Cluster analysis is another exploratory multivariate statistical technique used to describe dietary patterns. While factor analysis aggregates *food groups* based on their correlation with one another, cluster analysis aggregates *individuals* based on the similarity of their diets. The purpose is to sort people into relatively uniform groups, (i.e. clusters), so that members of each cluster have dietary intakes similar to one another, but *not* similar to the dietary intakes of members of other clusters (Darlington, 1973).

The ability of cluster analysis to separate individuals into distinct groups is an advantage over factor analysis in which an individual may score high on more than one factor and thus results may be more difficult to interpret. Another advantage of cluster analysis is that it can be applied to any sort of similarity measures, such as ratings of the similarity of likert scale responses, whereas factor analysis is usually only applied to a correlation matrix. On the other hand, cluster analysis is not designed to recognize certain unique properties of correlation matrices. For example, factor analysis is able to recognize the directionality of correlations (-1 to 1) and changes the signs of the factor loadings but does not change anything else in the factor analysis output, whereas negative correlations completely change the output of a cluster analysis. Additionally, factor analysis can recognize other properties of correlations that cluster analysis has no ability to recognize, since the associations in cluster analysis are treated only as generic

similarity measures rather than as correlations. For instance, if variables A and B each correlate 0.7 with variable C, and correlate 0.49 with each other, factor analysis can recognize that A and B correlate zero when C is held constant because $0.7^2 = .49$ (Darlington, 1973).

Both factor analysis and cluster analysis have distinctive features and the study question at hand must guide the decision regarding which technique to use. Each method has the ability to uncover associations and structure that are both sensible and useful, even though not previously apparent. The results of both may contribute to the definition of a formal classification scheme of dietary patterns.

3. Advantages

Assessment of the total diet by any method has several advantages over single nutrient analyses. For one thing, we have many examples of known interactions between nutrients (e.g. enhanced calcium absorption in the presence of vitamin D) (Devine *et al.*, 2002). Moreover, dietary intake is inherently complex with the possibility of unknown components in foods that may interact with known nutrients or yet other unknown food components. Similarly, single nutrient effects may be small, but their cumulative effect may be beneficial (or harmful) to human health. Dietary pattern analysis incorporates all nutrient interactions in the diet and allows us to capture diet-disease or diet-biomarker relationships without knowing the exact nutrient or food component involved (Jacobs & Murtaugh, 2000).

Additionally, since many nutrients are highly correlated within foods, it is difficult to examine their effects separately. For example, other beneficial compounds found in high fiber foods such as antioxidants and other phytochemicals confound

conclusions from early studies regarding dietary fiber intake. Likewise, nutrient intakes may be confounded by entire dietary patterns. As previously discussed, beta-carotene supplementation failed to prevent chronic disease in prospective trials that were conducted after the observation that fruits and vegetables (i.e. beta-carotene containing foods) were associated with lower incidence of disease. This has been partially attributed to a combination of the other beneficial components of diets high in fruits and vegetables, including folate, fiber, magnesium, potassium, flavonoids, and plant sterols (Bazzano *et al.*, 2002).

The diet as a whole in free-living populations must be considered in assessing both diet-disease relationships and the effects of dietary recommendations because the addition or deletion of certain foods within a diet tends to displace other foods. There is a move toward inclusive food-based recommendations in the prevention of chronic disease, however, the addition of all foods thought to be beneficial may lead to the consumption of excess energy (Kris-Etherton *et al.*, 2002). While it is the goal of most dietary recommendations to have healthy foods displace less healthy foods in the diet, it is critical to examine the entire dietary pattern in order to determine the actual effects of dietary changes. Additionally, certain foods (e.g. eggs) may contain both beneficial (i.e. unsaturated fats, essential amino acids, folate, other B vitamins) and detrimental nutrients (i.e. saturated fat and cholesterol) and thus the individual nutrient effects may actually cancel each other out.

Finally, it has been suggested that interventions based on total diet approaches are easier to implement. It is the position of the American Dietetic Association that healthful eating messages to the public should emphasize the total diet, or overall pattern of food

eaten, rather than any one food or meal (Freeland-Graves & Nitzke, 2002). Assessing dietary patterns that reflect actual consumption may provide insights into possibilities for dietary changes and also may help facilitate the findings into public health recommendations (van Dam *et al.*, 2002). Two notable clinical trials have set precedents not only in demonstrating that dietary patterns can have a marked beneficial impact on important risk factors for cardiovascular disease, but also in their ability to persuade subjects to adopt and comply with new dietary habits (Appel *et al.*, 1997) (de Lorgeril *et al.*, 1999).

4. Limitations

As with all nutrition research, dietary pattern analysis can only be as good as the dietary assessment method it is based upon. Dietary recalls, food records, or food frequency questionnaires (FFQ) are generally used to assess dietary intake. FFQs require subjects to recall their usual frequency of consumption of each food or food group from a list of foods for a given period of time. Because the FFQ gathers less information regarding specific foods, cooking methods, and portion size than 24-hour dietary recalls or food records, the quantification of nutrient intake is not considered as accurate. Measurement errors inherent in this retrospective method of dietary assessment include possible under or over-reporting of general food intake, or selective under or over-reporting of certain foods, or both. Validation studies comparing FFQs with repeated dietary recalls generally show correlations in the range of 0.4 to 0.7 for most foods and nutrients (Subar *et al.*, 2001). In validation studies comparing FFQs with biomarkers of nutrient intake, plasma triacylglycerol, vitamin C, and carotenoid concentrations also correlate similarly with food frequency estimates of both macronutrients (Willett *et al.*,

2001) and micronutrients (Block *et al.*, 2001). FFQs have been shown to correlate moderately well with 24-h urinary nitrogen (r=0.21-0.29) and urinary potassium (r=0.32-0.34), even though 7 day food diaries provided better estimates of nitrogen and potassium intakes (McKeown *et al.*, 2001). However, the FFQ is easier to administer and less costly to analyze, and because it is designed to assess usual intake, most large epidemiological studies have utilized food frequency questionnaires. Accordingly, the limitations of FFQs also apply to dietary pattern analyses that are based on dietary information collected from a FFQ.

5. Validity and Reliability

Dietary patterns resulting from a factor analysis of FFQ items have been validated against dietary patterns derived from diet records (Hu *et al.*, 1999). Hu et al examined the reproducibility and validity of dietary patterns defined by factor analysis by comparing results from a food-frequency questionnaire administered twice, 1 year apart, and two 1-week diet records among a subset of subjects in the Health Professionals Follow-up Study (Hu *et al.*, 1999). They identified two major eating patterns which they labeled "Prudent" and "Western." The Prudent pattern was characterized by a higher intake of vegetables, fruits, legumes, whole grains, and fish, whereas the Western pattern was characterized by a higher intake of processed meat, red meat, butter, high-fat dairy products, eggs, and refined grains. The correlations between the FFQ and the diet records (0.45 to 0.74) for the two patterns and the correlations between factor scores and plasma biomarkers, which were in the expected directions, indicated reasonable reproducibility and validity of the major dietary patterns defined by factor analysis. Similar studies exist validating dietary patterns derived from cluster analysis by

comparing nutrient or biochemical profiles between the clusters (Tucker *et al.*, 1992; Millen *et al.*, 2001).

The reproducibility of dietary patterns across populations, however, has not been examined and doing so brings up the subjective nature of factor analysis. In conducting factor analysis, the researcher must decide which variables to enter into the correlation matrix. A bias in either the inclusion or exclusion of certain variables can cause problems. The inclusion of unrelated variables can have the effect of redefining factors because of shared extraneous variance, whereas exclusion of variables in order to simplify the factorial structure can lead to erroneous conclusions (Kim, 1978). Although dietary patterns would not be expected to be uniform across all populations, the use of factor analysis has been criticized because of its subjective nature (Martinez *et al.*, 1998). However, similar findings across studies would support the use of factor analysis in nutrition epidemiology. The present study attempted to replicate or refute existing literature by deliberately using the same food groups that have previously been reported (Hu *et al.*, 1999) and determining if similar dietary patterns exist in a nationally representative sample. Further, we sought to ascertain if the relationship between dietary patterns and risk factors for CVD is consistent across study populations.

5. Studies of Dietary Patterns

It is now recognized that observational studies regarding dietary patterns are needed because the description of dietary patterns that exist in free-living populations may allow us to discover new associations between diet and disease risk (van Dam *et al.*, 2002). Although the study of dietary patterns is not novel, as Schwerin et al. (1982) described dietary patterns from national nutrition survey data in 1982 using factor

analysis, the use of factor analysis gained greater credibility in nutritional epidemiology after the study conducted by Slattery et al. (1998) associated dietary patterns with colon cancer risk.

The associations between dietary patterns and the risk of CHD were examined in two large ongoing cohort studies, the Nurses' Health Study (NHS) and the Health Professionals' Follow-up Study (HPFU) (Hu *et al.*, 2000; Fung *et al.*, 2001b). In both studies, after adjustment for confounding variables, risk for CHD was higher in persons consuming the Western dietary pattern, while risk for CHD was lower in those individuals consuming the prudent dietary pattern. In further analyses of a subset of men from the HPFS, dietary patterns were significantly associated with some biomarkers of CHD and obesity (tissue plasminogen antigen, fasting insulin, C-peptide, leptin, C-reactive protein, homocysteine, and plasma folate), but not with others (total plasma cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, apolipoprotien A-I, lipoprotein(a), fibrinogen, von Willebrand factor, factor VII antigen, and glycosylated hemoglobin concentrations) (Fung *et al.*, 2001a).

Several other studies also observed dietary patterns that were similar to the two major dietary patterns seen in men (HPFU) and women (NHS) (Slattery *et al.*, 1998; Osler *et al.*, 2001; Tseng & DeVellis, 2001). In a study in Denmark, similar dietary patterns were observed and the prudent dietary pattern was associated with lower risk of CVD and total mortality, but the association with the western dietary pattern was not significant. Tseng and DeVillis (2001) examined major dietary patterns in U.S. whites and found similar patterns. In a study conducted in Germany (Schulze *et al.*, 2001), the dietary patterns were somewhat different from U.S. patterns. Other investigators have

examined dietary intake and other lifestyle practices and found dietary patterns that are associated with high-risk health behaviors (Randall *et al.*, 1991); Tseng and DeVellis, 2001). An analysis of dietary patterns in relation to disease risk factors, however, has yet to be conducted in a nationally representative sample.

Dietary indices have also been used to predict disease risk or mortality. In elderly Greek subjects (Trichopoulou *et al.*, 1995), a composite score that reflects frequent consumption of traditional Mediterranean foods (i.e. vegetables, fruits, legumes, alcohol, dairy products, cereal, and a high ratio of monounsaturated to saturated fats) was associated with reduced all-cause mortality. Likewise, a dietary index based on the World Health Organization guidelines for the prevention of chronic diseases has been shown to be significantly associated with reduced all-cause mortality in a cohort of elderly men in Finland, Italy, and the Netherlands (Huijbregts *et al.*, 1997). The "recommended food score," which is the sum of the number of foods recommended by current dietary guidelines was associated with all-cause mortality in the Breast Cancer Detection Demonstration Project (Kant *et al.*, 2000). The Healthy Eating Index (HEI), which is a measure of adherence to the Dietary Guidelines for Americans, was examined in association with major chronic disease separately in men (HPFU) and women (NHS) and showed only a weak association with major chronic disease for men but no significant association with chronic disease for women (McCullough *et al.*, 2000a; McCullough *et al.*, 2000b). An "alternative healthy eating index" was developed by McCullough et al. (2002) and shown to be strongly associated with CVD mortality in both men and women.

The synergy of nutrients and non-nutrients within foods has also been investigated in experimental regimens. Several studies have used an intervention in which a specific dietary pattern was implemented and then the effects were observed. The Dietary Approaches to Stop Hypertension (DASH) (Appel *et al.*, 1997) and the Lyon Diet Heart studies (de Lorgeril *et al.*, 1999) are the most well-known of these types of interventions. Other controlled feeding trials with short-term (8 week) changes in dietary patterns have also shown favorable effects on measures of serum antioxidant capacity and lipid peroxidation (Miller *et al.*, 1998), and serum homocysteine concentrations (Appel *et al.*, 2000).

The DASH study was the first study to illustrate clearly that a dietary pattern that was high in fruits and vegetables (8-10 servings/day), with low-fat dairy products (2-3 servings/day), and low in total fat (27% energy) and saturated fat (7% energy) significantly improved multiple risk factors for CVD (i.e., systolic and diastolic blood pressure, total cholesterol, LDL-cholesterol) when compared to a control diet with nutrient content similar to the average diet in the US. It should be noted, however, that the DASH diet also reduced HDL-cholesterol (Appel *et al.*, 1997). In the DASH-Sodium trial, the DASH diet in combination with sodium restriction resulted in even greater reductions in blood pressure (Sacks *et al.*, 2001)). The DASH studies demonstrated that dietary interventions could effectively combine food and nutrient advice to achieve changes in dietary patterns and risks for CVD.

The Lyon Diet Heart Study was a randomized controlled trial designed to test a Mediterranean dietary pattern in the prevention of CHD recurrence in individuals who had already suffered a myocardial infarction. Subjects in the experimental group were

instructed to consume a dietary pattern with more bread, root vegetables, green vegetables, and fish, fruit ≥ 1/day, margarine high in alpha-linolenic acid (supplied by the study) to replace butter and cream, rapeseed and olive oil, and less red meat (replaced with poultry). The trial was actually terminated early because of the remarkable benefits in the experimental group including a 70% reduction in all-cause mortality. Although total blood cholesterol concentrations were significantly and independently associated with recurrence, there were no differences in total blood cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, or lipoprotein(a) concentrations between the control and experimental groups at the beginning or the end of the study (de Lorgeril *et al.*, 1999). This indicates that there are important risk factors other than serum lipids mediating the relationship between diet and CHD.

The study of dietary patterns is emerging as an informative and powerful means to augment our understanding of the role of diet in chronic disease in free-living populations. Dietary patterns associated with disease risk have been identified in specific population groups; however, nothing has been reported describing dietary patterns using national data in relation to risk factors for CVD.

C. Daily Eating Frequency

1. Description

The definition of an eating occasion varies widely in the literature depending on the purpose of the investigation. Often, eating occasions are classified as either meals or snacks, but the definition of what constitutes a meal or snack is not uniform. The majority of investigators have defined a meal versus a snack based on the criteria of time of consumption and/or nutrient composition of the eating occasion. Meals are generally

described as one of the main eating occasions of the day, with breakfast occurring in the morning, lunch occurring mid-day and dinner occurring in the evening. Snacks refer to other eating occasions, and are usually smaller and less structured than meals. Other researchers use caloric cut-points to differentiate between meals and snacks. The definition is important because it may significantly influence the outcome and interpretation of studies in which various definitions have been used. The relationship between energy intake and eating frequency has been shown to be dependent on the definition of what constituted a meal (Gatenby, 1997). Self-reporting of eating occasions reveals that subjects have conflicting views about the difference between a meal and a snack. For example, subjects may report a beverage and a cookie eaten at 12:30 p.m. either as a snack or a meal depending on whether the subject uses time of day or food-type as a means of classification. In the present study, the self-reported definitions of eating occasions collected with the 24-h dietary recall in NHANES III were used and consisted of: breakfast/brunch; lunch; dinner; and snack/beverage (National Center for Health Statistics, 1996b).

2. Studies of Daily Eating Frequency

Epidemiological studies have consistently shown an inverse association between daily eating frequency and blood lipid concentrations (Edelstein *et al.*, 1992; Redondo *et al.*, 1997; Titan *et al.*, 2001). Many of these studies adjusted for energy and macronutrient intakes but none further assessed the diet quality or micronutrient intakes associated with increasing meal frequency even though snacks have been shown to contribute significant energy and nutrients to overall dietary intakes in selected

populations (Cross *et al.*, 1994; Cross *et al.*, 1995; Haveman-Nies *et al.*, 1998; Siega-Riz *et al.*, 1998).

Most experimental studies in healthy adults comparing serum lipid concentrations while consuming different meal frequencies show reduced blood concentrations of total and LDL-cholesterol (Mann, 1997). Several of these studies, however, include comparisons of 3 large meals per day to 9 or more smaller meals per day (Arnold *et al.*, 1994; Arnold *et al.*, 1997), which may not be possible to translate into practical recommendations. Furthermore, these studies are all of limited duration and it is not known whether physiological adaptation occurs over time.

There is less consistency in the outcomes of studies comparing effects of eating frequency on other measures of CVD risk. Some investigators (Arnold *et al.*, 1993; Murphy *et al.*, 1996) have found decreased HDL-cholesterol concentrations with increased meal frequency, while others have not (Jenkins *et al.*, 1989). Similarly, some studies have found differences in triacylglycerol and free fatty acid concentrations (Jenkins *et al.*, 1992), while others have not (Wolever, 1991). In a case-control study of 291 smokers and 828 control subjects, increased meal frequency was associated with a reduced risk of developing symptomatic peripheral atherosclerosis (Powell *et al.*, 1999).

Evidence from short-term studies suggests that increased daily eating frequency may be beneficial for blood glucose control in healthy individuals and those with type 2 diabetes by reducing peak insulin secretion while maintaining blood glucose concentrations. This finding may help define a mechanism for the association of decreased serum lipid concentrations with increased meal frequency. Insulin stimulates hydroxymethyglutaryl-CoA (HMGCoA) reductase, which is the rate-limiting enzyme in

hepatic cholesterol synthesis. Additionally, removal of cholesterol via reverse cholesterol transport occurs only in the postprandial phase and therefore, may be facilitated when meal frequency is increased (Mann, 1997).

Epidemiological studies have shown an inverse relationship between meal frequency and body weight (Fabry *et al.*, 1966; Kant *et al.*, 1995). However, a review of related literature concluded that this finding is likely due to changes in dietary intake as a consequence of weight gain or dietary under-reporting of overweight persons (Bellisle *et al.*, 1997). The conclusion is based in part on the outcome of studies using the rigorous methods of whole-body calorimetry and doubly labelled water, which found no difference in 24h energy expenditure between nibbling and gorging. It appears that any effects of daily eating frequency on the regulation of body weight are likely to be because those who eat more often tend to consume fewer calories, but this has not been confirmed.

Because there are methodological challenges in describing meal and snack patterns of populations (Gatenby, 1997), we have very little information regarding the meal and snack patterns of U.S. adults (Oltersdorf *et al.*, 1999). Results from one 24-hour recall and two 1-day diet records from the 1987-1988 Nationwide Food Consumption Survey (NFCS) in the U.S. showed a mean daily eating frequency of 3.47 (SD 0.90) with 90% of the sample eating between 2 and 4 times per day (Longnecker *et al.*, 1997). The NFCS data were not grouped by type of eating occasion (e.g., breakfast, lunch, dinner, snack/beverage) or sociodemographic subgroups, nor were nutrient intake data available by daily eating frequency. Furthermore, the NFCS had a response rate of 31% and the results are not necessarily generalizable to all U.S. adults. In a population-

based sample in Rancho Bernardo, CA of 2,034 white men and women aged 50-89 y, 19% of the sample had a daily eating frequency of ≥ 4 (Edelstein *et al.*, 1992). Very little additional information is available regarding frequency of meals in free-living U.S. populations.

However, it is important to describe daily eating frequency and to determine if it is related to macronutrient or micronutrient intakes in order to ascertain whether or not daily eating frequency is a marker of some other variable (e.g. nutrient composition) that is affecting lipid profiles and/or glucose metabolism. Accurate descriptions of meal and snack patterns (including daily eating frequency) in free-living populations will assist in designing appropriate clinical experiments and aid in understanding both the metabolic and behavioral effects of different meal and snack patterns (Gibney & Wolever, 1997).

D. Summary

For decades, scientists have tried to show the cause and effect relation between dietary intake and CVD risk at individual and population levels. Dietary intake research has mainly focused on specific nutrients (e.g. saturated fat) or other food components (e.g. dietary cholesterol) in relation to certain risk factors (e.g. serum cholesterol concentration). Foods and nutrients are not eaten individually, however, but within the context of the total diet and at various frequencies throughout the day. Dietary pattern research evaluates individual dietary intake at the macro level, accounting for natural and common clusters of foods consumed. Research findings within specific sub-population groups support the hypothesis that dietary patterns can be identified from food intake data collected with FFQs and that selected biomarkers are associated with intake of certain food components, dietary patterns, and daily eating frequencies.

The study of dietary patterns as a method of evaluating dietary intake is emerging as an informative and powerful tool to better understand the role of diet in risk for CVD. Dietary patterns are easier to implement (if confirmed beneficial) than individual foods or nutrients and the use of dietary patterns in disease prevention efforts may assist in decreasing CVD incidence through promotion of optimal dietary intake. Dietary patterns associated with disease risk have been identified in specific population groups; however, nothing has been reported describing dietary patterns using national data in relation to risk factors for CVD in the U.S.

Chapter 3

DIETARY PATTERNS ASSOCIATED WITH RISK FACTORS FOR CARDIOVASCULAR DISEASE IN HEALTHY U.S. ADULTS

A. Abstract

Certain nutrients are well established as dietary risk factors for cardiovascular disease (CVD), but dietary patterns may be a better predictor of CVD risk. This study tested the hypothesis that the complex dietary behaviors of U.S. adults can be grouped into major dietary patterns that are related to risk factors for CVD. Using the food-frequency questionnaire from the third National Health and Nutrition Examination Survey (NHANES III), dietary patterns of healthy U.S. adults (≥20y; n=13,130) were identified by factor analysis. Log-transformed biomarker data were associated with major dietary patterns while controlling for confounding variables in regression analyses. All statistical analyses accounted for the survey design and sample weights. Of six dietary patterns identified, two patterns emerged most prominently and were characterized by high intakes of 1) processed meats, eggs, red meats, and high-fat dairy products ("Western"); and 2) green, leafy vegetables, salad dressings, tomatoes, other vegetables, cruciferous vegetables, and tea ("American-healthy"). The Western pattern was associated ($p<0.05$) positively with serum C-peptide, serum insulin, and glycosylated hemoglobin and inversely with RBC folate concentrations after adjusting for confounding variables. The American-healthy pattern had no linear relations with any of the biomarkers examined. The successful identification of common dietary patterns among free-living persons is promising in characterizing high-risk groups at the U.S. population level. The dietary patterns identified here are similar to those reported in other non-

representative samples and are associated with biomarkers of CVD risk which confirms that dietary pattern analysis can be an important method for assessing dietary intakes when predicting CVD risk.

B. Introduction

Cardiovascular disease (CVD) is a major public health problem in the United States (U.S.) and dietary intake is thought to exert a great influence on the risk for CVD. The known risk factors for CVD, however, including specific dietary components (e.g. excess saturated fat) explain only a portion of morbidity and mortality from CVD. Since dietary intake is such a complex exposure variable, it is now recognized that we must develop and refine methods of assessing dietary intake that focus on the total diet and not just individual dietary components (e.g. nutrients). As a result, the study of *patterns* of nutrients, foods, and food-group intakes has begun to emerge in nutrition research.

Assessment of the total diet takes into account all nutrient interactions and allows us to capture diet-disease or diet-biomarker relationships without knowing the exact nutrient or food component involved (Jacobs & Murtaugh, 2000). Furthermore, many nutrients are highly correlated within foods, making them difficult to examine separately. Likewise, nutrient intakes may be confounded by entire dietary patterns (Hu, 2002). For example, beta-carotene supplementation failed to prevent chronic disease in prospective trials that were conducted after the observation that fruits and vegetables (i.e. beta-carotene containing foods) were associated with lower incidence of disease (ATBC Study Group, 1994; Hennekens *et al.*, 1996; Omenn *et al.*, 1996). These results have been partially attributed to a combination of the other beneficial components of diets high in

fruits and vegetables, including folate, fiber, magnesium, potassium, flavonoids, and plant sterols (Bazzano *et al.*, 2002).

Two large prospective studies have recently reported that dietary patterns defined by factor analysis using dietary data collected with a food-frequency questionnaire (FFQ) are associated with various sociodemographic characteristics, coronary heart disease (CHD) risk (nonfatal myocardial infarction and fatal CHD), and biomarkers of CVD risk (Hu *et al.*, 2000; Fung *et al.*, 2001a; Fung *et al.*, 2001b). Findings from these studies, however, are limited to men and women who participated in the Health Professionals Follow-up Study and the Nurses' Health Study. Subjects were mostly white, over 40 years of age, and of higher socioeconomic status, and thus we do not know if and how dietary patterns differ in the overall U.S. adult population inclusive of all sociodemographic strata. Furthermore, if dietary patterns of the U.S. adult population are identifiable, we do not know if they are associated with CVD risk, as the risk for CVD differs by age, gender, and race.

While dietary patterns have been identified and associated with CVD risk in specific sub-population groups, they need to be identified in nationally representative populations in order to better understand the impact of diet on CVD risk at the population level. Therefore, the purposes of this study were to test the hypotheses that 1) dietary intake data of the healthy U.S. population can be systematically classified into distinct dietary patterns and 2) risk factors for CVD are associated with specific dietary patterns.

C. Subjects and methods

Dataset

Subjects in this study were participants in the third National Health and Nutrition Examination Survey (NHANES III), 1988-94. The National Center for Health Statistics (NCHS) conducted the survey to obtain nationally representative information on the health and nutritional status of the U.S. population. The NHANES III sample represents the total civilian, non-institutionalized population, two months of age or over, in the 50 states and the District of Columbia of the United States. In NHANES III, 39,695 persons were originally sampled over the six years. Of those, 33,994 (86% of sampled subjects) were interviewed in their homes and provided information for the Household Adult Questionnaire and the Dietary Food Frequency Questionnaire (ages 17 years and over). All interviewed persons were invited to the Mobile Examination Center, where blood and urine specimens were obtained, and a number of tests and measurements were performed including body measurements and blood pressure testing (National Center for Health Statistics, 1994).

Analytic sample

All adults aged ≥ 20 y were eligible for inclusion (n=18,125) in this study. From this eligible sample, subjects excluded were: pregnant (n=288) and lactating (n=95) women, subjects on drugs for hyperlipidemia or unspecified heart disease (n=1,251), subjects who were told by a physician that they have diabetes (n=1,498), and subjects who reported changing their diet in the past year for any reason (n=3,227). The final analytic sample in this study consisted of 13,130 individuals, aged 20 years and older, who completed both the home questionnaire and the medical examination.

Dietary Assessment methods

Within the framework of the Food Frequency Questionnaire (FFQ), subjects were asked how often over the past month they had eaten specified food items. The foods were listed in groups, targeting those high in vitamins A and C and calcium, but representing all major food groups consumed by Americans (see Table 1 for food groups). Interviewers also probed for other food and beverage items, which were included in the present analyses. It is important to note that portion sizes were not defined, and responses represent "number of times" as determined by the respondent. Foods were reported as number of times consumed per day, per week, per month, or never. All frequency of consumption variables were standardized as "times per month" using the conversion factors 4.3 weeks/month and 30.4 days/month rounded to the nearest whole number. Usual food intake to establish dietary patterns was based on the FFQ. The food groups from the FFQ were collapsed to 35 predefined food groups (Table 1) in order to closely approximate food groups that have previously been used in the literature. This was done in order to limit the subjectivity in defining food groups and allow us to replicate or refute existing literature.

Laboratory methods

Laboratory data in NHANES III were available from whole blood and sera. Prior to the phlebotomy (venipuncture), a questionnaire was administered to determine an examinee's eligibility for all phlebotomy procedures. It included questions to determine if it was safe to perform the venipuncture, to document and determine fasting compliance, and to aid in analyzing the results of the laboratory tests performed. Examinees were instructed to fast for 10-16 hours prior to the morning examination or for

six hours before the afternoon or evening examination (National Center for Health Statistics, 1996c). Detailed specimen collection and processing instructions are discussed in the Manual for Medical Technicians. The analytical methods used by each of the participating laboratories are described in the Laboratory Procedures Used for NHANES III (National Center for Health Statistics, 1996a).

Data for all adults were available for serum total cholesterol, HDL-cholesterol, and triacylglycerol concentrations. LDL-cholesterol was calculated for sample persons who reported fasting for ≥ 9 hours and who had triacylglycerol concentrations ≤ 400 mg/dL (4.52 mmol/L) by using the equation developed by Friedewald et al (Friedewald *et al.*, 1972). In the present study, we included triacylglycerol data only in subjects who reported fasting for ≥ 9 hours, which is consistent with the guidelines recommended by the third National Cholesterol Education Program Adult Treatment Panel for lipoprotein analysis (2001). RBC folate, serum C-peptide, serum insulin, and serum C-reactive protein concentrations, as well as glycosylated hemoglobin (%) and blood pressure were available for all adults. Serum homocysteine data were available only in phase 2 of the survey (1991-1994) and plasma fibrinogen data were available only in subjects aged 40 years and older.

Data analyses

Statistical software. Data preparation was performed using SAS (Inc., 2000) software (version 8.1). Because NHANES III was conducted in a stratified, multi-stage probability design, traditional methods of statistical analysis based on the assumption of a simple random sample are not applicable. As recommended by the NCHS, SUDAAN (Shah BV, 2001) software (version 8.0) was used to estimate descriptive and inferential

statistics of interest and the associated variances. Sample weighting was used in NHANES III to account for the unequal probability of selection, non-coverage, and non-response bias. Older persons (>60 y), African-Americans, and Mexican Americans were over-sampled to allow for more precise estimates of health and nutritional characteristics for these specific population subgroups. Appropriate sample weights were applied in all statistical analyses to produce estimates of means and percentiles that can be generalized to the healthy adult U.S. population.

Statistical methods. Because the distribution of the dietary data was extremely non-normal, data values were truncated at four standard deviations above the mean and then log transformed. Factor analysis (principal component) was used to derive food patterns based on the frequency of consumption of each of the 35 food groups collapsed from the FFQ. The analysis was conducted using the FACTOR PROCEDURE in SAS. In order to account for the complex survey design of NHANES III, a correlation matrix was created from the weighted data on the 35 food groups after pooling the variance within the sample strata using PROC GLM in SAS. Next, a data step was performed to read the correlation matrix directly into the FACTOR PROCEDURE in SAS. The factors were orthogonally transformed using varimax rotation to achieve a structure with independent (nonoverlapping) factors.

In determining the number of factors to retain, a step-wise process was utilized. Eigenvalues >1.25 and the interpretability of the factors were used as the initial cut-point to report dietary patterns, which resulted in six dietary patterns. Next, the Scree test was considered which clearly identified two major dietary patterns, which were then used in further analyses with CVD risk factors. The factors were labeled on the basis of

interpretation of the data as well as on prior literature (Yarnold *et al.*, 1995). A factor score was created for each individual based on the monthly intake frequencies of the 35 food groups and the standardized scoring coefficient of each food group for each factor. Thus, each individual has a factor score for each factor that emerged from the data.

Percentage and standard error of means were calculated by the linearization (Taylor series) variance estimation method for population parameters. Categorical variable associations were assessed using a chi-square test. Ratio scale variables were assessed using Wald F tests for determination of significance between means of biomarkers by quintile of dietary pattern scores, using the first quintile as the reference group. Linear regression analyses were conducted between biomarkers of CVD risk and dietary pattern scores while controlling for confounding variables including: age, gender (M/F), ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American), smoking status (yes/no), alcohol intake (nondrinker—0 drinks/day, light—>0 to ½ drink/day, moderate—½ to <2 drinks/day, or heavy drinker—≥2 drinks/day), vitamin/mineral supplement use (yes/no), BMI, physical activity (summation of the frequency of multiple leisure-time activities multiplied by the respective estimated oxygen consumption of each activity), and income (poverty income ratio calculated as the ratio of family income to a Census Bureau-determined poverty threshold). Univariate statistics of all dependent variables were assessed prior to regression analyses and log transformed where extremely non-normal in order to more closely approximate a normal distribution.

D. Results

Figure 1 depicts each of the six major and minor dietary patterns (i.e. factors) in a pyramid shape with the foods that are highly correlated with the factor at the base of the pyramid (factor loadings ≥ 0.60), the foods that are moderately correlated in the center of the pyramid (factor loadings 0.40-0.59), and those that are correlated to a lesser extent near the tip of the pyramid (factor loadings 0.20-0.39). Foods that were correlated to the factor at a factor loading magnitude of <0.20 are not listed, while foods that are negatively correlated with the factor (also at a magnitude of ≥ 0.20) are listed outside the tip of the pyramid to represent their extreme infrequency of consumption in relation to the factor. Table 2 reveals the complete factor-loading matrix for the 2 major and 4 minor dietary patterns. These six dietary patterns represent 37% of the explained variance.

Further statistical analyses are only presented for the 2 major dietary patterns, which were subjectively labeled as "Western," and "American-healthy" and together accounted for 20% of the explained variance. Subjects in the highest quintile of the Western dietary pattern (Table 3) were more likely to be male, non-Hispanic Black or Mexican-American, smokers, heavy drinkers, less educated, and less likely to take vitamin/mineral supplements, while those in the highest quintile of the American-healthy dietary pattern were more likely to be female, white, moderate drinkers, more educated, and more likely to take vitamin/mineral supplements. Additionally, when compared to subjects in the lowest dietary pattern quintile, those in the highest quintile of the Western dietary pattern were younger, had a higher mean BMI, lower income, and reported less

physical activity, while those in the highest quintile of the American-healthy dietary pattern were older, with higher income, and more physical activity.

In age-adjusted models, serum total cholesterol, serum LDL-cholesterol, serum triacylglycerol, serum homocysteine, glycosylated hemoglobin (%), and serum insulin concentrations were higher, while serum HDL-cholesterol, serum C-peptide, and RBC folate concentrations were significantly lower in the higher quintiles (compared to the first quintile) of the Western dietary pattern (Table 4). Conversely, serum HDL-cholesterol, and RBC folate concentrations were higher, while serum homocysteine and serum insulin concentrations were lower in the higher quintiles (compared to the first quintile) of the American-healthy dietary pattern (Table 5).

Since the dietary pattern scores were related to sociodemographic and lifestyle characteristics, further statistical analyses controlled for the effect of sex, ethnicity, smoking status, alcohol intake, vitamin/mineral supplement use, BMI, physical activity, and income. Table 6 shows significant differences in serum LDL-cholesterol, serum HDL-cholesterol, RBC folate, glycosylated hemoglobin (%), serum C-peptide and serum insulin concentrations across quintiles of Western dietary pattern scores even after controlling for confounding variables. To test for linear relationships, factor scores were modeled as continuous variables and positive associations were found between the Western dietary pattern and glycosylated hemoglobin ($p<0.0001$), serum C-peptide ($p<0.0001$), and serum insulin concentrations ($p<0.001$). An inverse association was seen between the Western dietary pattern and RBC folate concentrations ($p=0.0001$). Table 7 shows significant differences in serum total cholesterol and HDL-cholesterol concentrations, and glycosylated hemoglobin (%) across quintiles of the American-

healthy dietary pattern, but none showed a significant linear relationship (data not shown). Additionally, systolic blood pressure, serum lipoprotein(a), plasma fibrinogen, and serum C-reactive protein were not found to be associated with the dietary patterns (data not shown).

E. Discussion

The underlying assumption of statistical data reduction in regard to food intake is that foods eaten together (i.e. within the total diet) can be characterized as part of a dietary pattern that is more epidemiologically meaningful than its individual components (Slattery *et al.*, 1998). The use of factor analysis to define dietary patterns, however, has been criticized for its subjective nature with the concern that results are unable to be replicated across populations or even within the same population (Martinez *et al.*, 1998). Similar findings across studies would support the use of factor analysis in nutrition epidemiology. Therefore, this research attempted to replicate dietary patterns that have been reported in other epidemiological studies by using similar steps in the subjective decision-making process while using data representative of the healthy U.S. adult population. Remarkably similar results to those reported previously (Slattery *et al.*, 1998; Hu *et al.*, 2000; Fung *et al.*, 2001b; Tseng & DeVellis, 2001) were found, given the fact that the NHANES III sample is extremely diverse in sociodemographic and lifestyle characteristics and previously reported data has been in age, gender, and race specific populations. These results are in support of Tseng's description of 2 fundamental U.S. dietary patterns that she posits are a result of British culinary heritage (Western) and nutrition science, industry, and government efforts to promote diets that prevent illness (American-healthy) (Tseng, 1999).

The relation of the dietary patterns to sociodemographic and lifestyle characteristics is in support of the theory that healthy food choices are a part of a larger pattern of health-related characteristics and behaviors (Randall *et al.*, 1991). Our results indicated associations between a Western dietary pattern and being nonwhite, male, less educated, with lower income, smoking, decreased physical activity, and less vitamin/mineral supplementation. Dietary patterns may either be independent from, or interacting with other known risk factors for CVD, although many sociodemographic and lifestyle characteristics were statistically controlled for in the multivariate analyses. Additionally, the description of dietary patterns by using only food intake and not additional dietary behaviors such as meal and snack patterns has been criticized (Tseng, 1999) and may indicate an area for further research.

It is not surprising that the Western dietary pattern is associated with lower RBC folate concentrations, because of the lower consumption of folate containing foods in the Western dietary pattern. It is somewhat surprising, although not inconsistent with prior literature, that the Western dietary pattern is not associated with serum lipid concentrations. An analysis of dietary intake in free-living persons using NHANES III data revealed a positive relationship between dietary fat intake and serum total cholesterol in men but not in women (Yang *et al.*, 2003). Results similar to those reported in this study were found in the Health Professionals Follow-up Study in which a Western dietary pattern characterized by high intakes of red meat, processed meat, high fat dairy products, and refined grains was associated with CHD mortality but was not associated with serum lipid concentrations (Hu *et al.*, 2000; Fung *et al.*, 2001a). In addition, the Lyon Diet Heart Study revealed a strong protective effect of a

Mediterranean dietary pattern in the prevention of CHD recurrence even though there were no differences in total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol, or lipoprotein(a) concentrations between the control and experimental groups at the beginning or the end of the study (de Lorgeril *et al.*, 1999). This indicates that there are important risk factors other than serum lipids mediating the relationship between diet and CHD. Perhaps the association shown here between markers of glucose metabolism and the Western dietary pattern are indicative of the importance of the relationship between dietary patterns and metabolic syndrome, which is in turn related to CVD risk (Lakka *et al.*, 2002).

The American-healthy dietary pattern identified in this research is somewhat similar to the prudent dietary pattern reported in both the Health Professionals Follow-up Study and the Nurses' Health Study, however, it does not include high intakes of potentially beneficial food groups such as fish, legumes, and low-fat dairy products. This led us to label the pattern "American-healthy" implying an effort toward a healthy dietary pattern, even if not exactly "prudent." Because the dietary patterns described here are based on actual food intake of free-living individuals and do not represent ideal dietary patterns, strong associations with specific biomarkers were not expected. Additionally, the two major dietary patterns described in this research represent only 20% of the between-person variance in dietary patterns, thus further analyses on the minor dietary patterns may reveal additional associations between dietary patterns and biomarkers of CVD risk. Also described here are four minor dietary patterns and future research should explore associations between minor dietary patterns and chronic disease risk in order to more fully explore the diet-disease risk relationship at the population level.

Dietary pattern analysis is likely to be a more robust and longer-term measure of dietary habits and more likely to be accurate than estimating intake of specific nutrient(s) and their interactions. As with all nutrition research, however, dietary pattern analysis can only be as good as the dietary assessment method it is based upon. Measurement errors inherent in using FFQs for dietary assessment include possible under or over-reporting of general food intake, or selective under or over-reporting of certain foods, or both. Accordingly, the limitations of FFQs also apply to dietary pattern analyses that are based on dietary information collected from a FFQ. However, since the FFQ is designed to assess usual intake, and because of lower cost and relative ease of administration, most large epidemiological studies have utilized FFQs.

As previously mentioned, subjectivity was limited by intentionally choosing variables (food groups) to include in the factor analysis that have previously been used, however, the exact re-creation of previously reported food groups was not possible. For example, because of the food grouping system used in NHANES III (e.g. oatmeal and other hot cereals), our best attempt to replicate food groupings in other studies led us to include all hot cereals in our food group titled, "Whole grains." The inclusion of unrelated variables in a factor analysis can have the effect of redefining factors because of shared extraneous variance, whereas exclusion of variables in order to simplify the factorial structure can lead to erroneous conclusions. Fine-tuning the food groups entered into a factor analysis may improve associations between dietary patterns and markers for disease risk.

A strength of this study is the statistical technique used to account for the complex survey design of NHANES III by pooling the variance within the strata prior to creating a

correlation matrix based on weighted data that could then be used in a factor analysis procedure using SAS software. Since the software programs generally available that will account for the sample design of NHANES III in producing correct variance estimates (e.g. SUDAAN) do not have the capability of conducting factor analyses this was imperative in conducting an appropriate factor analysis. To our knowledge, the use of this technique has not been reported elsewhere.

The use of multivariate data reduction techniques (e.g. factor analysis) to define dietary patterns is emerging in nutritional epidemiology as an important method of describing a snapshot of the entire diet that can then be associated with risk for chronic disease. Dietary pattern analysis is an extension of the many years of research involving specific dietary components and it will remain critically important to continue investigating singular dietary components for many reasons, including the correct interpretation of dietary pattern research. In the present study, no attempt has been made to surmise cause and effect relationships both because of the cross-sectional design of NHANES III and because certain dietary patterns may be part of a larger pattern of healthy or unhealthy behaviors and thus may simply be a surrogate measure for other variables. However, even after we statistically controlled for many potentially confounding variables, dietary patterns alone were significant predictors of biomarkers for CVD risk. The dietary patterns identified here in this nationally representative sample are associated with biomarkers of CVD risk. These data support the use of dietary patterns in guiding public health recommendations for dietary prevention of chronic disease.

Table 1. Food groupings used in the dietary pattern analysis

Food or food groups	Food groups from the NHANES III FFQ[1]
Processed meats	Bacon, sausage (chorizo) and luncheon meats such as hot dogs, salami, and bologna
Red meats	Beef, including hamburger, steaks, roast beef, and meatloaf; Pork and ham, including roast pork, pork chops, and spare ribs
Organ meats	Liver and other organ meats such as heart, kidney, tongue, and tripe (menudo)
Fish and other seafood	Fish including fillets, fish sticks, fish sandwiches, and tuna fish; Shrimp, clams, oysters, crab, and lobster
Poultry	Chicken, all types, including baked, fried, chicken nuggets, and chicken salad; Turkey
Eggs	Eggs including scrambled, fried, omelets, hard-boiled eggs, and egg salad; Egg substitutes
Butter	Butter
Margarine	Margarine
Low-fat dairy products	2%/low fat; 1%; Skim/nonfat; Buttermilk; Evaporated; and Other milk; Chocolate milk and hot cocoa; Yogurt and frozen yogurt
High-fat dairy products	Whole/regular milk; Half and half; Cheese, all types including American, Swiss, cheddar, and cottage cheese; Cheese dishes such as macaroni and cheese, cheese nachos, cheese enchiladas, and quesadillas; Ice cream, ice milk, and milkshakes
Liquor	Hard liquor such as tequila, gin, vodka, scotch, rum, whiskey and liqueurs, either alone or mixed
Wine	Wine, wine coolers, sangria, and champagne
Beer	Beer and lite beer; Non-alcoholic beer/wine
Tea	Regular tea with caffeine; Decaffeinated/herbal tea
Coffee	Regular coffee with caffeine; Decaffeinated coffee and espresso
Fruits	Citrus fruits including oranges, grapefruits, and tangerines; Melons including cantaloupe, honeydew, and watermelon; Peaches nectarines, apricots, guava, mango, and papaya; Other fruits such as apples, bananas, pears, berries, cherries, grapes, plums, and strawberries
Fruit juices	Orange juice, grapefruit juice and tangerine juice; Other fruit juices such as grape juice, apple juice, cranberry juice, and fruit nectars
Cruciferous vegetables	Broccoli; Brussels sprouts and cauliflower; Cabbage, coleslaw, and sauerkraut
Dark-yellow vegetables	Carrots and vegetable mixtures containing carrots; Sweet potatoes, yams, and orange squash including acorn, butternut, hubbard, and pumpkin
Tomatoes	Tomatoes including fresh and stewed tomatoes, tomato juice, and salsa
Green, leafy vegetables	Spinach, greens, collards, and kale; Tossed salad

Table 1 (cont'd).

Food or food groups	Food groups from the NHANES III FFQ
Legumes	Beans, lentils, chickpeas/garbanzos, including kidney, pinto, refried, black, and baked beans; Soy products, including tofu, soy milk, soy ice cream, soy cheese, soy hamburgers, soy hot dogs, soy flour, and textured vegetable protein
Other vegetables	Hot red chili peppers; Peppers including green, red, and yellow peppers; Any other vegetables such as green beans, corn, peas, mushrooms, and zucchini
Potatoes	White potatoes, including baked, mashed, boiled, french-fries, and potato salad
Cold breakfast cereals	All-Bran, All-Bran Extra Fiber, 100% Bran, and Fiber One; Total, Product 19, Most, and Just Right; All other cold cereals like corn flakes, Cheerios, Rice Krispies, and presweetened cereals
Whole grains	Cooked, hot cereals like oatmeal, cream of wheat, cream of rice, and grits; Dark breads and rolls, including whole wheat, rye, and pumpernickel
Refined grains	White bread, rolls, bagels, biscuits, English muffins, and crackers; Corn bread, corn muffins, and corn tortillas; Flour tortillas; Rice; Spaghetti and pasta with tomato sauce
Pizza	Pizza, calzone, and lasagna
Snacks	Salted snacks such as potato chips, taco chips, corn chips, and salted pretzels and popcorn
Nuts	Peanuts, peanut butter, other types of nuts, and seeds
High-energy drinks	Regular colas and sodas, not diet; Hi-C, Tang, Hawaiian Punch, Kool-Aid, and other drinks with added vitamin C; Sports drinks, fruit drinks (excluding juices but including tamarind drinks), Popsicles
Low-energy drinks	Diet colas, diet sodas, and diet drinks such as Crystal Light; Water, including tap, mineral, spring, seltzer, soda
Salad dressings	Oil and vinegar, mayonnaise and salad dressings such as Italian and Thousand Island, including those added to salads and sandwiches
Soup	Stew or soup containing vegetables including minestrone, tomato, and split pea
Sweets	Cakes, cookies, brownies, pies, doughnuts, and pastries; Chocolate candy and fudge

[1]FFQ=Food frequency questionnaire

Table 2. Factor-loading matrix for major and minor dietary patterns[1] in healthy U.S. adults[2]

	Factor					
	Major		Minor			
	1	2	3	4	5	6
Food groups	Western	American -healthy	Californ- nian	Breakfast	South- western	Convenience
Processed meats	0.69[3]	-0.02	0.02	-0.05	-0.09	0.08
Red meats	0.64	0.07	0.07	-0.09	0.07	0.26
Organ meats	0.20	-0.21	0.41	-0.16	0.20	-0.27
Fish and other seafood	0.01	0.13	0.64	0.01	0.00	0.07
Poultry	0.09	0.11	0.55	0.08	0.00	0.16
Eggs	0.65	-0.07	0.12	0.07	0.07	-0.23
Butter	0.18	0.12	-0.07	0.02	-0.05	0.00
Margarine	0.23	0.19	-0.03	0.10	-0.06	-0.07
Low-fat dairy products	-0.16	0.07	0.05	0.61	0.02	0.14
High-fat dairy products	0.42	0.10	-0.08	0.09	0.08	0.15
Liquor	0.06	0.04	0.02	0.00	-0.05	-0.03
Wine	-0.15	0.13	0.13	0.07	-0.05	0.05
Beer	0.18	-0.08	-0.02	-0.09	0.13	0.14
Tea	-0.10	0.41	0.04	-0.15	-0.17	0.05
Coffee	0.20	0.18	-0.20	-0.22	0.14	-0.38
Fruits	0.20	0.23	0.41	0.25	0.15	-0.02
Fruit juices	0.02	0.11	0.27	0.57	0.04	-0.05
Cruciferous vegetables	-0.18	0.42	0.45	0.06	0.16	-0.22
Dark-yellow vegetables	-0.13	0.37	0.36	0.21	0.25	-0.26
Tomatoes	0.03	0.51	0.04	0.10	0.38	-0.01
Green, leafy vegetables	-0.07	0.67	0.30	0.11	0.06	-0.03
Legumes	-0.02	0.05	0.02	0.06	0.70	-0.04
Other vegetables	-0.01	0.47	0.17	0.05	0.43	0.02
Potatoes	0.35	0.34	-0.15	0.03	0.25	0.09
Cold breakfast cereals	0.07	0.02	-0.07	0.72	0.05	-0.03
Whole grains	-0.10	0.28	0.21	0.34	-0.18	-0.37
Refined grains	0.32	-0.02	0.04	-0.14	0.52	0.32
Pizza	0.07	0.05	0.06	0.08	0.01	0.62
Snacks	0.22	0.09	0.00	-0.11	-0.05	0.49
Nuts	-0.01	-0.01	0.09	0.14	0.17	0.00
High-energy drinks	0.29	-0.09	-0.06	-0.10	0.09	0.37
Low-energy drinks	-0.12	0.07	0.13	0.07	-0.07	0.14
Salad dressings	0.19	0.63	0.03	0.05	-0.09	0.08
Soup	0.01	0.07	0.34	0.06	0.44	-0.21
Sweets	0.22	0.07	-0.07	0.08	0.02	0.17
Variance explained (%)	11.06	8.85	5.20	4.68	3.86	3.63

[1]Factor retention criteria of scree plot (major) and eigenvalue cutoff of 1.25 (minor)

[2]Based on n=13,130 FFQ data; weighted N=128,068,510 (includes only cases with no missing values on FFQ and who met other criteria as described in methods

[3]Factor loadings represent magnitude and direction of association with factors (dietary patterns) and can range from -1.0 to +1.0

Table 3. Population percentages in sociodemographic and lifestyle categories by quintile of dietary pattern scores in healthy U.S. adults[1]

		Western dietary pattern						American-healthy dietary pattern					
		Q1[2]	Q2	Q3	Q4	Q5	*P*[3]	Q1	Q2	Q3	Q4	Q5	*P*
Age group	20-39 y	20	18	20	21	21	0.0003	26	20	19	18	16	<0.0001
	40-59 y	26	21	18	18	17		19	19	21	21	21	
	60+ y	28	20	17	17	18		22	16	16	19	26	
Gender	Male	18	16	20	21	25	<0.0001	27	21	19	18	15	<0.0001
	Female	29	23	18	18	13		20	17	19	20	23	
Ethnicity	White	23	19	20	20	18	<0.0001	20	17	20	21	22	<0.0001
	Black	23	17	17	18	25		35	25	18	13	9	
	Mex-Amer	17	20	20	21	23		39	24	16	13	8	
Education Level	< 12 yrs	20	17	18	20	26	<0.0001	32	22	18	14	14	<0.0001
	12 yrs	19	18	19	22	21		22	20	19	18	21	
	> 12 yrs	29	22	20	17	13		19	17	20	23	21	
Income[4]	≥ 1.85 PIR	19	17	18	21	25	<0.0001	31	22	18	14	16	<0.0001
	1.86-3.5 PIR	21	18	19	20	22		22	20	19	20	19	
	>3.5 PIR	28	22	20	17	13		17	17	20	23	23	
Smoking status	Smoker	16	15	19	22	28	<0.0001	25	20	19	18	19	0.1319
	Nonsmoker	27	21	19	18	15		23	19	19	20	19	
Alcohol intake	Nondrinker	25	20	18	18	19	<0.0001	23	19	18	19	21	0.0021
	Light	22	20	20	20	17		24	20	20	19	17	
	Moderate	26	21	18	17	19		16	21	20	20	23	
	Heavy	17	13	20	22	29		25	17	20	20	18	
Vit/Min Suppl Use	Yes	28	20	18	18	16	<0.0001	19	19	20	21	22	<0.0001
	No	21	18	20	20	21		26	20	19	18	17	
Physical activity[5]	<33rd %tile	19	18	19	21	22	<0.0001	25	20	18	19	18	0.0210
	33-66th %tile	22	20	19	21	18		22	19	20	19	20	
	>66th %tile	29	19	19	17	17		20	17	21	21	21	

[1]Based on n=13,763; Weighted N=133,758,856 [2]Quintile of dietary pattern score (Q1-Q5) [3]Chi-square test of independence [4]PIR=Poverty Income Ratio; represents a poverty index set by the federal government [5]Estimated oxygen consumption measured in metabolic equivalents

Table 4. Age-adjusted mean[1] biomarker values by quintile of Western dietary pattern scores in healthy U.S. adults[2]

Biomarker	n	Q1[3]	Q2	Q3	Q4	Q5	*P* (Wald F)[4]
BMI (kg/m^2)	12,163	25.4	26.1	26.3	26.2	26.1	0.0001
		(25.1 25.7)	(25.8 26.5)	(25.9 26.7)	(25.9 26.5)	(25.7 26.5)	
TC (mmol/L)	11,604	5.15	5.24	5.22	5.19	5.15	0.0186
		(5.08 5.21)	(5.19 5.30)	(5.14 5.30)	(5.10 5.28)	(5.09 5.20)	
HDL-C (mmol/L)	11,542	1.39	1.34	1.28	1.30	1.27	<0.0001
		(1.36 1.42)	(1.30 1.37)	(1.24 1.31)	(1.27 1.33)	(1.24 1.30)	
LDL-C (mmol/L)[5]	5,104	3.14	3.31	3.34	3.24	3.23	0.0093
		(3.06 3.22)	(3.22 3.39)	(3.27 3.41)	(3.15 3.33)	(3.17 3.29)	
TG (mmol/L)[6,7]	6,795	1.16	1.21	1.26	1.21	1.25	0.0051
		(1.11 1.21)	(1.15 1.28)	(1.19 1.33)	(1.14 1.29)	(1.20 1.30)	
Hcy (mmol/L)[7,8]	5,301	8.68	8.77	8.88	9.26	9.65	0.0008
		(8.38 8.99)	(8.43 9.13)	(8.61 9.17)	(9.01 9.50)	(9.31 10.01)	
RBC folate (nmol/L)[7]	11,643	405	403	396	383	353	<0.0001
		(392 419)	(387 421)	(385 408)	(372 396)	(341 367)	
Glycosylated Hgb (%)[7]	11,719	5.14	5.18	5.20	5.23	5.31	<0.0001
		(5.09 5.18)	(5.13 5.22)	5.16 5.24)	(5.18 5.27)	(5.27 5.36)	
C-peptide (nmol/L)[7]	11,652	0.49	0.51	0.52	0.55	0.56	0.0005
		(0.47 0.51)	(0.48 0.54)	(0.50 0.55)	(0.52 0.57)	(0.54 0.58)	
Insulin (pmol/L)[7]	11,620	46.4	48.8	50.9	50.4	53.2	0.0004
		(44.5 48.4)	(46.6 51.2)	(48.5 53.5)	(47.7 53.3)	(51.0 55.6)	

[1]Mean ± 95% confidence interval [2]n size varies by biomarker; all significance testing based on weighted data [3]Quintile of dietary pattern score (Q1-Q5); Range of dietary pattern score = -3.88 - 3.48; Q1 mean = -1.47; Q2 mean = -0.54; Q3 mean = -0.01; Q4 mean = 0.47; Q5 mean = 1.24 [4]Q1=Reference level [5]Calculated by the Friedewald equation only on subjects who fasted ≥ 9 hrs and had serum TG concentrations ≤ 400 mg/dL [6]Included only for subjects who fasted ≥ 9 hrs [7]Test for significance performed on log-transformed biomarker values [8]Measured only in Phase 2, NHANES III, 1991-94

Table 5. Age-adjusted mean[1] biomarker values by quintile of American-healthy dietary pattern scores in healthy U.S. adults[2]

Biomarker	n	Q1[3]	Q2	Q3	Q4	Q5	*P* (Wald F)[4]
BMI (kg/m^2)	12,163	26.0	26.1	26.2	26.1	25.7	0.2704
		(25.7 26.3)	(25.7 26.4)	(25.8 26.6)	(25.7 26.5)	(25.3 26.1)	
TC (mmol/L)	11,604	5.17	5.23	5.15	5.20	5.19	0.3631
		(5.11 5.24)	(5.16 5.29)	(5.08 5.21)	(5.13 5.27)	(5.11 5.27)	
HDL-C (mmol/L)	11,542	1.30	1.29	1.32	1.34	1.35	0.0249
		(1.28 1.33)	(1.26 1.31)	(1.29 1.35)	(1.31 1.36)	(1.30 1.39)	
LDL-C (mmol/L)[5]	5,104	3.28	3.28	3.21	3.23	3.23	0.6540
		(3.21 3.34)	(3.21 3.36)	(3.13 3.29)	(3.15 3.31)	(3.12 3.34)	
TG (mmol/L)[6,7]	6,795	1.22	1.24	1.24	1.21	1.17	0.5447
		(1.16 1.27)	(1.17 1.31)	(1.17 1.32)	(1.15 1.28)	(1.09 1.25)	
Hcy (mmol/L)[7,8]	5,301	9.69	8.85	8.98	8.85	8.75	0.0040
		(9.37 10.03)	(8.54 9.17)	(8.65 9.33)	(8.43 9.28)	(8.43 9.09)	
RBC folate (nmol/L)[7]	11,643	367	389	398	395	397	0.0025
		(354 380)	(378 400)	(383 414)	(382 409)	(382 413)	
Glycosylated Hgb (%)[7]	11,719	5.21	5.25	5.19	5.20	5.19	0.1219
		(5.17 5.26)	(5.20 5.29)	(5.14 5.24)	(5.15 5.24)	(5.15 5.23)	
C-peptide (nmol/L)[7]	11,652	0.54	0.53	0.53	0.52	0.50	0.2138
		(0.52 0.56	(0.50 0.55)	(0.51 0.56)	(0.49 0.55)	(0.47 0.53)	
Insulin (pmol/L)[7]	11,620	51.4	50.3	50.7	49.1	47.3	0.0132
		(49.4 53.5)	(47.8 53.0)	(48.6 52.8)	(46.8 51.6)	(44.9 49.7)	

[1]Mean ± 95% confidence interval [2]n size varies by biomarker; all significance testing based on weighted data [3]Quintile of dietary pattern score (Q1-Q5); Range of dietary pattern score = -3.52 - 3.11; Q1 mean = -1.16; Q2 mean = -0.28; Q3 mean = 0.25; Q4 mean = 0.75; Q5 mean = 1.47 [4]Q1=Reference level [5]Calculated by the Friedewald equation only on subjects who fasted ≥ 9 hrs and had serum TG concentrations ≤ 400 mg/dL [6]Included only for subjects who fasted ≥ 9 hrs [7]Test for significance performed on log-transformed biomarker values [8]Measured only in Phase 2, NHANES III, 1991-94

Table 6. Mulitvariate-adjusted[1] mean[2] biomarker values by quintile of Western dietary pattern scores in healthy U.S. adults[3]

Biomarker	n	Q1[4]	Q2	Q3	Q4	Q5	*P* (Wald F)[5]
BMI (kg/m^2)	8,407	25.4	26.1	26.3	26.1	25.9	0.0020
		(25.1 25.8)	(25.7 26.5)	(25.9 26.7)	(25.7 26.4)	(25.5 26.3)	
TC (mmol/L)	8,048	5.16	5.22	5.19	5.17	5.13	0.2013
		(5.09 5.23)	(5.16 5.28)	(5.11 5.27)	(5.08 5.26)	(5.07 5.19)	
HDL-C (mmol/L)	8,005	1.35	1.33	1.29	1.32	1.32	0.0230
		(1.32 1.39)	(1.30 1.36)	(1.26 1.32)	(1.29 1.35)	(1.29 1.35)	
LDL-C (mmol/L)[6]	3,533	3.18	3.29	3.28	3.18	3.17	0.0499
		(3.08 3.28)	(3.19 3.39)	(3.20 3.36)	(3.07 3.28)	(3.10 3.25)	
TG (mmol/L)[7,8]	4,707	1.19	1.22	1.20	1.19	1.19	0.9848
		(1.14 1.25)	(1.16 1.28)	(1.14 1.27)	(1.11 1.28)	(1.13 1.26)	
Hcy (mmol/L)[8,9]	3,651	9.17	8.99	8.79	9.03	9.07	0.5964
		(8.77 9.59)	(8.69 9.29)	(8.56 9.04)	(8.78 9.30)	(8.68 9.48)	
RBC folate (nmol/L)[8]	8,062	402	401	399	393	373	0.0098
		(389 415)	(387 416)	(388 410)	(382 406)	(360 386)	
Glycosylated Hgb (%)[8]	8,115	5.15	5.15	5.15	5.20	5.24	0.0011
		(5.10 5.20)	(5.11 5.19)	(5.11 5.19)	(5.15 5.24)	(5.19 5.29)	
C-peptide (nmol/L)[8]	8,081	0.50	0.49	0.50	0.51	0.53	0.0172
		(0.48 0.52)	(0.47 0.51)	(0.48 0.53)	(0.49 0.54)	(0.51 0.55)	
Insulin (pmol/L)[8]	8,062	46.4	46.9	49.0	48.3	51.8	0.0002
		(44.6 48.2)	(45.2 48.7)	(47.0 51.1)	(45.7 51.0)	(49.8 53.8)	

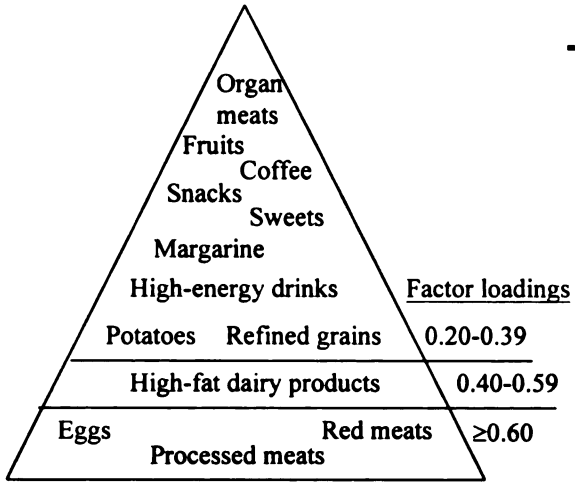
[1]Adjusted for: sex (M/F); ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American); smoking status (yes/no); alcohol intake (nondrinker, light drinker, moderate drinker, heavy drinker); vitamin/mineral supplement use (yes/no); age; BMI (all models except where BMI is outcome); physical activity; income [2]Mean ± 95% Confidence Interval [3]n size varies by biomarker; all significance testing based on weighted data [4]Quintile of dietary pattern score (Q1-Q5); Range of dietary pattern score = -3.88 - 3.48; Q1 mean = -1.47; Q2 mean = -0.54; Q3 mean = -0.01; Q4 mean = 0.47; Q5 mean = 1.24 [5]Q1=Reference level [6]Calculated by the Friedewald equation only on subjects who fasted ≥ 9 hrs and had serum TG concentrations ≤ 400 mg/dL [7]Included only for subjects who fasted ≥ 9 hrs [8]Test for significance performed on log-transformed biomarker values [9]Measured only in Phase 2, NHANES III, 1991-94

Table 7. Mulitvariate-adjusted[1] mean[2] biomarker values by quintile of American-healthy dietary pattern scores in healthy U.S. adults[3]

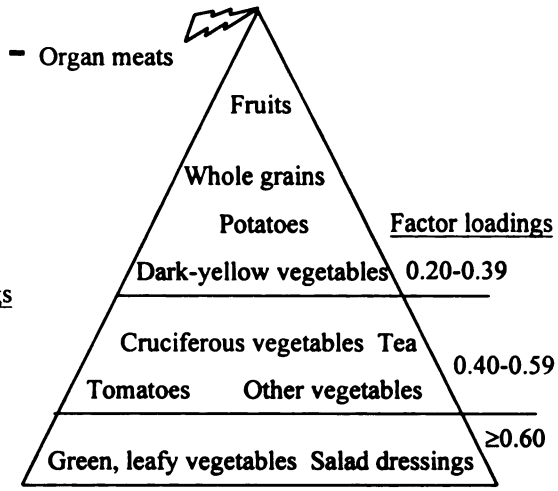
Biomarker	n	Q1[4]	Q2	Q3	Q4	Q5	*P* (Wald F)[5]
BMI (kg/m^2)	8,407	25.6	25.6	26.3	26.1	26.0	0.0067
		(25.3 25.9)	(25.3 25.9)	(25.9 26.7)	(25.7 26.4)	(25.6 26.4)	
TC (mmol/L)	8,048	5.19	5.22	5.09	5.19	5.18	0.0470
		(5.11 5.27)	(5.14 5.30)	(5.03 5.15)	(5.11 5.26)	(5.10 5.26)	
HDL-C (mmol/L)	8,005	1.33	1.30	1.33	1.34	1.32	0.3690
		(1.30 1.35)	(1.28 1.33)	(1.30 1.35)	(1.31 1.37)	(1.29 1.35)	
LDL-C (mmol/L)[6]	3,533	3.24	3.23	3.18	3.18	3.25	0.7017
		(3.17 3.32)	(3.14 3.31)	(3.11 3.26)	(3.09 3.27)	(3.14 3.36)	
TG (mmol/L)[7,8]	4,707	1.18	1.22	1.21	1.20	1.19	0.8895
		(1.10 1.25)	(1.15 1.30)	(1.14 1.28)	(1.15 1.26)	(1.12 1.27)	
Hcy (mmol/L)[8,9]	3,651	9.47	8.75	9.13	8.89	8.87	0.1340
		(9.09 9.87)	(8.45 9.06)	(8.79 9.47)	(8.53 9.26)	(8.53 9.23)	
RBC folate (nmol/L)[8]	8,062	390	401	400	390	388	0.3490
		(378 402)	(389 415)	(387 414)	(379 400)	(375 402)	
Glycosylated Hgb (%)[8]	8,115	5.15	5.19	5.15	5.19	5.21	0.0289
		(5.09 5.20)	(5.15 5.23)	(5.11 5.19)	(5.14 5.24)	(5.17 5.25)	
C-peptide (nmol/L)[8]	8,081	0.51	0.50	0.51	0.51	0.51	0.9859
		(0.49 0.53)	(0.48 0.53)	(0.49 0.53)	(0.48 0.53)	(0.48 0.53)	
Insulin (pmol/L)[8]	8,062	48.7	48.2	48.3	48.4	48.1	0.9935
		(46.2 51.3)	(45.9 50.6)	(46.5 50.0)	(46.6 50.3)	(46.3 50.0)	

[1]Adjusted for: sex (M/F); ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American); smoking status (yes/no); alcohol intake (nondrinker, light drinker, moderate drinker, heavy drinker); vitamin/mineral supplement use (yes/no); age; BMI (all models except where BMI is outcome); physical activity; income [2]Mean ± 95% Confidence Interval [3]n size varies by biomarker; all significance testing based on weighted data [4]Quintile of dietary pattern score (Q1-Q5); Range of dietary pattern score = -3.52 - 3.11; Q1 mean = -1.16; Q2 mean = -0.28; Q3 mean = 0.25; Q4 mean = 0.75; Q5 mean = 1.47 [5]Q1=Reference level [6]Calculated by the Friedewald equation only on subjects who fasted ≥ 9 hrs and had serum TG concentrations ≤ 400 mg/dL [7]Included only for subjects who fasted ≥ 9 hrs [8]Test for significance performed on log-transformed biomarker values [9]Measured only in Phase 2, NHANES III, 1991-94

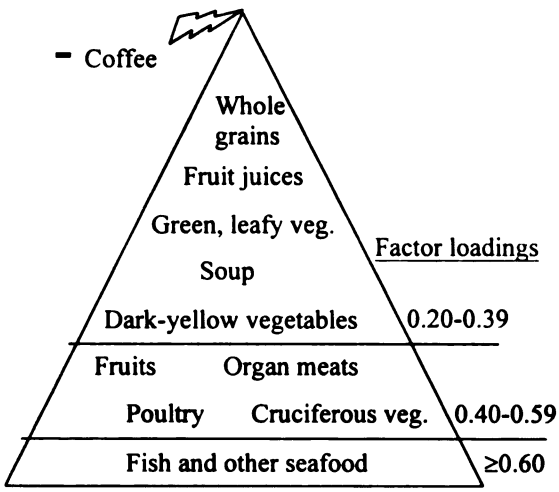
Figure 1. Heirarchical depiction (within each pyramid) of the factor loadings associated with each food group in the two major (A, B) and four minor (C, D, E, F) dietary patterns detected in healthy U.S. adults using factor retention criteria of a scree plot (major) and eigenvalue cutoff of 1.25 (minor). Food groups shown outside the tip of the pyramid represent negative factor loadings within the absolute value range of 0.20-0.39.



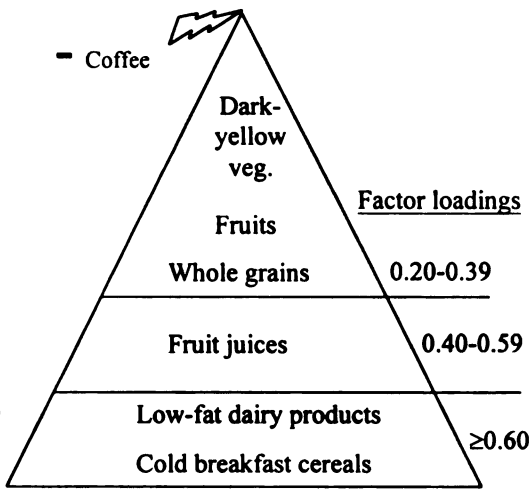
A. "Western" dietary pattern (factor 1)



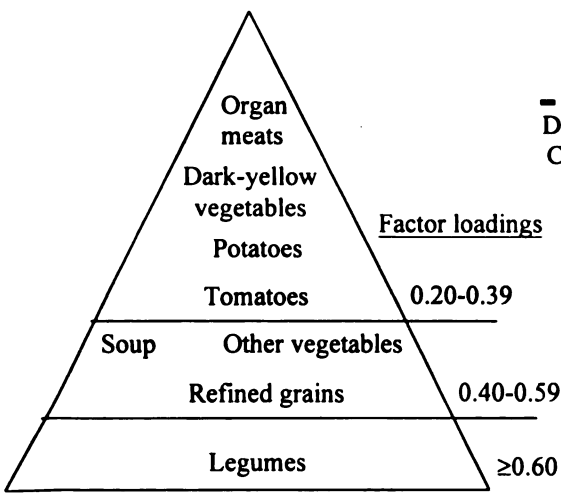
B. "American-healthy" dietary pattern (factor 2)



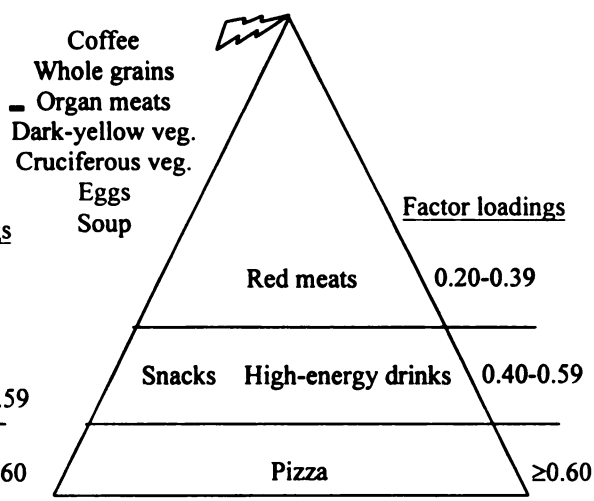
C. "Californian" dietary pattern (factor 3)



D. "Breakfast" dietary pattern (factor 4)



E. "Southwestern" dietary pattern (factor 5)



F. "Convenience" dietary pattern (factor 6)

Chapter 4

MEAL AND SNACK PATTERNS ARE ASSOCIATED WITH DIETARY INTAKE OF ENERGY AND NUTRIENTS IN U.S. ADULTS

A. Abstract

This research tested the hypothesis that meal and snack patterns are associated with nutrient intakes in U.S. adults. Using the 24-h dietary recall from the third National Health and Nutrition Examination Survey (NHANES III), meal and snack patterns of U.S. adults (≥20 y; n=15,978) were described in relation to nutrient intakes while controlling for confounding variables in regression analyses. All statistical analyses accounted for the survey design and sample weights. Daily eating frequency was positively related to carbohydrate (% energy), folic acid, vitamin C, calcium, magnesium, iron, potassium, and fiber intakes and inversely related to protein (% energy), total fat (% energy), cholesterol, and sodium intakes. Meal patterns were further categorized into the 5 most commonly reported meal and snack combinations by population percentages including: Breakfast (B), lunch (L), dinner (D) and ≥ 2 Snacks (S) (31.6%); B, L, D, and 1 S (15.4%); B, D and ≥ 2 S (13.1%); B, L, D (8.3%); and L, D and ≥ 2 S (7.6%). The groups reporting B, L, D, and ≥ 1 S had the highest intakes of all micronutrients examined except cholesterol, vitamin B6, and sodium. Breakfast skippers had the lowest intakes of all micronutrients examined except sodium. Findings from this cross-sectional survey suggest that meal and snack patterns may be markers for nutrient intakes and therefore nutrient intakes should be considered in investigations of meal patterns and health outcomes.

B. Introduction

Meal and snack patterns including the frequency of daily eating occasions are suspected to affect health outcomes including cardiovascular disease (CVD) risk and glucose intolerance (Fabry *et al.*, 1968; Jenkins *et al.*, 1989; Favero *et al.*, 1998). Epidemiological studies have consistently shown more favorable lipid profiles with increasing number of meals (Edelstein *et al.*, 1992; Redondo *et al.*, 1997; Titan *et al.*, 2001). Experimental dietary studies show variable responses in lipid profiles and carbohydrate tolerance to changes in meal and snack patterns in different study populations (Jenkins, 1997; Mann, 1997). Many of these studies adjusted for energy and macronutrient intakes but none further assessed the diet quality or micronutrient intakes associated with increasing meal frequency even though snacks have been shown to contribute significant energy and nutrients to overall dietary intakes in selected populations (Cross *et al.*, 1994; Cross *et al.*, 1995; Haveman-Nies *et al.*, 1998; Siega-Riz *et al.*, 1998).

Because there are methodological challenges in describing meal and snack patterns of populations (Gatenby, 1997), we have very little information regarding the meal and snack patterns of U.S. adults (Longnecker *et al.*, 1997; Oltersdorf *et al.*, 1999). However, it is important to determine if meal and snack patterns are related to macronutrient or micronutrient intakes in order to ascertain whether or not the patterns are markers of some other variable (e.g. nutrient composition) that is affecting lipid profiles and/or glucose metabolism. Accurate descriptions of meal and snack patterns in free-living populations will assist in designing appropriate clinical experiments and aid in understanding both the metabolic and behavioral effects of different meal and snack

patterns (Gibney & Wolever, 1997). Therefore, the purpose of this research was to provide descriptive information on the meal and snack patterns of U.S. adults and test the hypothesis that meal and snack patterns are associated with energy and nutrient intakes in U.S. adults.

C. Subjects and methods

Dataset

Subjects in this study were participants in the third National Health and Nutrition Examination Survey (NHANES III), 1988-94. The National Center for Health Statistics (NCHS) conducted the survey to obtain nationally representative information on the health and nutritional status of the U.S. population. The NHANES III sample represents the total civilian, noninstitutionalized population, two months of age or over, in the 50 states and the District of Columbia of the United States. In NHANES III, 39,695 persons were originally sampled over the six years. Of those, 33,994 (86% of sampled subjects) were interviewed in their homes and provided information for the household adult questionnaire. All interviewed persons were invited to the mobile examination center, where the 24-hour dietary recall was administered (National Center for Health Statistics, 1994).

Analytic sample

All adults aged ≥ 20 y were eligible for inclusion in this study (n=18,125). From this eligible sample, subjects excluded from the main analyses were those whose 24-hour dietary recall data were not reliable and complete (as coded by NCHS) (n=2,147). Therefore, the final analytic sample consisted of 15,978 adults aged ≥ 20 y who

completed both the home questionnaire and the 24-hour dietary recall (a component of the medical examination).

Dietary Assessment

The 24-hour dietary recall records (from NHANES III) and the United States Department of Agriculture (USDA) Survey Nutrient Database were used to determine meal pattern variables (number and self-reported meal or snack/beverage name) and daily intake of energy and selected nutrients (i.e. macronutrient distribution; dietary cholesterol; vitamin B6, folic acid; vitamin C; calcium; magnesium; iron; sodium; potassium; and dietary fiber). Respondents reported all foods and beverages consumed except plain drinking water for the previous 24-hour time period (midnight to midnight). Eating occasions were self-reported from a list of possible options and included: breakfast; brunch; lunch; dinner; snack/beverage and, Spanish language equivalents. Breakfast and brunch were collapsed into one group hereafter referred to as breakfast and the Spanish language groups were combined with the English language equivalent groups. Subjects were divided into categories of daily eating frequency (1-2; 3; 4; 5; ≥ 6) based on the distribution of data obtained and prior literature. Categories based on self-reported meal and/or snack/beverage intakes were also identified based on the population distribution and nutrient intakes were compared among groups.

Data Analyses

Statistical software. Data preparation was performed using SAS software (version 8.1). Because NHANES III was conducted in a stratified, multi-stage probability design, traditional methods of statistical analysis based on the assumption of a simple random sample are not applicable. As recommended by the NCHS, SUDAAN (Shah BV, 2001)

software (version 8.0) was used to estimate descriptive and inferential statistics of interest and the associated variances. Sample weighting was used in NHANES III to account for the unequal probability of selection, non-coverage, and non-response bias. Older persons (<60 y), African-Americans, and Mexican Americans were over-sampled to allow for more precise estimates of health and nutritional characteristics for these specific population subgroups. Appropriate sample weights were applied in all statistical analyses to produce estimates of means and percentiles that can be generalized to the healthy adult U.S. population.

Statistical methods. Percentage and standard error of means were calculated by the linearization (Taylor series) variance estimation method for population parameters. Categorical variable associations were assessed using a chi-square test. Linear regression analyses were conducted between nutrients and both daily eating frequency and meal pattern groups, while controlling for confounding variables including: age, gender (M/F), ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American), smoking status (yes/no), alcohol intake (nondrinker—0 drinks/day, light—>0 to ½ drink/day, moderate—½ to <2 drinks/day, or heavy drinker—≥2 drinks/day), vitamin/mineral supplement use (yes/no), BMI, physical activity (summation of the frequency of multiple leisure-time activities multiplied by the respective estimated oxygen consumption of each activity), income (poverty income ratio calculated as the ratio of family income to a Census Bureau-determined poverty threshold), and energy intakes. Ratio scale variables were assessed using Wald F tests for determination of significance between means of nutrient intakes by daily eating frequency or meal pattern groups. The fewest eating occasions per day (1-2) was used as the reference group in the daily eating frequency

analyses, while breakfast, lunch, and dinner only (B, L, D only) was used as the reference group in the meal and or snack/beverage pattern analyses.

D. Results

On average, subjects reported a daily eating frequency of 4.90 (SE 0.04) with a range of 1-18 (median=4.18; mode=4). Daily eating frequency was categorized into 5 groups (1-2; 3; 4; 5; 6+) based on the distribution of the data and prior literature. More frequent eaters were more likely to be middle-aged (40-59 y), white, smokers, heavy drinkers, vitamin/mineral supplement users, with higher income and education levels than less frequent eaters (Table 1). Because daily eating frequency was associated with many sociodemographic and lifestyle characteristics, further statistical analyses controlled for the effect of age, sex, ethnicity, smoking status, alcohol intake, vitamin/mineral supplement use, BMI, physical activity, income, and energy intakes. After controlling for these confounding variables, more frequent eaters had higher intakes of carbohydrate (% energy), folic acid, vitamin C, calcium, magnesium, iron, potassium, and dietary fiber and lower intakes of dietary fat (% energy), protein (% energy), cholesterol, and sodium than less frequent eaters (Table 2).

When subjects were categorized in terms of self-reported specific meal (breakfast, lunch, dinner) and snack/beverage consumption, the prevalence of reported meal skipping was highest for lunch (26.1%) and lowest for dinner (10.4%), while 17.7% of the population reported skipping breakfast. The majority of subjects reported consuming at least two snacks (62.3%), while 25.2% of the population reported consuming one snack and 12.5% reported consuming no snacks. Twenty-three different meal/snack

combinations were reported, but five of these described 75.9% of the population and thus were the only five considered in further analyses regarding nutrient intake.

The most common meal pattern was reported by 31.6% of the population and consisted of breakfast, lunch, dinner and at least 2 snacks (B, L, D + ≥ 2 S). Subjects who reported consuming this meal pattern were more likely to be female, middle-aged (40-59 y), white, nonsmokers, moderate drinkers, vitamin/mineral supplement users with higher education and income levels and moderate activity levels. Also of note is that within the non-Hispanic Black and Mexican-American subpopulation groups, over 40% reported consuming a meal pattern type other than the five most commonly reported meal patterns (Table 3). Subgroup analyses revealed a wide distribution of meal pattern types within each race/ethnicity category, rather than a few prominent meal patterns in each (data not shown).

After controlling for confounding variables in the multivariate analyses, those reporting no snacks consumed the least amount of energy and carbohydrate (% energy) and the highest amount of protein (% energy) and total fat (% energy). Those consuming B, L, D + ≥ 2 S had the highest energy and carbohydrate (% energy) and lowest total fat (% energy) intakes. Breakfast skippers had the lowest intakes of all micronutrients examined except sodium. The groups reporting breakfast, lunch, dinner and at least one snack had the highest intakes of all micronutrients examined except cholesterol, vitamin B6 and sodium (Table 4).

E. Discussion

The results of this study provide descriptive information regarding meal and snack patterns of U.S. adults. We report 30% of U.S. adults aged 20 y and older

consumed ≥ 6 daily meals and snacks based on self-reported designation of eating occasions from one 24-h dietary recall. By comparison, results from one 24-hour recall and two 1-d diet records from the 1987-1988 Nationwide Food Consumption Survey (NFCS) in the U.S. showed a mean daily eating frequency of 3.47 (SD 0.90) with 90% of the sample eating between 2 and 4 times per day (Longnecker *et al.*, 1997). The higher number of meals and snacks in the NHANES III data reported here is likely due in part to the fact that the classification scheme in the NFCS for eating occasions was not based on self-report but rather the researcher considered everything eaten within a one-hour time period to be part of the same eating occasion. The NFCS data was not broken down by type of eating occasion (e.g., breakfast, lunch, dinner, snack/beverage) or sociodemographic subgroups, nor were nutrient data available by daily eating frequency. Furthermore, the NFCS had a response rate of 31% and the results are not necessarily generalizable to all U.S. adults.

In a population-based sample in Rancho Bernardo, CA of 2,034 white men and women aged 50-89 y, 19% of the sample had a daily eating frequency of ≥ 4 (Edelstein *et al.*, 1992). Similarly, in the Norfolk cohort of the European prospective investigation into cancer (EPIC-Norfolk), involving over 14,000 men and women aged 45-75 y; approximately 10% of the sample had a daily eating frequency of ≥ 6 (Titan *et al.*, 2001). In both of these studies, daily eating frequency was assessed by a single question asking participants to estimate the number of daily meals and snacks they consume each day. Again, the discrepancy between the NHANES III results reported here may be due in part to differences in methods for deriving daily eating frequency and also due in part to the differences in geographic location and age of study populations. In our study, 22.8% of

participants aged ≥ 60 y had a daily eating frequency ≥ 6, which is similar to results reported by Edelstein et al. (1992).

The definition of an eating occasion varies widely in the literature depending on the purpose of the investigation. Often, eating occasions are classified as either meals or snacks, but the definition of what constitutes a meal or snack is not uniform. Before the effects of different food consumption patterns on health outcomes can be fully explored, there is a need to develop appropriate methods for identifying and assessing eating patterns within and between populations (Oltersdorf *et al.*, 1999). In the present study, the self-reported definitions of eating occasions collected with the 24-h dietary recall in NHANES III were used, however, perception of meals versus snacks may be different in different populations, which could explain differences between studies.

This study also answers important questions regarding the relationship of differing meal and snack patterns to macronutrient and micronutrient intakes. Because one-day dietary recall information is insufficient to represent usual dietary intake, we were careful to never compare individual intake data to other individual characteristics. We examined whether daily eating frequency is related to nutrient intake within diets, not within individuals. While others have reported macronutrient intakes by daily eating frequency, we are not aware of other studies that report micronutrient intakes by daily eating frequency.

In the study by Edelstein et al. (1992), total fat (% energy), dietary cholesterol and dietary fiber were higher with increasing daily eating frequency. In the EPIC-Norfolk study, with increased daily eating frequency higher fat (% energy) and carbohydrate (% energy) and lower protein (% energy) intakes were seen in men, while no difference in fat

(% energy) but higher carbohydrate (% energy) and lower protein (% energy) intakes were seen in women. Similarly, our study shows lower protein (% energy) and higher carbohydrate (% energy) intakes with increasing daily eating frequency, however, our results indicate lower fat intake (% energy).

The issue of diet composition by daily eating frequency is very important in studying the effects of daily eating frequency on health outcomes. Most experimental studies in healthy adults comparing serum lipid concentrations while consuming different meal frequencies show reduced concentrations of total and LDL-cholesterol (Mann, 1997). In one study in which subjects were identified as men whose usual eating habits included either six meals per day or three meals per day, and were then instructed to convert to the alternate eating pattern for 3 weeks duration, increasing meal frequency was accompanied by a significant reduction in total and LDL-cholesterol. Interestingly, however, changes in nutrient intake also occurred in the group who decreased their meal frequency. Specifically, even though subjects were instructed to change their eating frequency but not to change the composition of their diet, nutrient analysis of food records indicated an increase in protein, fat, saturated fat and alcohol (all as % energy), which may explain the changes in lipid concentrations (McGrath & Gibney, 1994).

Finally, this study is an important first step in describing meal patterns consumed by U.S. adults and understanding the nutritional importance of specific meal patterns. In particular, breakfast skippers (L, D + ≥ 2 S) had the lowest intakes of all nutrients except sodium indicating that the breakfast meal provides significant daily nutrients. This was true regardless of daily eating frequency as evidenced by the fact that all five of the most commonly reported meal patterns included a daily eating frequency of at least three, but

the breakfast skippers actually reported four or more eating occasions, whereas one of the meal patterns was comprised of only three daily eating occasions (B, L, D). This indicates that meal patterns may be better described by specific meal type rather than simple daily eating frequency. In conclusion, findings from this cross-sectional survey suggest that meal and snack patterns may be markers for macronutrient and micronutrient intakes and therefore diet quality and/or complete nutrient intakes should be considered in investigations of meal patterns and health outcomes.

Table 1. Sociodemographic and lifestyle characteristics by daily eating frequency in U.S. adults[1,2]

		Daily eating frequency					
	All	1-2	3	4	5	≥ 6	P^3
Population (%)		4.2	16.5	25.0	24.3	30.0	
Gender							
Male	47.4	4.8	16.1	24.7	23.5	30.9	0.0958
Female	52.6	3.7	16.9	25.4	24.8	29.2	
Age Group							
20-39 y	46.3	5.8	16.4	24.7	23.3	29.8	<0.0001
40-59 y	31.3	2.7	13.7	21.6	26.5	35.5	
60+ y	22.5	3.2	20.5	30.6	22.9	22.8	
Ethnicity							
White	82.7	3.0	14.2	24.0	25.1	33.7	<0.0001
Black	11.8	10.8	24.9	27.5	19.0	17.9	
Mex-Amer	5.5	8.7	26.9	28.6	19.7	16.1	
Education Level							
<12 yrs	24.7	6.9	22.5	29.4	21.1	20.2	<0.0001
12 yrs	33.8	4.3	16.2	25.1	24.3	30.1	
>12 yrs	41.5	2.6	13.1	22.4	26.1	35.8	
Income Level							
≥ 1.85 PIR[4]	30.0	7.4	21.7	28.9	19.6	22.4	<0.0001
1.86-3.5 PIR	34.0	3.6	14.3	24.5	24.9	32.7	
>3.5 PIR	36.1	2.0	13.3	22.1	27.2	35.4	
Smoking status							
Smoker	28.3	5.2	15.2	22.0	23.7	33.9	0.0001
Nonsmoker	71.7	3.9	17.0	26.3	24.4	28.5	
Alcohol intake							
Nondrinker	44.5	4.9	18.8	28.2	23.2	25.0	<0.0001
Light drinker	37.0	3.3	15.9	23.7	24.7	32.4	
Moderate	9.3	4.8	12.8	19.9	25.9	36.5	
Heavy drinker	9.3	3.8	11.9	20.5	25.5	38.4	
Vit/MinSupplement							
Yes	42.3	2.7	14.4	24.0	26.4	32.5	<0.0001
No	57.7	5.4	18.0	25.8	22.6	28.2	
Activity Level[5]							
<33rd percentile	33.5	3.6	17.3	24.8	23.8	30.5	0.0973
33-66th percentile	33.1	4.5	14.5	23.9	25.0	32.1	
>66th percentile	33.4	3.2	15.3	24.2	25.8	31.5	

[1]n=15,978; N=171,457,892 [2]Population percentage; rows may not add up to 100% because of rounding [3]Chi square test of independence [4]PIR=Poverty Income Ratio; represents a poverty index set by the federal government [5]Estimated oxygen consumption measured in metabolic equivalents

Table 2. Multivariate-adjusted[1] mean[2] nutrient intakes by daily eating frequency in U.S. adults[3]

	Daily eating frequency										
	1-2		3		4		5		≥6		*P* (Wald F)[3]
Population (%)	4.2		16.5		25.0		24.3		30.0		
Energy[4] (kcals)	1446±	60	1910±	32	2140±	25	2288±	23	2540±	35	<0.0001
Protein (% energy)	16.5±	0.5	15.9±	0.2	15.5±	0.2	15.2±	0.2	14.9±	0.1	0.0002
Carbohydrate (% energy)	44.9±	1.0	47.3±	0.4	48.8±	0.3	49.3±	0.4	51.1±	0.4	<0.0001
Total fat (% energy)	36.7±	0.9	34.9±	0.3	34.1±	0.3	34.3±	0.3	32.7±	0.3	<0.0001
Cholesterol (mg)	322±	17	311±	9	294±	7	291±	7	261±	5	0.0001
Vitamin B6 (mg)	1.86±	0.06	1.96±	0.03	1.96±	0.03	1.89±	0.02	1.96±	0.02	0.0794
Folic acid (mcg)	258±	9	286±	6	302±	7	289±	5	302±	4	0.0007
Vitamin C (mg)	91.7±	5.8	102.5±	3.3	109.2±	3.3	105.1±	3.3	111.3±	3.3	0.0222
Calcium (mg)	778±	32	851±	13	848±	17	866±	19	887±	12	0.0304
Magnesium (mg)	279±	5	296±	3	306±	3	312±	3	330±	3	<0.0001
Iron (mg)	14.5±	0.4	15.3±	0.3	16.3±	0.4	16.0±	0.3	16.4±	0.3	0.0014
Sodium (mg)	3765±	105	3690±	47	3659±	43	3627±	34	3500±	27	0.0011
Potassium (mg)	2751±	58	2850±	37	2916±	28	2944±	25	3088±	28	<0.0001
Dietary fiber (gm)	15.5±	0.4	16.6±	0.2	17.2±	0.3	17.2±	0.3	17.6±	0.2	0.0002

[1]Adjusted for: sex (M/F); ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American); smoking status (yes/no); alcohol intake (nondrinker, light drinker, moderate drinker, heavy drinker); vitamin/mineral supplement use (yes/no); age; BMI; physical activity; income; and energy intake [2]Mean ± SE [3]n=10,893 ;N=127,782,267 [4]Adjusted for all variables listed in footnote 1 except energy intake

Table 3. Sociodemographic and lifestyle characteristics by selected meal patterns in U.S. adults[1,2,3]

	All	B, L, D + ≥ 2 S	B, L, D +1 S	B, L + ≥ 2 S	B, L, D only	L, D + ≥ 2 S	Other	p^4
Population (%)		31.6	15.4	13.1	8.3	7.6	24.1	
Gender								
Male	47.4	29.4	13.7	14.3	7.8	8.5	26.3	<0.0001
Female	52.6	33.6	16.9	12.0	8.6	6.8	22.2	
Age Group								
20-39 y	46.3	28.5	13.0	12.5	6.9	9.9	29.3	<0.0001
40-59 y	31.3	37.2	14.2	14.0	6.9	8.0	19.7	
60+ y	22.5	30.2	21.8	13.0	13.0	2.3	19.8	
Ethnicity								
White	82.7	35.7	16.0	13.4	7.9	8.0	19.0	<0.0001
Black	11.8	16.1	12.1	13.8	8.1	6.5	43.3	
Mex-Amer	5.5	15.3	13.4	8.0	10.7	6.9	45.6	
Education Level								
<12 yrs	24.7	19.9	16.3	13.6	10.3	5.8	34.2	<0.0001
12 yrs	33.8	30.4	15.5	13.4	8.2	8.3	24.1	
>12 yrs	41.5	39.5	14.8	12.6	7.0	8.1	18.1	
Income Level								
≥ 1.85 PIR[5]	30.0	20.3	15.3	12.4	9.3	7.1	35.8	<0.0001
1.86-3.5 PIR	34.0	33.8	14.9	14.2	7.7	8.2	21.3	
>3.5 PIR	36.1	40.3	15.7	12.4	7.5	8.2	16.0	
Smoking status								
Smoker	28.3	27.8	10.3	15.8	6.3	10.8	28.9	<0.0001
Nonsmoker	71.7	33.1	17.4	12.0	9.0	6.3	22.2	
Alcohol intake								
Nondrinker	44.5	29.9	17.2	11.6	10.1	6.0	24.6	<0.0001
Light drinker	37.0	31.8	14.5	13.7	6.9	9.2	24.0	
Moderate	9.3	37.6	10.5	15.9	7.3	8.2	20.6	
Heavy drinker	9.3	33.4	11.7	15.2	6.1	8.2	25.3	
Vit/MinSupplement								
Yes	42.3	36.6	15.8	14.2	8.1	6.4	18.9	<0.0001
No	57.7	27.9	15.1	12.3	8.4	8.5	27.9	
Activity Level[6]								
<33rd percentile	33.5	31.2	14.7	12.8	9.1	8.4	23.9	0.0003
33-66th percentile	33.1	34.6	14.5	12.1	6.9	8.6	23.2	
>66th percentile	33.4	33.3	15.8	15.3	7.9	6.4	21.4	

[1]n=15,978; Weighted N=171,457,892 [2]Population percentage; rows may not add up to 100% because of rounding [3]B=breakfast; L=lunch; D=dinner; S=snack/beverage [4]Chi square test of independence [5]PIR=Poverty Income Ratio; represents a poverty index set by the federal government [6]Estimated oxygen consumption measured in metabolic equivalents

Table 4. Multivariate-adjusted[1] mean[2] nutrient intakes by selected meal patterns in U.S. adults[3,4]

	B, L, D + ≥ 2 S	B, L, D + 1 S	B, D + ≥ 2 S	B, L, D only	L, D + ≥ 2 S	*P* (Wald F)[5]
Population (%)	31.6	15.4	13.1	8.3	7.6	
Energy (kcals)[5]	2461 ± 25.4	2214 ± 32.9	2248 ± 47.7	2009 ± 46.2	2263 ± 57.9	<0.0001
Protein (% energy)	15.3 ± 0.11	16.0 ± 0.19	14.3 ± 0.16	16.4 ± 0.26	15.4 ± 0.27	<0.0001
Carbohydrate (% energy)	50.6 ± 0.35	48.9 ± 0.38	49.3 ± 0.56	47.2 ± 0.49	48.6 ± 0.6	<0.0001
Total fat (% energy)	33.4 ± 0.3	34.4 ± 0.36	34 ± 0.46	35 ± 0.43	34.1 ± 0.45	0.0015
Cholesterol (mg)	269 ± 4.46	299 ± 8.8	321 ± 9.21	323 ± 10.2	256 ± 8.05	<0.0001
Vitamin B6 (mg)	2.07 ± 0.03	2.09 ± 0.05	1.93 ± 0.03	2.10 ± 0.05	1.83 ± 0.04	<0.0001
Folic acid (mcg)	322 ± 4.69	327 ± 9.66	299 ± 6.41	314 ± 10.3	252 ± 7.29	<0.0001
Vitamin C (mg)	116 ± 3.12	117 ± 4.04	111 ± 4.46	112 ± 4.94	97.6 ± 4.19	0.0055
Calcium (mg)	942 ± 13.5	921 ± 26.5	848 ± 15.6	923 ± 17.9	818 ± 26.8	<0.0001
Magnesium (mg)	339 ± 3.01	327 ± 4.32	320 ± 4.77	312 ± 3.92	310 ± 5.79	<0.0001
Iron (mg)	17.5 ± 0.29	17.6 ± 0.46	16 ± 0.4	16.7 ± 0.43	14.5 ± 0.25	<0.0001
Sodium (mg)	3685 ± 28.5	3889 ± 53.3	3536 ± 56.4	3946 ± 48.4	3810 ± 66.5	<0.0001
Potassium (mg)	3177 ± 23.4	3112 ± 35.5	3026 ± 41.9	3025 ± 41.3	2995 ± 67.4	0.0001
Dietary fiber (gm)	18.6 ± 0.2	18.6 ± 0.41	16.9 ± 0.36	17.4 ± 0.36	16.8 ± 0.37	<0.0001

[1]Adjusted for: sex (M/F); ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American); smoking status (yes/no); alcohol intake (nondrinker, light drinker, moderate drinker, heavy drinker); vitamin/mineral supplement use (yes/no); age; BMI; physical activity; income; and energy intake [2]Mean ± SE [3]n=7,502; N=100,232,781 [4]B=breakfast; L=lunch; D=dinner; S=snack/beverage [5]B,L,D only group used as reference group [5]Adjusted for all variables listed in footnote 1 except energy intake

Chapter 5

THE RELATION OF MEAL AND SNACK FREQUENCY TO DIETARY PATTERNS AND CARDIOVASCULAR DISEASE RISK FACTORS IN HEALTHY U.S. ADULTS

A. Abstract

Dietary patterns based on food groupings have been associated with risk factors for cardiovascular disease (CVD), but may not represent the entire relationship between dietary intake and CVD risk. Daily eating frequency has also been associated with risk factors for CVD in specific populations but may be a marker for nutrient intake, dietary patterns, or both. Hence, this study examined the associations between daily eating frequency and both risk factors for CVD and dietary patterns in healthy U.S. adults. Subjects included were from the third National Health and Nutrition Examination Survey (NHANES III) (≥20y; n=10,427). Subjects excluded were: pregnant (n=288) and lactating (n=95) women, individuals on drugs for hyperlipidemia or unspecified heart disease (n=1,251), individuals told by a physician that they have diabetes (n=1,498), those who reported changing their diet in the past year for any reason (n=3,227), those with implausibly low (n=1,512) or high (n=6) reported energy intakes, and those without complete and reliable dietary data as reported by the NCHS (n=2,147). The survey design and sample weights were accounted for in all statistical analyses. Self-reported eating occasions were categorized by daily frequency (1-2; 3; 4; 5; and ≥ 6). After controlling for confounding variables in multivariate analyses, daily eating frequency was positively associated with serum C-peptide concentrations ($p<0.05$). Daily eating frequency was

positively associated with an "American-healthy" dietary pattern but not related to a "Western" dietary pattern. After further controlling for the American-healthy dietary pattern, the relationship between daily eating frequency and serum C-peptide concentrations was no longer statistically significant (p=0.0871). In this cross-sectional national probability sample, daily eating frequency was associated with serum C-peptide concentrations but may only be a marker for a healthy dietary pattern.

B. Introduction

Dietary patterns based on food groupings have been associated with cardiovascular disease (CVD) and CVD risk factors in specific sub-population groups (Hu *et al.*, 2000; Fung *et al.*, 2001a; Fung *et al.*, 2001b) and in nationally representative populations (Chapter 3). Dimensions of dietary patterns other than food intake frequencies and their intercorrelations (e.g., daily eating frequency), however, may provide additional information regarding the relationship between dietary intake and CVD risk (Tseng, 1999). Daily eating frequency has also been associated with risk factors for CVD in specific populations (Edelstein *et al.*, 1992; Redondo *et al.*, 1997; Titan *et al.*, 2001) but may be a marker for nutrient intake, dietary patterns, or both.

The issue of diet composition by daily eating frequency is very important in studying the effects of daily eating frequency on health outcomes. In free-living populations, nutrient intakes may vary depending on meal or snacking habits (e.g., eating frequency, breakfast skipping). In fact, increased daily eating frequency was associated with higher intakes of energy, fat, carbohydrate, and protein in the Norfolk population of the European prospective investigation into cancer (EPIC-Norfolk), however, more favorable lipid concentrations were still associated with increasing meal frequency after

adjusting for macronutrient intake and other confounding variables in multivariate analyses. Many of these aforementioned studies adjusted for energy and macronutrient intakes but none further assessed the diet quality or micronutrient intakes associated with increasing meal frequency even though snacks have been shown to contribute significant energy and nutrients to overall dietary intakes in selected populations (Cross *et al.*, 1994; Cross *et al.*, 1995; Haveman-Nies *et al.*, 1998; Siega-Riz *et al.*, 1998).

In U.S. adults, more frequent eaters have been shown to be more likely to be white, older, smokers, heavy drinkers, vitamin/mineral supplement users, with lower BMI, higher education levels, and higher incomes than less frequent eaters. After controlling for confounding variables, daily eating frequency has also been shown to be positively related to carbohydrate (% energy), folic acid, vitamin C, calcium, magnesium, iron, potassium, and fiber intakes and inversely related to protein (% energy), total fat (% energy), cholesterol, and sodium intakes (Chapter 4). Most observational and experimental studies of daily eating frequency and health outcomes control for the effects of macronutrient composition, but not the effects of dietary patterns, diet quality and/or micronutrient composition. This study tested the hypothesis that daily eating frequency is associated with biomarkers for CVD and specific dietary patterns.

C. Subjects and methods

Dataset

Subjects in this study were participants in the third National Health and Nutrition Examination Survey (NHANES III), 1988-94. The National Center for Health Statistics (NCHS) conducted the survey to obtain nationally representative information on the health and nutritional status of the U.S. population. The NHANES III sample represents

the total civilian, noninstitutionalized population, two months of age or over, in the 50 states and the District of Columbia of the United States. In NHANES III, 39,695 persons were selected over the six years. Of those, 33,994 (86%) were interviewed in their homes and provided information for the Household Adult Questionnaire and the Dietary Food Frequency Questionnaire (ages 17 years and over). All interviewed persons were invited to the Mobile Examination Center, where blood and urine specimens were obtained, and a number of tests and measurements were performed including body measurements and blood pressure testing (U.S. DHHS, 1996).

Analytic sample

Individuals included in this study were adults ≥ 20 yrs (n=10,427). Subjects excluded were: pregnant (n=288) and lactating (n=95) women, individuals on drugs for hyperlipidemia or unspecified heart disease (n=1,251), individuals told by a physician that they have diabetes (n=1,498), those who reported changing their diet in the past year for any reason (n=3,227), those with implausibly low (n=1,512) or high (n=6) reported energy intakes, and those without complete and reliable dietary data as reported by the NCHS (n=2,147). Cut-off values for implausibly low or high energy intakes were determined based on the distribution of the data. Reported energy intakes of less than 15 kcals per kg body weight or higher than 10,000 kcals per day were considered implausibly low and high intakes, respectively.

Dietary Assessment

Assessment of daily eating frequency has been described in detail elsewhere (Chapter 4). Briefly, the 24-hour dietary recall records (from NHANES III) were used to determine daily eating frequency. Respondents reported all foods and beverages

consumed except plain drinking water for the previous 24-hour time period (midnight to midnight). Subjects were divided into groups based on total daily eating occasions (1-2; 3; 4; 5; ≥ 6). The plausibility of energy intakes was determined based upon the distribution of the data and prior literature. Subjects who consumed less than 15 kcals per kg of body weight were thought to have implausibly low energy intakes, while those reporting more than 10,000 kcals were considered to have implausibly high energy intakes.

Dietary Pattern Assessment

Methods for derivation of major dietary patterns have been reported in detail elsewhere (Chapter 3). Briefly, dietary patterns of healthy U.S. adults (≥20y; n=13,130) were described by factor analysis of the food-frequency questionnaire (FFQ) from the third National Health and Nutrition Examination Survey (NHANES III) while accounting for the survey design and sample weights. Major dietary patterns included high intakes of: 1) processed meats, eggs, red meats, and high-fat dairy products ("Western"); and 2) green, leafy vegetables, salad dressings, tomatoes, other vegetables, cruciferous vegetables, and tea ("American-healthy").

Factor analysis (principal component) was used to derive food patterns based on 35 predefined food groups collapsed from the 62 food groups available from the FFQ. The analysis was conducted using the FACTOR PROCEDURE in SAS. In order to account for the complex survey design of NHANES III, a correlation matrix was created from the weighted data on the 35 food groups after pooling the variance within the sample strata using PROC GLM in SAS. Next, a data step was performed to read the correlation matrix directly into the FACTOR PROCEDURE in SAS. The factors were

orthogonally transformed using varimax rotation to achieve a structure with independent (nonoverlapping) factors. Each individual has a factor score for both the Western and the American-healthy dietary patterns.

Blood Collection

Laboratory testing in NHANES III included determination of serum total cholesterol, HDL-C, and triacylglycerol concentrations in all adults. LDL-C was calculated for sample persons who reported fasting for ≥ 9 hours and who had triacylglycerol concentrations ≤ 400 mg/dL (4.52 mmol/L) by using the equation developed by Friedewald et al. In these analyses, triacylglycerol measurements were included only in subjects who reported fasting for ≥ 9 hours. This is consistent with the guidelines recommended by the third National Cholesterol Education Program Adult Treatment Panel for lipoprotein analysis. Serum homocysteine, serum folate, red blood cell folate, serum C-peptide, serum insulin, and serum C-reactive protein concentrations, as well as glycosylated hemoglobin and blood pressure were measured in all adults. Serum homocysteine was determined only in phase 2 of the survey (1991-1994). Lipoprotein(a) and plasma fibrinogen were measured only in subjects aged 40 years and older.

Data Analyses

Statistical software. The survey was conducted in a stratified, multi-stage probability design, thus traditional methods of statistical analysis based on the assumption of a simple random sample are not applicable. As recommended by the NCHS, SUDAAN (Shah, 1995) software (version 8.0) was used to estimate statistics of interest and the associated variance. Appropriate sample weights were applied in all

analyses to produce estimates of means and percentiles that can be generalized to the U.S. population. Data preparation was performed using SAS software (version 8.01). Descriptive and inferential statistical analyses were performed using SUDAAN software (version 8.0).

Statistical methods. Percentage and standard error of means were calculated by the linearization (Taylor series) variance estimation method for population parameters. Ratio scale variables were assessed using Wald F tests for determination of significance between means of biomarkers by daily eating frequency.

Linear regression analyses were conducted between biomarkers of CVD risk and daily eating frequency while controlling for confounding variables. Possible confounding variables included based on the current literature were: age, gender (M/F), ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American), smoking status (yes/no), alcohol intake (nondrinker—0 drinks/day, light—>0 to ½ drink/day, moderate—½ to <2 drinks/day, or heavy drinker—≥2 drinks/day), vitamin/mineral supplement use (yes/no), BMI, physical activity (summation of the frequency of multiple leisure-time activities multiplied by the respective estimated oxygen consumption of each activity), income poverty income ratio calculated as the ratio of family income to a Census Bureau-determined poverty threshold), and energy intake. Linear regression analyses were also conducted between factor scores of the Western and American-healthy dietary patterns and daily eating frequency while controlling for age and energy intakes.

D. Results

After controlling for confounding variables in multivariate analyses, daily eating frequency was positively associated with serum C-peptide concentrations (Table 1), but

not other biomarkers of CVD risk. Additionally, systolic blood pressure, serum lipoprotein(a), plasma fibrinogen, serum C-reactive protein, serum triacylglycerol, and serum homocysteine were not found to be associated with daily eating frequency (data not shown). Daily eating frequency was not related to a "Western" dietary pattern after adjusting for age and energy intake (Table 2), however, daily eating frequency was positively associated with an "American-healthy" dietary pattern ($p<0.0001$) (Table 3). After further controlling for the American-healthy dietary pattern, the relationship between daily eating frequency and serum C-peptide concentrations was no longer statistically significant ($p=0.0871$).

E. Discussion

Evidence from short-term studies suggests that increased daily eating frequency may be beneficial for blood glucose control in healthy individuals and those with type 2 diabetes by reducing insulin secretion while maintaining blood glucose concentrations (Jenkins *et al.*, 1989; Jenkins *et al.*, 1992). This finding may help define a mechanism for the association of decreased serum lipid concentrations seen with increased meal frequency in epidemiologic observations (Edelstein *et al.*, 1992; Redondo *et al.*, 1997; Titan *et al.*, 2001). Insulin stimulates hydroxymethyglutaryl-CoA (HMGCoA) reductase, which is the rate-limiting enzyme in hepatic cholesterol synthesis. Additionally, removal of cholesterol via reverse cholesterol transport occurs only in the postprandial phase and therefore, may be facilitated when meal frequency is increased (Mann, 1997). However, after controlling for confounding variables, our results indicate a significant relationship between daily eating frequency and serum C-peptide concentrations only. None of the other biomarkers examined were significantly related to daily eating frequency.

Furthermore, after we controlled for the effects of a healthy dietary pattern, the relationship between daily eating frequency and serum C-peptide concentrations was no longer statistically significant. This implies that daily eating frequency may be a marker of nutrient intakes (Chapter 4) and entire dietary patterns.

Epidemiological studies have shown an inverse relationship between meal frequency and body weight. However, a review of related literature concluded that this finding is likely due to changes in dietary intake as a consequence of weight gain or dietary under-reporting of overweight persons (Bellisle *et al.*, 1997). The conclusion is based in part on the outcome of studies using the rigorous methods of whole-body calorimetry and doubly labelled water, which found no difference in 24h energy expenditure between nibbling and gorging. It appears that any effects of daily eating frequency on the regulation of body weight are likely to be because those who eat more often tend to consume fewer calories, but this has not been examined in depth.

Since these issues could also confound a relationship between daily eating frequency and biomarkers for CVD risk, we addressed the issue of possible confounding effects of post hoc dietary changes by excluding all individuals who reported changing their diet in the past year for any reason. Additionally, we addressed the issue of confounding by dietary-underreporting by excluding all individuals who reported consuming implausibly low caloric intakes for their body weight. Further, we controlled for characteristics that are associated with dietary under-reporting in our multivariate analyses (i.e. gender and BMI) (Briefel *et al.*, 1997). The present analyses only utilized group data in any comparisons between the one day dietary recall data and individual

characteristics (i.e. sociodemographics, lifestyle characteristics, biomarkers, dietary pattern scores).

In this cross-sectional national probability sample, daily eating frequency was associated with serum C-peptide concentrations, which is not inconsistent with experimental studies of eating frequency and markers of glucose metabolism (Jenkins, 1997). However, daily eating frequency was also associated with a dietary pattern characterized by high intakes of green, leafy vegetables, salad dressings, tomatoes, other vegetables, cruciferous vegetables, and tea and when analyses were further controlled for this dietary pattern the relationship between daily eating frequency and serum C-peptide concentrations was attenuated. Thus, daily eating frequency may be a marker for a healthy dietary pattern.

Table 1. Mulitvariate-adjusted[1] mean[2] biomarker values by daily eating frequency in healthy U.S. adults[3]

Biomarker	n	1-2	3	4	5	≥ 6	*P* (Wald F)
BMI (kg/m^2)	7,205	26.3	25.8	25.6	25.4	25.4	0.4924
		(24.7 28.0)	(25.3 26.4)	(25.3 26.0)	(25.1 25.7)	(25.1 25.7)	
TC (mmol/L)	6,908	5.13	5.08	5.16	5.16	5.18	0.1659
		(4.93 5.33)	(4.99 5.17)	(5.08 5.24)	(5.08 5.24)	(5.14 5.23)	
HDL-C (mmol/L)	6,867	1.38	1.33	1.31	1.33	1.33	0.4218
		(1.31 1.44)	(1.30 1.36)	(1.29 1.34)	(1.30 1.36)	(1.31 1.36)	
LDL-C (mmol/L)[4]	3,057	3.10	3.20	3.26	3.19	3.20	0.6760
		(2.89 3.32)	(3.08 3.31)	(3.16 3.35)	(3.11 3.27)	(3.11 3.29)	
RBC folate (nmol/L)[5]	6,923	381	387	388	401	399	0.1259
		(360 403)	(371 404)	(376 400)	(387 414)	(389 409)	
Glycosylated Hgb (%)[5]	6,973	5.14	5.14	5.15	5.16	5.18	0.6448
		(5.05 5.23)	(5.10 5.19)	(5.10 5.20)	(5.11 5.22)	(5.14 5.22)	
C-peptide (nmol/L)[5]	6,941	0.51	0.52	0.50	0.50	0.48	0.0455
		(0.46 0.57)	(0.50 0.54)	(0.48 0.52)	(0.48 0.52)	(0.46 0.49)	
Insulin (pmol/L)[5]	6,924	47.8	48.4	48.0	47.5	46.2	0.2299
		(45.3 50.5)	(45.9 51.0)	(46.2 49.8)	(45.5 49.6)	(44.5 47.9)	

[1]Adjusted for: sex (M/F); ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American); smoking status (yes/no); alcohol intake (nondrinker, light drinker, moderate drinker, heavy drinker); vitamin/mineral supplement use (yes/no); age; BMI (all models except where BMI is outcome); physical activity; income [2]Mean ± 95% confidence interval [3]n size varies by biomarker; all significance testing based on weighted data [4]Calculated by the Friedewald equation only on subjects who fasted ≥ 9 hrs and had serum TG concentrations ≤ 400 mg/dL
[5]Test for significance performed on log-transformed biomarker values

Table 2. Regression model for Western dietary pattern by eating frequency

Category	Variable	$\beta^1 \pm$ SE		P^2
Eating frequency	1-2	0		
	3	0.01	0.06	0.8189
	4	-0.02	0.05	0.7617
	5	-0.03	0.06	0.6411
	≥ 6	-0.02	0.06	0.7633
Age (yrs)		<-0.01	<0.01	0.2849
Calories (kcal)		<0.01	<0.01	< 0.0001

[1]Regression coefficient

[2]*P* for comparison between group with β=0 and other groups within category

Table 3. Regression model for American-healthy dietary pattern by eating frequency

Category	Variable	$\beta^1 \pm$ SE		P^2
Eating frequency	1-2	0		
	3	0.07	0.09	0.4065
	4	0.27	0.08	0.0009
	5	0.32	0.08	0.0001
	≥ 6	0.43	0.08	<0.0001
Age (yrs)		0.01	<0.01	<0.0001
Calories (kcal)		<-0.01	<0.01	0.0246

[1]Regression coefficient

[2]*P* for comparison between group with β=0 and other groups within category

Chapter 6

CONCLUSION

A. Implications

Major and minor dietary patterns of U.S. adults were identified using multivariate data reduction techniques. After statistically controlling for many potentially confounding variables, dietary patterns alone were significant predictors of biomarkers for CVD risk. The dietary patterns identified here are similar to those reported in other nonrepresentative samples and are associated with biomarkers of CVD risk which supports the use of dietary patterns in guiding public health recommendations for dietary prevention of chronic disease.

These results are also in support of Tseng's description of 2 fundamental U.S. dietary patterns that she posits are a result of British culinary heritage (Western) and nutrition science, industry, and government efforts to promote diets that prevent illness (American-healthy) (Tseng & DeVellis, 2001). The concept of two fundamental U.S. dietary patterns is also in agreement with other research conducted in the Food and Nutrition Database Research Center by Yang (unpublished data) on dietary intake of Korean Americans. Korean Americans were shown to have dietary patterns classified as "westernized" or "American-healthy," both of which showed acculturation from the traditional Korean diet, but in different qualitative directions. The extension of this theory is that nutrition intervention efforts do make a difference. The challenge to researchers is to identify core aspects of dietary patterns as targets and then practitioners can develop menus around those targets.

While the Western dietary pattern was associated with biomarkers for CVD, the American-healthy dietary pattern was not, which is not surprising because the dietary patterns described here were based on actual intake and not ideal intake patterns. The American-healthy diet described here is less than ideal in part because it does not contain high amounts of foods thought to be beneficial in the prevention of chronic disease (e.g., fish, legumes, dairy products). We labeled this diet "American-healthy," because we think it is a good representation of the foods that Americans perceived as healthy during the time period of data collection (1988-1994). The perception of a healthy diet versus an actual "ideal" diet may be related to the fact that 77% of Americans believe that there are "good" and "bad" foods (Freeland-Graves & Nitzke, 2002), which may in turn stem from "reductionism and the narrowing nutrition perspective" (Messina *et al.*, 2001). This implies that there is a disconnect between the messages nutrition educators are trying to disperse and the messages that consumers are perceiving.

The results presented here also provide descriptive information regarding meal and snack patterns (including daily eating frequency) of U.S. adults. After controlling for confounding variables in multivariate analyses, daily eating frequency was significantly related to macronutrient and micronutrient intakes in healthy U.S. adults. Daily eating frequency was positively associated with serum C-peptide concentrations and also with an "American-healthy" dietary pattern but not related to a "Western" dietary pattern. After further controlling for the American-healthy dietary pattern, the relationship between daily eating frequency and serum C-peptide concentrations was no longer statistically significant, indicating that daily eating frequency may only be a marker for a healthy dietary pattern. Diligence is required to capture accurate diet-disease

relationships without assuming a causal relationship between a dietary factor and a diet-associated biomarker of risk.

B. Recommendations for future research

Further research should explore the minor dietary patterns identified here. Even though the dietary patterns that emerge may be similar enough in all adult subgroups to use one factor analysis, the *relationship* between the factor scores and the biomarkers of CVD risk may be different. Exploration of dietary patterns in relation to biomarkers for CVD risk in sub-population groups, including adolescents and children may provide new insights into the relationships between dietary patterns and chronic disease risk.

Using current nutrition knowledge, an in-depth examination of each pattern in terms of what it does or does not contain that may be harmful or beneficial and how the harmful and beneficial components displace each other in diets of free-living people is warranted. The importance of not only the presence of harmful foods, but also the absence of beneficial foods in the Western dietary pattern is confirmed by the low RBC folate concentrations. Additional research on dietary patterns may explore the possibility that animal food is displacing plant food in U.S. diets. Furthermore, any description of an ideal dietary pattern using the inclusionary food approach must consider if all foods will fit into a diet without excessive energy content (Kris-Etherton *et al.*, 2002).

Additionally, we need to explore whether or not the dietary pattern itself is a marker for an entire healthy lifestyle. Even though we statistically controlled for many confounding variables, we still acknowledge the possibility in observational research that other unknown confounders exist. As such, we may find a psychological determinant that predicts the healthy lifestyle behaviors, but ultimately, we need to establish a

definition of healthy dietary patterns so that we can educate people about the composition and benefits. Yet other research may seek a way to convert consumers of the traditional dietary pattern to a healthier dietary pattern.

Even though it appears that eating frequency may be related to biomarkers for CVD risk simply because it is a surrogate marker for nutrient intake and/or dietary patterns, other factors such as who you eat with and where you eat should be explored in order to fully characterize dietary patterns. Future research should include further analysis of specific foods eaten at the same meal together (e.g. iron fortified cold breakfast cereal with orange juice) and the effects on health consequences.

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