AN ABSTRACT OF THE THESIS OF

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Title: Correlation of Habitual Diet with Plasma Risk Factors for Coronary Heart Disease

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The statistical correlations between habitual diet and plasma risk factors for coronary heart disease CHD were analyzed using multiple regression. Thirty-one male subjects between 30-56 years kept complete dietary records for 7 days. Daily means of nutrient consumption were calculated using a computerized data base. Fourteen independent variables (total kilocalories, protein %, carbohydrate %, fat %, ethanol %, caffeine, P/S ratio, cholesterol, age, weight, height , weight**.75, Body Mass Index, kilocalories/weight**.75) were created. Plasma samples were analyzed and the following simple (total plasma cholesterol (TC), VLDL-C, LDL-C, HDL2-C, HDL3-C, apo A-I, apo A-II, apo B) and derived (VLDL-C+LDL-C, LDL-C/TC, LDL-C/HDL-C, HDL-C, HDL2-C/HDL3-C, HDL-C/TC, apo B/apo A-I, apo B/apo A-II, apo A-II/apo A-I) dependent variables were created. Dependent variables were individually regressed

against the entire set of independent variables. An F-value of 4.00 to enter an independent variable in the model and of 3.99 to remove one were used to achieve significance of $p<05$.

Age appeared in 5 regression models (TC, apo B, apo B/apo A-I, apo B/apo A-II, apo A-II/apo A-I) and was positively correlated with increased risk for CHD. Total kilocalories appeared in 4 models (LDL-C, apo B, VLDL-C+LDL-C, LDL/TC) and was negatively correlated with risk. Fat % appeared in 4 models (VLDL-C+LDL-C, LDL/TC, LDL-C/HDL-C, HDL-C/TC) and was associated with increased risk. Body Mass Index was entered in 2 models (HDL2-C, HDL-C) and was positively correlated with risk. P/S ratio was negatively correlated with risk in the three models (HDL2, apo A-I, HDL-C) in which it appeared. The independent variable carbohydrate % was negatively associated with risk in 2 models, LDL-C and apo B. Kcal/wt**.75 was also negatively correlated with risk in the VLDL-C, LDL-C/TC and HDL-C/TC models. One independent variable, EtOH %, was positively associated with risk in the apo A-II and apo A-II/apo A-I models. Two dependent variables did not have any independent predictors (HDL3-C, HDL2-C/HDL3-C) entered in their regression models.

Six independent variables did not appear in any regression model (protein %, caffeine, cholesterol, weight, height, weight**.75). Independent variables positively correlated with increased risk for CHD were therefore fat %, ethanol %, age, and Body Mass Index. Independent variables correlated with decreased risk for CHD were total kilocalories, carbohydrate %, P/S ratio, and kilocalories/weight**.75.

Correlation of Habitual Diet with Plasma Risk Factors for Coronary Heart Disease

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TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS USED IN THIS THESIS

ANOVA	-	analysis of variance
Apo A-I	-	apolipoprotein A-I
Apo A-II	-	apolipoprotein A-II
Apo B	-	apolipoprotein B
BMI	-	body mass index
CHD	-	coronary heart disease
ETOH	-	ethanol
HDL	-	high density lipoprotein
HDL-C	-	high density lipoprotein cholesterol
Kcal	-	kilocalories
LCR-CPPT	-	Lipid Research Clinics Coronary Primary Prevention Test
LDL	-	low density lipoprotein
LDL-C	-	low density lipoprotein cholesterol
MI	-	myocardial infarction
PS	-	polyunsaturated fatty acids
P/S	-	polyunsaturated fatty acids / saturated fatty acids
SEM	-	standard error of the mean
TC	-	total blood cholesterol
VLDL-C	-	very low density lipoprotein cholesterol

CORRELATION OF HABITUAL DIET WITH PLASMA RISK FACTORS FOR CORONARY HEART DISEASE

INTRODUCTION

The development of analytical laboratory techniques has resulted in progressively more precise assessment of risk for coronary heart disease (CHD) and related circulatory diseases. As these diseases presently are the leading cause of mortality in Western societies (Levy 1981), routine assesment of risk by clinically available analysis is desirable (Castelli 1984). Intervention to alter these risk factors has been shown to result in an actual reduction of risk.

Total serum or plasma cholesterol and the relative amounts of cholesterol in various plasma lipoprotein fractions have been used as discriminators of risk. More recently the apoprotein moieties of lipoproteins have been used as better discriminators between persons with or without CHD (Avogaro et al. 1979; DeBacher et al. 1982). Using various ratios and combinations of the above parameters has resulted in improved discrimination of risk and/or presence of disease.

Many studies have been done on large populations to assess the correlation of habitual dietary intake with plasma risk factors for CHD. Usually, however, dietary intake assessment has been indirect, either accomplished through dietary recall methods or food

disappearance data. Many metabolic studies have likewise been performed with precise regulation of nutrient intake and measurement of plasma parameters.

The new analytical techniques of apoprotein measurement, in addition to plasma and lipoprotein cholesterol measurements, coupled with precise assessment of habitual dietary intake would yield new data on the relationships between habitual diet and plasma risk factors for CHD. The present study is an attempt to produce such data. New relationships suggested would then be available for mechanistic explanations by future investigators.

The purpose of this study was to correlate plasma risk factors for CHD with habitual dietary intake of middle-aged men. Further, any such correlations could refute or support recent recommendations to 1) lower total fat intake, 2) increase the P/S ratio of this dietary fat, and 3) to maintain ideal body weight to protect populations from CHD (Senate Select Committee on Nutrition and Human Needs 1977; NAS-NRC 1980). The data used were obtained from the baseline data on middle-aged men participating in one or two studies on the effects of egg supplementation on plasma lipids (Sanders 1984).

REVIEW OF LITERATURE

I. Plasma Risk Factors

A. Total Plasma Cholesterol

The first plasma risk factor for CHD to be identified was total cholesterol. Many epidemiological studies have proven that populations with higher plasma cholesterol have increased risk for CHD (Gotto and Jackson 1978). However, the overlap in cholesterol levels between those who develop CHD and individuals who do not is considerable. In the Framingham study, the means and standard deviations of serum cholesterol values in subjects who were either free from or were CHD patients were 219+41 mg/dl and 244+51 mg/dl, respectively (Castelli 1984). The large standard deviations and closeness of the means reduce the usefulness of total plasma cholesterol (TC) as a clinical discriminator of CHD in this study.

B. LDL Cholesterol

1. Epidemiological Studies

Since low density lipoprotein cholesterol (LDL-C) closely mirrors TC, its level is also a good predictor of CHD. It is also an independent predictor. Rhoads et al. (1976) found a positive correlation between LDL-C and CHD in a population of Hawaiian

Japanese men 50 to 72 years old. In the Framingham study, Gordon et al. (1977a) found a positive association ($p<.05$) of LDL-C with CHD in a study of 2815 men and women aged 49 to 82 years. Of these subjects, 79 of the 1025 men and 63 of th 1445 women had CHD. The same researchers found a regression coefficient of .332 for LDL with CHD in the same study. Miller et al.(1977) found that cholesterol content of $d<1.063$ (includes VLDL-C) was positively correlated with incidence of CHD in a case-control follow-up study of 6595 men aged 20-49 years living in Tromso, Norway.

2. Clinical Studies

Another approach to risk discrimination was to clinically assess CHD and find correlations between presence of CHD and its severity and plasma lipid values. Of 63 patients who underwent coronary angiography, 38 had stenoses. Those with the disease had higher LDL levels (Reisen et al. 1980). Sniderman et al. (1980) found significant differences in LDL cholesterol betweeen the 59 patients with angiographically defined CHD and 31 controls. Miller and Miller (1978) also found higher LDL-C levels in those with angiographically defined CHD of 104 men aged 35-65 years. Whayne et al. (1981) found LDL was one of the variables which is useful for discriminating between those with and without CHD in male patients who were angiographically diagnosed. However the author found it was not as significant in those over 50 years old. Finally in Japan, Noma et al. (1983) found LDL-C was a good discriminator of the severity of CHD in

100 subjects referred for angiography for chest pain.

C. HDL Cholesterol

The next major plasma risk factor useful in prediction of risk for CHD is high density lipoprotein cholesterol (HDL-C). There were early reports of low HDL-C in atherosclerosis, but attention had been given to the cholesterol distribution in the lipoprotein classes that were elevated, since it seemed logical that they would be responsible for cholesterol delivery and subsequent deposition in the arterial intima (Gofman et al. 1966; Brunner and Lobl 1958). With the advent of the "HDL hypothesis" (Miller and Miller 1975), attention was focused on this density class. In contrast to both total cholesterol and LDL-C this risk factor was inversely correlated with CHD.

1. Myocardial infarction survivors versus controls

Berg and Borresen (1976) found the mean serum HDL-C concentrations were lower in 49 middle-aged male survivors of myocardial infarction compared to 102 healthy controls. Albers et al. (1978) reported lower HDL-C values of 39+1 mg/dl (SEM) in 90 myocardial infarction survivors than in lipid-matched control subjects with values of 43+1 mg/dl in a population whose mean HDL-C value (n=172) was 45+1 mg/dl. In myocardial infarction survivors matched for age, sex, and body mass index, DeBacher et al. (1982) found HDL-C contributed independently to risk using multivariate analysis in both

younger and older subgroups.

2. Epidemiological population studies

Miller and Miller (1975) noted that HDL-C was reduced in hypercholesterolemia, hypertriglyceridemia, male sex, obesity, and diabetes, all conditions associated with increased risk of CHD. Rhoads et al. (1976) found a higher risk in those in the upper than those in the lower quartiles of alpha (HDL) cholesterol in a population-based sample of 1859 Hawaiian Japanese men aged 50-72 years, and thus questioned the protective value of the HDL subfraction against CHD. However Castelli et al. (1977) in the cooperative lipoprotein phenotyping study, using 5 US population groups totalling nearly 7000 men and women over 39 years of age, found decreased risk of CHD with increasing HDL-C in all categories of CHD and in every age group.

In Tromso, Norway 6595 men 20-49 years old were followed for 2 years at which time 21 had coronaries and 17 were used for a prospective case-control study. The researchers found significant differences ($p<0.005$) in HDL-C, 26 and 39 mg/dl respectively between cases and controls matched for age, weight, physical activity level, smoking, and race. Their analysis showed that HDL-C was 3 times as effective as LDL-C in distinguishing between cases and controls (Miller et al. 1977).

In the Framingham study (Gordon et al. 1977b), 2815 men and women aged 49-82 years were followed between 1969 and 1971. CHD

developed in 79 of the 1025 men and in 63 of the 1445 women. The most potent risk factor to emerge was HDL-C with a significance level of $p<0.001$.

Castelli (1984) in an analysis of the epidemiology of CHD based on the Framingham study found that individuals with higher values of HDL-C run the lower rates of disease.

3. Clinical studies

Several studies have shown that both the presence and sometimes the severity of extant CHD were inversely correlated with HDL-C. Barboriak et al. (1979a) measured the HDL-C of 400 male patients referred for diagnostic angiography for either 1.unstable angina pectoris, 2. stable angina, 3. previous myocardial infarction, or 4. recurrent chest pain of unknown etiology. Dividing the HDL-C values into quartiles, they found that those with HDL-C values over 50 mg/dl had significantly lower coronary occlusion score than the patients with values less than 35 mg/dl. They also found a good correlation between HDL-C and severity of CHD. In contrast Reisen et al. (1980) observed no discriminative value of HDL-C in 63 patients undergoing coronary angiography. However, he stated that HDL-C is still of value in epidemiological studies. On the other hand, Swanson et al. (1981) stated that serum HDL-C correlates with presence but not severity of CHD. This is difficult to explain since their results show that there were significant differences in HDL-C in groups having 1, 2, or 3 vessel disease and between those with and without disease in 189

patients who underwent diagnostic coronary angiography.

In Britain, 104 men aged 35-65 years suspected of having myocardial ischemia underwent coronary angiography. The researchers found significant differences p=0.01 in HDL-C between those with high coronary scores and those with lower scores (Miller et al. 1981). Whayne et al. (1981) compared 161 male patients with angiographically documented CHD and 72 patients with normal coronary arteries. They found no difference in the mean HDL-C in these two groups of patients but did find that those with HDL-C less than 40 mg/dl tended to have more CHD. In Japan 100 male patients referred because of chest pain and suspected of myocardial infarction underwent coronary angiography. Those with disease had significantly lower plasma levels of HDL-C than those without. The coronary scores were not, however, correlated with HDL-C levels (Noma et al., 1983).

D. Risk factors using combinations of lipoprotein fractions

Since total plasma or serum cholesterol is often higher in populations at risk and the cholesterol content of various lipoprotein fractions provides further discriminative power, attention has naturally focused on ratios of total cholesterol to LDL-C or to HDL-C as well as LDL-C/HDL-C ratios.

Possibly because of limitations of methodology (paper electrophoresis for determination of fraction cholesterol content), Keys (1963) found no better discrimination with alpha/beta (HDL-C/LDL-C) lipoprotein ratios than with total cholesterol in

upper-class Minnesota business men observed prospectively. In several recent studies trying to determine relationships between angiographically documented CHD and plasma risk factors in men, Swanson et al. (1981) found that the HDL/Total cholesterol ratios were correlated with presence but not severity of CHD; Whayne et al. (1981) found no significant correlation with either TC/HDL-C or LDL-C/HDL-C. Noma et al. (1983) found LDL-C/HDL-C was a powerful discriminator for both the presence and the severity of CHD. DeBacher et al. (1982) using 70 male survivors of myocardial infarction and 70 healthy controls matched for age and body mass index, attained a 72% exact classification using HDL-C/TC. Castelli (1984) found TC/HDL-C as good a clinical tool as any in assessing risk and he suggests that patients with this value higher than 4.5 should be treated but said that LDL-C/HDL-C is probably equally as useful.

E. Apolipoproteins as risk factors

The most recent approach to assess risk for CHD is to measure the apolipoproteins (apoproteins), the protein moieties of the lipoproteins. It is believed that these values may be more consistent over time and less influenced by short term influences such as transient dietery perturbations and illness.

1. Apolipoprotein B

Apo-B, the major apoprotein of LDL, is positively correlated

with risk of CHD. In study of 218 survivors of myocardial infarction and 160 controls, Avogaro et al. (1979) found that apolipoproteins were as good as lipoproteins in discriminating the risk of developing CHD up to age 50 and better for older subjects.

Sniderman et al. (1980) studied 100 consecutive patients undergoing diagnostic angiography and found that 59 patients had significant CHD and 31 did not. The patients with CHD had Apo-B levels of 118+22 mg/dl while those without had levels of 82+22 mg/dl. They concluded that Apo-B is a better separator of those with and without CHD than other lipid parameters. Of 63 patients undergoing coronary angiography, 38 had significant (50% or higher grade) stenosis. There was a significant difference in the Apo-B levels of these 38 patients compared to normal controls (Reisen et al. 1980). In a contingency table approach, Whayne et al. (1981) found Apo-B less significant than TC or VLDL-C in discriminating between 161 male patients with angiographically documented CHD and 72 male controls. For the subset of subjects under 50 years old, Apo-B and LDL-C were the most significant variables ($p<0.05$); while in those with plasma cholesterol less than 265 mg/dl, the most reliable variable was Apo-B ($p<0.05$). They concluded that Apo-B cholesterol added important predictive value for CHD especially in those patients with lower plasma cholesterol. In a similar study, Noma et al. (1983) concluded Apo-B was a better indicator for severity than detection of CHD in 100 Japanese patients angiographically screened for presence or absence of CHD. Finally Vander Heiden et al. (1984), studying 110 male subjects referred for diagnostic angiography, found that Apo-B levels were

correlated with the extent of coronary occlusion.

2. Apolipoproteins A-I and A-II

Apolipoproteins A-I and A-II are the major apoproteins of the HDL and are therefore correlated with HDL-C levels. Albers et al. (1976) found an r=0.72 between Apo A-I and HDL-C in a sample of 263 Bell telephone company employees. The same researchers also found lower Apo A-I levels (107+16 mg/dl, $p<.01$, n=24) in myocardial infarction survivors than in normal male controls (120+20 mg/dl). Berg and Borresen (1976) found significantly lower Apo A-I levels in 49 survivors of myocardial infarction than in 102 healthy, middle-aged men from Northern Sweden. In a later study, Albers et al. (1978) found lower Apo A-I, Apo A-II and HDL-C in 90 male myocardial infarction survivors (Apo A-I 112+2 mg/dl, Apo A-II 29+1 mg/dl, HDL-C 39+1 mg/dl) than in lipid-matched controls (Apo A-I 121+2 mg/dl, Apo A-II 33+1 mg/dl, and HDL-C 43+1 mg/dl) or in a population-based control group (n=172) who had values of Apo A-I 121+2 mg/dl, Apo A-II 33+1 mg/dl and HDL-C 45+1 mg/dl.

Vergani et al. (1978) determined Apo A in 33 survivors of myocardial infarction and found significantly lower Apo A levels in cases than in controls. In a study of myocardial infarction survivors (n=25, age 40-44 years, sampled 6 months to 3.5 years post-infarction), Fager et al. (1980) found significantly lower Apo AI values in survivors than in the controls. In a later study comparing 70 male myoinfarction survivors with controls matched for age, sex and

body mass index, DeBacher et al. (1982) found significant differences between Apo A-I, 111.1+24.5 mg/dl and 124.7+21.4 mg/dl; and Apo A-II, 46.3+12.6 mg/dl and 43.0+6.9 mg/dl, respectively.

Thus both Apo A-I and possibly Apo A-II appear to be lower in myocardial infarction survivors than in controls. In studies comparing normal or matched controls to subjects with angiographically diagnosed CHD, this same trend prevails. Reisen et al. (1980) studied 63 patients undergoing coronary angiography. Of these, 38 had 50% or higher grade stenosis. They found significant differences in Apo A-I and Apo A-II concentrations between those with and those without documented CHD. However, Miller et al. (1981) found no association between coronary scores reflecting the number, degree and length of stenoses in the seven major coronary arteries and either the Apo A-I or Apo A-II levels of 104, 35-65 year old men undergoing angiography for suspected CHD. Noma et al. (1983) found significant predictive value of the apoproteins A-I and A-II in 100 patients undergoing coronary angiography for suspected myocardial infarction. They suggested that HDL-C and Apo A-I were good discriminators for CHD, while the LDL-C and Apo-B were better discriminators for diagnosing severity.

Maciejko et al. (1983), in a study designed to test whether Apo A-I or HDL-C is a better discriminator of CHD in male subjects, used 83 patients with angiographically documented CHD and compared these with 25 patients without CHD. The patients with the disease were further classified into those with single, double, or triple vessel disease. They found there were significant differences in both ApoA-I

and HDL-C between controls and those with CHD but not between the different patient classes. Both stepwise discriminative analysis and ANOVA showed the superiority of Apo A-I to HDL-C in separating cases from controls.

3. Apolipoprotein combination variables

Since Apo B is positively correlated with risk and presence of CHD and Apo A-I is negatively associated with risk, it is reasonable to use ratios of the above to see if they give more discriminative power. In a study of 218 survivors of myocardial infarction and 160 controls, Avogaro et al. (1979) found that ratios of Apo A-I/Apo B gave a bimodal distribution effectively distinguishing patients from controls. They concluded that these apolipoproteins were better discriminators of risk than lipid parameters. In another study comparing 70 male myocardial infarction survivors and 70 controls, DeBacher et al. (1982), using univariate analysis, obtained 72% exact classification with Apo B/Apo A-I ratio. Using multivariate analysis, they attained 82% exact classification using HDL-C, Apo B/Apo A-I and Apo A-I/Apo A-II ratios in the model. Using angiographically documented CHD subjects and controls Noma et al. (1983) found Apo B/Apo A-I and Apo B/Apo A-II to be powerful discriminators of either presence or severity of disease.

II. The Lipid Hypothesis

There are few public health issues that have been the center of more heated debate than the "lipid hypothesis", i.e. that decreasing plasma lipid levels through dietary intervention and/or pharmacological agents decreases CHD incidence. At stake is not only the individual's health but also large financial interests such as the livestock, egg, dairy, and food processing industries. Although it has been generally shown that dietary manipulations do affect plasma lipid levels, the effects of changes in these levels on actual risk is still being assessed. One approach to study the effects of diet on plasma lipid parameters is through epidemiological studies. Samuel et al. (1983) listed four epidemiological data sources useful in evaluating the diet/CHD relationship; 1) analysis of dietary patterns and disease incidence among nations, 2) analysis of autopsy data, 3) studies of populations within various nations, 4) analysis of the effects of migration on dietary patterns and CHD incidence . A more definitive approach is through feeding studies in man or experimental animals. Lastly, an understanding of metabolism can elucidate effects of diet on risk.

A. Epidemiological Studies

General results from epidemiological studies showed increased incidences of CHD with increases in total kilocalories, total fat, saturated fat, animal protein, cholesterol, or sugar intake. Two

problems occur in this kind of analysis. First, the variables are often interrelated; second, comparisons are often made between populations with very different lifestyles (e.g. sedentary overconsumers vs. manual laboring underconsumers), as well as dietary differences.

Keys (1970) in his massive 7 country study of 12,000 men aged 40-59 years found positive relationships between CHD and saturated fat intake, ($r=0.81$), and fat as a percentage of calories ($r=0.40$). No other correlations between dietary components and CHD were found in that study. Most of the dietary data was collected in subsamples using 7 day diet records with weighing of all food consumed. There was a good correlation between these dietary data and data from 24 hour recall used for the bulk of the subjects. In the Ni Hon-San study, Kato et al. (1973) found serum cholesterol regressed positively with intake of saturated fat, cholesterol and animal protein in Japanese living in Japan, Hawaii, and the U.S although the regression coefficients were always smaller than $r=0.085$. Herscopf et al. (1982) found serum cholesterol dropped 6% between July 1963 and June 1971 in the Baltimore Longitudinal Study of Aging. They were unable to correlate changes in cholesterol with either changes in dietary variables or in body mass index.

B. Clinical Intervention Trials

With the recent publication of the Lipid Clinic Coronary Prevention Trial results, (Lipid Research Clinics Program 1984a), the

"lipid hypothesis" appears to be answered in the affirmative. Using a multisited, randomized, double blind study, the group definitively showed that reduction of plasma cholesterol was associated with a reduction in "hard" endpoints (i.e. death by myocardial infarction). Specifically, a 19% reduction in risk was associated with an 8.5% greater total plasma cholesterol reduction, and a 12.6% greater LDL-C reduction through the use of the drug cholestyramine than with diet alone. They also observed a 2% reduction in risk associated with increases in HDL-C of 1.6+0.19 mg/dl over the 7 years of the study.

The control group was also placed on a lipid lowering diet but were given a placebo in place of the cholestyramine. The rationale as given by the authors was:

> Although the cholesterol lowering expected from the diet given to both study groups had the potential to diminish the statistical power of the trial by reducing the subsequent incidence of CHD, it was hoped that such a diet, along with a nutritional counseling program, would facilitate recruitment of participants. ... The maintenance of both treatment groups on the diet after randomization minimized the opportunity for confounding of the study because of differential dietary intakes. Dietary intake was assessed semiannually by means of a 24-hour dietary recall.

There is some danger in extrapolating the results of this study to dietary intervention alone for the subjects were all men 35-59 years old with type II hyperlipoproteinemia, and the reduction of plama cholesterol was achieved by the drug cholestyramine, a bile acid sequesterant. They conclude:

> The LRC-CPPT was not designed to assess directly whether cholesterol lowering by diet prevents CHD. Nevertheless, its findings, taken in conjunction with the large volume of evidence relating diet, plasma cholesterol levels, and CHD, support the view that cholesterol lowering by diet also would be beneficial. The findings of the LRC-CPPT take on additional significance if it is acknowledged that it is unlikely that a conclusive study of dietary-induced cholesterol lowering for the prevention of CHD can be designed or implemented.

The authors of the aforementioned study analyzed 8 studies which fit the following criteria; 1) random assignment to treatment group, 2) endpoints of either MI or CHD death were reported, 3) post treatment total plasma cholesterol reported, and 4) no interventions on other risk factors besides diet and/or drugs, 5) at least 100 subjects per treatment group, 6) at least 3 years study duration, 7) CHD status of all subjects at end of study known. In the studies a

lowering of CHD incidence from between 9.4% and 33.5% was associated with each decrement in total serum or plasma cholesterol of 20.7 mg/dl. Risk reduction was also associated with changes in lipoprotein cholesterol in the Lipid Research Clinics Program study (Lipid Research Clinics Program 1984b). Using Cox proportion hazards models, the relative effects of changes in the various lipid classes were assessed. Decrease in LDL-C was associated with a risk reduction of 18%, increases in HDL-C with a 3.2% risk reduction, HDL-C/Total cholesterol increases with an 18.2% reduction, and HDL-C and LDL-C included in same model with a 20.2% risk reduction. There is now extant strong evidence that favorable changes in risk factors result in significant reduction of risk.

III. Dietary Effects on Plasma Risk Factors

A host of studies have been performed to analyze the effects of dietary perturbations on plasma risk factors for CHD. The interplay of diet, cholesterol synthesis, excretion and tissue storage, subject selection/compliance, and cholesterol catabolism on these factors has resulted in many conflicting reports. The interplay between the various nutrients is also important and as John Muir once said "if one tries to pick up anything by itself, one finds the whole universe connected to it." Thus, decreasing percent fat in the diet requires an increase in either carbohydrates or protein, an increase in polyunsaturated fat requires an increase in total fat or a decrease in monounsaturated or saturated fat.

A. Dietary Cholesterol

While it is generally accepted that dietary cholesterol is directly related to plasma cholesterol, as recently as 1980, Alfin-Slater and Aftergood stated " the serum concentration of cholesterol in man is essentially unaffected by cholesterol feeding," (Alfin-Slater and Aftergood 1980). Two inputs, dietary cholesterol and endogenous synthesis, and two outputs, bile acids and fecal sterols, influence the level of cholesterol in the plasma. In addition, tissue stores of cholesterol can be either an input or an output depending on metabolic state. Adipose tissue storage is particularly important as this pool may account for up to 50% of whole

body cholesterol (in obese humans) and takes from 1 to 5 months to equilibrate based on isotope dilution studies, (Krause and Hartman 1984). In fact, cholesterol can accumulate in body pools while plasma cholesterol remains constant (Quintao et al. 1971).

Mattson et al. (1972) in a study designed to measure only changes in dietary cholesterol, placed 56 men on diets with identical fat composition. After 21 days of a cholesterol-free baseline diet, the subjects were placed in 4 groups with varying cholesterol levels of 0, 106, 212, and 317 mg of cholesterol/1000 kilocalories. The plasma cholesterol levels dropped during the baseline period and rose differentially with the different cholesterol intakes. The authors concluded that plasma cholesterol is linearly dependent on cholesterol intake. Lin and Conner (1980) fed two subjects a liquid diet containing 15% protein, 40% fat, and 45% carbohydrate for a period of 25 weeks. During the first 10-14 weeks the dietary cholesterol content was 45 mg/day and during the second 11 weeks it was 1000 mg/day supplied as egg yolk. Both subjects' plasma cholesterol decreased for the first period and increased during the cholesterol feeding period. In addition the LDL-C, the HDL-C, and the LDL-C/HDL-C were increased during the cholesterol feeding period. Subject 1, a type IIa hyperlipidemic, had a change in plasma cholesterol of 280mg/dl to 427mg/dl, while her LDL/HDL ratio rose from 3.34 to 4.69. The second subject, a 31 year old normolipemic male, had an increase in plasma cholesterol from 123 to 166 mg/dl and an increase in LDL/HDL ratio of 2.04 to 2.32 resulting from cholesterol feeding. The long term design and carefully monitored conditions helped to insure that

this was, indeed, definitive proof that cholesterol feeding does increase plasma risk factors for CHD.

B. Modification of P/S ratio and total fat content of diet.

One important issue is whether lipid-lowering fat modified diets, with increased P/S ratio and/or decreased total fat content, selectively decrease atherogenicity by decreasing the LDL/HDL ratio or alternately do these diets decrease these two lipoproteins simultaneously and proportionally (Vessby et al. 1982). Since, in experimental studies atherosclerotic lesions in primates regress when lipid-lowering diets are fed (Armstrong et al. 1970), a change in the P/S ratio would be congruent with proposed mechanisms of atherogenesis.

Vessby et al. (1982) reviewed recent findings on the effects of dietary modification on HDL and LDL. Reviewing only studies where ordinary foodstuffs were used and only intraindividual changes were used for comparison, they attempted to resolve this debate. They concluded that both LDL-C and HDL-C were lowered in PUFA modified diets without any improvement (lowering) in LDL/HDL ratio. HDL did not increase with increases in P/S ratio unless dietary modification was accompanied by weight loss. Nearly all studies showed a decrease in LDL of 10-20%.

Vessby et al. (1980) fed 9 hyperlipoproteinemic patients with a lipid lowering diet for 4 weeks in a metabolic ward. The diet contained 35% fat and a P/S ratio of 2.0. They reported a decrease in

LDL-C of 17%, apoB of 27%, HDL of 15%, apoA-I of 9%. The apoB/apoA-I ratio was reduced by 19%. They suggest that a combination of P/S ratio change and reduction in fat intake contributed to these changes.

Keys et al. (1957) fed a variety of fats to institutionalized subjects and derived the following regression equation for the hypercholesteremic effects of dietary saturated and PS fats:

$$\text{cholesterol} = 2.74\ S - 1.31\ PS$$

Hegsted et al. (1965) using a similar approach and including dietary cholesterol derived the following equation:

$$\text{cholesterol} = 2.16\ S - 1.65\ PS + 6.66C - 0.53.$$

In a review of the status of PS fats in the prevention of CHD, Illingworth and Connor (1980) note 3 points about these equations:

> 1) saturated fatty acids exert twice as much influence on blood cholesterol levels as PS but each has an independent effect, 2) monounsaturated fatty acids have no effect, and 3) the hypercholesteremic action of saturated fatty acids is greater with C12 to C16 acids whereas shorter chain fatty acids appear to have no significant influence.

The authors further note that VLDL-C, LDL-C and apo B were reduced when P/S ratios were increased. Burslem et al. (1978) examined 58 vegetarians who ate no animal products. Thirty-two per cent of their kcal were derived from fat and their intake of cholesterol was less than 10 mg/day. P/S ratio was 1.9. All lipoprotein and apoprotein levels were decreased but the ratio of apo B/apo A-I was lower in controls (males .65, females .62) than vegetarians (males .72, females .71). LDL-C/HDL-C ratios were higher in controls (males 2.83, females 2.37) than in vegetarians (males 1.90, females 2.13). Jackson et al. (1984) in a study designed to measure changes in P/S ratio that were practical for free-living individuals fed 3 males and 3 females eucaloric diets containing 40% fat and P/S ratios of .4, 1.0, and 2.0 each for 2 weeks. Total cholesterol fell 6% and 12% as the P/S ratio was increased from .4 to 1.0 and 2.0, respectively. HDL-C, LDL-C, and apo A-I all decreased with increasing P/S ratios. LDL-C/HDL-C and HDL-C/TC ratios did not change significantly.

C. Dietary Protein and Carbohydrate

Little research has been performed on the effects of the relative amounts of either protein or carbohydrate on plasma risk factors for coronary heart disease. Terpstra, Hermus and West (1983) reviewed the available research and found conflicting results. Confounding of variables such as duration of the study and amount and type of fat and cholesterol make conclusions impossible to draw.

Sacks et al. (1983) state:

> From the literature on dietary protein and blood lipid levels and from the present data, it appears that neither the amount of protein in the diet nor whether the protein comes from animal or vegetable sources has an important effect on plasma LDL and HDL levels in humans when consumed in physiologic amounts.

While type of carbohydrate may be important in the development of CHD, relative per cent of carbohydrate intake as a source of kcals has not been shown to affect risk factors (Wolf and Grundy 1983).

D. Alcohol and risk factors

Alcohol consumption has been shown to be positively correlated with plasma HDL-C in several studies (Miller 1980). Fraser et al. (1983) used 11 male subjects and a carefully controlled diet; HDL, apo A-I, apo A-II all rose significantly when alcohol was fed compared to an isocaloric substitute of sucrose. Barboriak et al. (1979b) found that those patients with HDL-C over 50 mg/dl had less coronary artery occlusion (angiographically assessed) and consumed more alcohol than those with lower HDL-C values.

E. Caffeine

While past evidence does not implicate coffee consumption as impinging on risk factors for CHD, a recent report by Thelle et al. (1983) raised some interesting questions. In a study of 7213 women and 7368 men aged 20 to 54 years, the authors found a linear relationship betweeen coffee consumption and total cholesterol. From the lowest to the highest coffee consumption levels, plasma cholesterol rose 14% in both men and women. This relationship remained even after adjusting for the confounding variables of age, logarithm of BMI, physical activity, cigarette smoking and alcohol consumption. HDL-C showed a linear decrease with increased coffee consumption in women and an uncertain pattern in men. All the results were highly significant ($p<.001$). This contrasts with previous studies where no such interrelationship has been observed (Dawber et al.1974; Heyden et al. 1978; Yano et al. 1977; Little et al. 1966). One explanation is that the coffee consumption in the significant study was high (60% of the subjects drank 5 or more cups per day) whereas in the Framingham study this figure was 20%. Another possible explanation for the discrepancy in results could be the brewing method. Most populations imbibe drip or instant coffee; in Norway, where these results were obtained, coffee is usually prepared by boiling.

F. Age, weight, and body mass index

It has been well established that blood risk factors for CHD correlate with age, weight and indices of body mass (Castelli 1984;

Gordon et al. 1977a; Hershcopf et al. 1982). Body mass index is correlated with increased cholesterol, LDL-C and decreased HDL (Laskarzewski et al. 1980, Krause and Hartman 1984). Avogaro et al. (1978) found HDL and apo A-I increased with increasing age and decreased with increasing BMI. Apo B showed opposite effects with respect to age and BMI.

MATERIALS AND METHODS

I. General

A. Subjects

Thirty-one subjects were selected from a pool of 32 subjects who participated in one or both of two diet studies conducted at Oregon State University. Data on the one subject was omitted because we were unsure of its accuracy. The subject had a period of illness during the period of the study. The studies were designed to measure the effects of dietary supplementation with egg on cholesterol metabolism. However, for the purposes of the present study only baseline data were used. The subjects were healthy, middle aged men between the ages of 30 and 56 years. Mean values of age, weight, height, and plasma cholesterol appear in Table 1.

Recruitment was by poster in the Corvallis area. The subjects were screened by the use of a questionnaire (Appendix A) and were apparently healthy, had no documented history of CHD, and took no medications known to affect plasma cholesterol levels. All subjects gave consent and signed a form approved by the Human Subjects Committee of Oregon State University (Appendix B). Twelve subjects participated in both studies, data from only the first study were used for these subjects.

B. Experimental Design

During the baseline period of the two studies, subjects consumed self-selected diets and kept complete dietary records for a period of 7 days (Sanders 1984). On the morning of day 8 a fasting blood sample was drawn. A computerized analysis of nutrient intake was performed for each individual. Daily dietary intakes were calculated for total kilocalories, protein kilocalories, fat kilocalories, carbohydrate kilocalories, PS ratio, ethanol, and caffeine. Subjects' height and weight were measured. Using these data, 14 independent variables were created as listed in Table 2.

Plasma samples were analyzed for total cholesterol, VLDL-C, LDL-C, HDL2-C, HDL3-C, apolipoprotein A-I, apolipoprotein A-II, and apolipoprotein B. Using these data 17 dependent variables were created as listed in Table 3. Stepwise multiple regression analysis of each dependent variable with all independent variables was performed to determine correlations between them.

C. Blood Drawing Procedure

Subjects were instructed to abstain from foods and drink, except for water, from 8:00 PM the previous night until after the blood draw between 7:00 and 9:00 AM the following morning. Licensed medical technologists employed by the Department of Foods and Nutrition at Oregon State University drew 35 ml of blood into sterile

evacuated tubes containing 1 mg sodium EDTA/ml of blood as an anticoagulant. Plasma was separated from whole blood by centrifugation at 4 C for 30 minutes at 2500 RPM.

II. Analytical Methods

A. Lipoprotein Isolation

A Beckman Model L5-75 ultracentrifuge with a fixed angle rotor (Ti50) spun at 50,000 RPM was used for all lipoprotein isolations. Centrifugation temperature was 5 C. The tube slicing technique was used for fraction separation. Density was adjusted using solutions of KBr in normal saline and by adding granular KBr to the plasma. VLDL was centrifuged for 12 hr at plasma density ($d<1.006$ gm/ml) and then washed by recentrifugation in normal saline ($d=1.006$gm/ml) for 12 hr to remove albumin. LDL was then isolated by adjustment of the density to 1.063 gm/ml and centrifugation for 18 hr. Similarly HDL2 and HDL3 were isolated by sequential centrifugation for 24 and 36 hr at densities of 1.125 gm/ml and 1.210 gm/ml, respectively. To remove traces of albumin, the HDL3 was washed by repeating centrifugation for 24 hr at $d=1.210$ gm/ml. This isolation technique is based on flotation and was described by Havel et al. (1955).

B. Cholesterol Quantification

Total cholesterol in whole plasma and lipoprotein fractions was determined using the method of Allain et al. (1974). The reagent mixture contains the enzymes cholesterol esterase, cholesterol oxidase, and peroxidase (Miles Laboratories. Elkhart, Indiana) as well as buffering salts, detergent (for dispersion of lipids), phenol, and

4-amino-antipyrine (Sigma Chemical Company. St. Louis, Missouri). The esterase hydrolyzes cholesterol esters present to free cholesterol. Then the oxidase oxidizes the free cholesterol to cholest-4-en-3-one and simultaneously produces hydrogen peroxide. Coupling of the hydrogen peroxide with the phenol and the 4-amino-antipyrine produces a chromagen that absorbs strongly at 500 nm.

C. Apolipoprotein Quantification

ApoA-I and Apo A-II were quantified using an immunological turbidimetric assay purchased from Boeringher-Mannheim, (Indianapolis, Indiana). Plasma diluted in buffer was added to a solution containing an excess of gamma globulin specific for the apoprotein antigen being measured and to a blank buffer solution containing no antibody. The solution was incubated for two hours and then the precipitate that was formed was measured at 336 nm. The sample blank absorbance was subtracted from the sample absorbance and this value was plotted against concentration. A standard curve was constructed using plasma samples of known concentration and sample concentration was interpolated from this curve.

Apolipoprotein B was quantified using Diffu-Gen radial immunodiffusion plates and reference sera (both from Tago Inc., Burlingame, California) using the Ouchterlony technique (Ouchterlony 1962) adapted for apoprotein B measurement by Rosseneu et al. (1983). Briefly, an agarose gel containing specific antibody to the antigen being measured was formed and small holes were punched out of the gel

matrix. Samples and standards containing known amounts of antigen were added to the wells and they were then incubated for 96 hr at 37 C. During this time small precipitan rings were formed and the diameters were proportional to antigen concentration. A standard curve was drawn and samples' concentrations were read from this curve.

III. Dietary Analysis

A. Dietary Records

Subjects were individually instructed how to record all beverage and food intakes on the forms shown in Appendix C. An instruction sheet (Appendix D) was also issued to each subject. Careful attention was given to insure subject compliance with this protocol, and further incentive was provided by informing the subjects that they would receive their complete individual dietary analysis at the study's conclusion.

The dietary recording and intake determinations for this study were provided by Terri Sanders (Sanders 1984). The dietary analyses were completed with extreme care, and all restaurant items and some purchased foods were investigated for recipe and ingredients. If composition of food menu items was unclear, wives were called and restaurants visited. If needed, ingredients and proportions of recipes were taken from the Betty Crocker Cookbook (General Mills, Inc. 1969) or the Joy of Cooking (Rombauer and Becker 1975). Dietary supplements were recorded but were not included in this study's analysis.

B. Computer analysis of diets

The 1981 update of the Ohio State Nutrient Data Base (Schaum et al. 1973) was used to determine the individual nutrient intake. All

diet items were coded for batch processing on the CDC Cyber 170/172 system at the Oregon State University Computer Center. Results were expressed as daily averages.

IV. Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) (Nie et al. 1975) on Oregon State University's Cyber 70/73 and Honeywell 440 computers. Descriptive statistics for each independent and dependent variable, (mean, variance, minimum, and maximum) were obtained. The dependent variables were regressed serially against the 14 independent variables using stepwise multiple regression, starting with no independent variables in the model. An F-value of 4.0 to enter a variable and an F-value of 3.9 to remove a variable were used to achieve significance at approximately the $p<.05$ level.

RESULTS AND DISCUSSION

I. Variables, values and comparisons

A. Independent Variables

Table 6 gives descriptive statistics for all independent variables. The RDA (NAS-NRC 1980) lists desirable weight for adult men ages 23-50 years who are 180 cm tall at 72 kg. At an average of 81.7 kg these subjects were heavier than the range given (65 - 81 kg) as desirable. The mean height and weight for American men was given as 70 kg and 178 cm with a recommended dietary intake of 2300-3100 kcal. The subjects' intake of 2646 kcal/day was within this range.

Protein in the U.S. food supply is 14.2% of available kcal (Stamler 1979) and the subjects' mean of 14.3% was very close to this. Fat intake ranged between 23.9% to 49.3% of calories with a mean of 36.8%. This mean is near the 35% of the RDA. Linoleic acid accounted for 0.6% of the subjects' intake, which is below the 10% recommended in the RDA (NAS-NRC 1980). P/S ratio in the American diet is reported to average 0.44 (Senate Select Committee on Nutrition and Human Needs 1977); the subjects' value of 0.51 was close to this but had a wide range between 0.21 and 1.02. Cholesterol intake (494 mg/dl) was very close to the 500 mg/day average found in the U.S. food supply (National Dairy Council 1979). Average ethanol intake of adult men in the U.S. is estimated to be between 200 to 400 kcal/day (McNutt 1979), or 7.5% of kcal (Senate Select Committee on Nutrition and Human Needs

1977). Our mean of 93.1 kcal or 3.3 % of kcal is lower, however 6 subjects consumed alcohol in the higher range. Alcohol intake ranged between 0 and 14.5% of calories.

Coffee contains about 100 mg caffeine/6 oz cup, tea contains about 65 mg/6 oz cup, and cola drinks contain 3-4.5 mg/oz (Bender 1982). The subjects consumed 288 mg/day, the equivalent of 2 to 3 cups of coffee, with the high being 1085 mg, the equivalent of nearly 11 cups of coffee.

B. Dependent Variables

According to the Lipid Research Clinics Prevalence Study (American Heart Association 1980) normal plasma and lipoprotein cholesterol values in 40 year old American men are total (205 mg/dl), VLDL (25 mg/dl), LDL (135 mg/dl), and HDL (44 mg/dl). The subjects' mean values were total (185 mg/dl), VLDL (8.8 mg/dl), LDL (112 mg/dl), and HDL (42 mg/dl). Thus the subjects of this study had lower mean values for total, VLDL and LDL cholesterol but near normal for HDL-C. Normal values for apolipoproteins A-I, A-II, and B have yet to be extablished. Schaefer et al. (1978) suggested approximate normal levels of apo A-I, 120 mg%; apo A-II, 40 mg%; and apo B, 90 mg%, which are comparable to our values of apo A-I, 130 mg%; A-II, 60 mg%; and apo B, 72 mg%.

II. Regression models

A. Description

The regression results are tabulated in Table 7. The first column lists the dependent variables used in each regression; the second column, the independent variables in the order they were entered in the model. Simple F is the F-value for testing an individual variable's eligibility to be entered or removed from the model. (Recall that an F-value of 4.00 to enter and 3.9 to remove a variable were set to achieve approximate p-values of less than 0.05). The next column, simple R, is the R value associated with the entry of the individual independent variables in the model. A positive value means that the independent variable is positively correlated with the dependent variable; a negative value means that as the independent variable increases in value, the dependent variable decreases and vice versa. Simple p refers to the probability of making a type I error when using that particular independent variable in the regression model. (A type I error refers to the probability of concluding that the independent variable in question is responsible for predicting the value of the dependent variable according to the given model when in reality it has not this association. Type II errors were not computed; this is the probability of concluding that any given independent variable does not belong in the model when in fact it does belong (Glantz 1980). The overall F in the next column is the F-value for including all variables up to and including the associated listed

variable in the model.

The multiple R2 statistic is perhaps the most interesting value. This is a measure of the proportional effect of a given variable or set of variables on the dependent variable being regressed. (E.G. in row one, the independent variable age has an R2 value of .135; this means that 13.5% of the variability in the dependent variable plasma cholesterol can be explained by the effect of variation in age.) Finally, overall p refers to the probability of making a type I error (see previous parenthesis) given the inclusion of the listed variable(s) in the model.

B. Collinearity

Interpretation of regression results can be confounded by collinearity (Neter and Wasserman 1974).

> ... the estimated regression coefficients tend to have large variablility when the independent variables are highly correlated. As a result, only imprecise information may be available about the individual true regression coefficients ...
>
> When the independent variables are highly correlated, not only do the estimated regression coefficients tend to be quite inprecise, but the true regression coefficients tend to lose their meaning.

Therefore a reparameterized regression coefficient matrix for each of the 9 models in which multiple independent variables appeared is given in Table 12 and a collinearity matrix for the entire set of independent variables is given in Table 13. (A coefficient of 1.00 means that the variables are perfectly correlated.)

Due to the aforementioned reasons (Neter and Wasserman 1974), the relatively small sample size, the large collinearity between some of the independent variables, and the probability that the multiplicity of regression models and independent variables used will result in some variables appearing in error, the results are only suggestive of real interrelationships.

C. Occurrences of independent variables in regression models.

All the dependent variables modelled had independent variables associated with them with the exception of HDL3-C and the HDL2-C/HDL3-C ratio. These variables had no predictors with sufficiently high F-values to be entered in the model. One dependent variable had 3 independent variables in its model, 8 had 2, and 5 had 1. Six out of the 14 independent variables did not appear in any of the models; these were protein % , caffeine, cholesterol intake, weight, height, and weight.75 (see Tables 8 and 9). One variable, age, appeared in five models; two variables, total kilocalories and fat % appeared in 4 models; P/S ratio and Body Mass Index each appeared in 3 models; carbohydrate %, ethanol %, and

kilocalories/wt.75 each appeared in 2 models.

Certain combinations of independent variables appeared in 2 models. These are Body Mass Index and P/S ratio which fit in the VLDL-C and HDL2-C models, total kilocalories and fat % which fit in the VLDL + LDL and LDL/total-C models, and kcal/wt.75 and fat % which appeared in the LDL/HDL and HDL/total-C models.

Table 10 shows which independent variables were associated with an increase or decrease in risk (see Table 11 for risk factor classification). Three independent variables, fat %, EtOH %, and BMI, were consistently associated with an increase in risk. Four independent variables, total kilocalories, carbohydrate %, P/S ratio, and kcal/wt.75, were consistently associated with a decrease in risk. One variable, age, was positively associated with increased risk in 4 out of 5 models; in 1 model it was associated with a decrease in risk.

III. Regression Results

A. Models

Total plasma cholesterol, the first dependent variable modelled, had only age in the model. This lack of inclusion of dietary values agrees with the analysis of Stamler (1979) whose review showed that individual correlations with dietary intake and serum cholesterol are not easily shown. He stated that interindividual metabolic differences in individuals (standard deviations in plasma cholesterol of 30 mg/dl) created problems and suggested that dietary intake records of 9 days are needed under American conditions to demonstrate a correlation. Age, however, is usually correlated with increasing plasma cholesterol (Levy 1981). This is true in Western populations. It is not true, however, in populations that habitually consume low fat diets (Illingworth and Conner 1980; Stamler 1979).

VLDL-C was also positively correlated with age; this relationship is also supported by past studies. The Lipid Clinics Program (1984b) showed VLDL-C rising from a mean of about 20 mg/dl at age 30 to approximately 27 mg/dl at age 55, after that VLDL levels fall. VLDL-C is not considered an important risk factor except in certain types of hyperlipoproteinemias (Levy 1981).

LDL-C is considered an important risk factor for CHD. Our regression results showed a negative correlation with total kcals and carbohydrate %. The US-USSR Steering Committee for Problem Area I

(1984) found carbohydrate the sole variable correlated with LDL-C in the U.S., whereas total kcal along with fat and sucrose were negatively correlated with LDL-C in the Russian subjects. It is generally believed that P/S ratio is correlated negatively with LDL-C (Vessby et al. 1982), this result was not confirmed in our study.

P/S ratio was a predictor for HDL2-C, total HDL-C and apo A-I in our models. Since apo A-I is the major apoprotein of HDL-2 and indeed of total HDL these results are congruent. Burslem et al. (1978) found vegetarians consuming a diet with a P/S ratio of 1.9 had lower apo A-I and HDL levels. These lipid levels were 60% of normal control groups' values. Illingsworth and Connor (1980) also found lower apo A-I and HDL levels in subjects fed an increased P/S ratio diet. Vessby et al. (1982) also confirmed this result. The US-USSR Steering Committee for Problem Area I (1984) found no significant correlations between P/S ratio and HDL-C. Our results showed a positive correlation with P/S ratio. This result differs from Illingworth and Connor (1980), Vessby et al. (1982), Burslem et al. (1978), and Jackson et al. (1978) who found lower HDL in diets with increased P/S ratios. However, these studies were either clinical intervention studies or studies of vegetarians versus controls; none of them were simple correlations between habitual diet and plasma values in omnivorous humans as was the present study.

BMI index was also a predictor for both HDL2-C and total HDL-C in our models. Krause and Hartman (1984) and Laskarzewski et al. (1980) found negative correlations between BMI and HDL. Avogaro et al. (1979) also found decreasing apo A-I with increased BMI. The

former but not the latter results were confirmed in our study.

Our sole predictor for apo A-II was % of kcal supplied by EtOH. Fraser et al. (1983) in a crossover study using 11 male subjects isocalorically substituted alcohol (32 g ethanol/day) for sucrose for 3 weeks each. They found increases in apo A-I, HDL-C, and apo A-II during the ethanol regimen. The inclusion of EtOH% as a variable in our regression equation for apo A-II/apo A-I ratio is probably due to its inclusion in the apo A-II regression mode. The inclusion of age in the apo A-II/apo A-I model in the present case is not confirmed elsewhere in the literature.

Apo-B is virtually the only apoprotein in LDL. Kcal and carbohydrate % were included in both these regression models in our study. Since apo B is also found in VLDL and age was a predictor for VLDL-C, this could account for the correlation of apo-B with age (Schaefer et al.1978). LDL and VLDL also are known to increase with age (Levy 1981); this adds confirmation to its present inclusion in the apo-B model.

The ratio of apo B/apo A-I and apo B/apo A-II were also significantly correlated with the variable, age. The strong correlation of age with apo B adequately explains these associations.

The ratios of LDL and HDL to each other and to total cholesterol may be good clinical tools for the evalulation of CHD (Lipid Research Clinics Program 1984b). Fat % was a predictor for all combinations (LDL-C/TC, LDL-C/HDL-C, and HDL-C/TC) in the present study. Most investigators have stated that the type of fat (i.e. polyunsaturated vs. saturated) is the most important determinant of

CHD risk, with results being divided as to whether P/S ratio modified diets selectively lower LDL-C, HDL-C or both (Jackson et al. 1978; Vessby et al. 1980). Our result is significant in light of the current recommendations to lower total fat intake (Levy 1982; Coates 1983).

The collinearity between kcal/wt.75 and kcal was .924 (Table 13). This high value indicates that they had a high probability of being interchangeably included in any given model. One or the other was included in all three ratio models, in which it was always negatively correlated. This is an interesting result that will be addressed later.

B. Effects of dietary variables on risk

Our results are supportive of the recommendations to reduce fat intake to 30% or 35% of dietary energy and increase P/S ratio to 1.0 (Senate Select Committee on Nutrition and Human Needs 1978, National Research Council 1980). Indeed, decreased total fat was associated with a decrease in VLDL + LDL, LDL/TC, LDL, HDL and an increase in HDL/total cholesterol. Increasing P/S ratio was correlated with increased HDL2, apo A-I, and HDL-C. All these results support the view that individuals whose habitual diet contained less total fat and had a higher P/S ratio are at less risk for CHD. The recommendation to reduce consumption of total cholesterol was not confirmed in this study. This does not contradict the finding that populations that consume more cholesterol

have higher risk for CHD and does support the observations of Stamler (1979) that there is too much intraindividual variation in plasma cholesterol to make statistical correlations with habitual dietary cholesterol. (Our cholesterol values ranged from 124 to 293 mg/dc.)

Carbohydrate % and total kcal were both negatively associated with risk in the LDL-C and apo-B regression models. In addition total kcal were negatively associated with risk in the VLDL + LDL and LDL/total cholesterol models. This unexpected result could be explained by the inadvertant selection of heavier individuals with lower cholesterol values, or alternately, by the observation that there was a low correlation between body weight and kcal intake. Thus those individuals who exercised more would have higher caloric intakes without necessarily having higher risk lipid profiles. We have no data to test this hypothesis in the present study. This explanation could also suffice to explain the negative correlation of kcal/wt.75 with the LDL-C/total cholesterol ratio and the positive correlation of kcal/wt.75 with the HDL-C/total cholesterol ratio.

BMI was included in the models for HDL2 and total HDL. Since BMI was a measure of body composition (relative obesity), these negative correlations were reasonable. Thus a person with a higher adipose tissue content had lower HDL2 and HDL-C values which is congruent with the results of Laskarzewski et al. (1980) and Krause and Hartman (1984).

EtOH % had positive correlations with apo AII and apo A-II/apo A-I ratio. Fraser et al., (1983) found positive correlations between alcohol and total HDL and apo A-I. They also

found a borderline positive correlation between EtoH and apo A-II. Our results were only positive for apo A-II, and possibly by transference, the apo A-II/apo A-I ratio.

CONCLUSIONS

The data supported the hypothesis that habitual diet is statistically correlated with plasma risk factors for CHD. This includes new data showing correlations with the apoprotein moieties of the lipoproteins with dietary variables as well. Therefore the synthesis and metabolism of these apoproteins are evidently under nutritional as well as genetic control. Mechanisms for these effects need to be determined.

Future investigators utilizing a similar study design would do well to measure energy expenditure if predictive variables including energy intake are to be correlated with plasma values. Perhaps then the puzzling correlations between increasing energy intake and decreasing risk could be explained. A further suggestion is to limit the number of independent variables with a high degree of collinearity used, in particular the variables involving weight and kcal intake, since their inclusion only confounded analysis of the results.

The effect of ethanol consumption on the HDL subfraction and its apoprotein moieties merits further elucidation. Which apoprotein is affected? Does moderate consumption really decrease risk?

The diet factor risk analysis supported current recommendations to decrease total dietary fat intake, to increase the P/S ratio, and to limit body weight (see Table 10) since these

variables were correlated with changes in plasma risk factors for CHD. Decreased fat intake was associated with decreased VLDL and LDL cholesterol, decreased LDL-C/HDL-C ratios, and increased HDL-C/TC ratios. Increases in P/S ratio were correlated with increased HDL2-C, HDL-C and apo A-I. Lower values for BMI were correlated with decreased VLDL-C, and increased HDL2-C and HDL-C. All these changes reflect probable lower risk for CHD in persons affecting them and thus should be encouraged in the general population.

Table 1. Statistical description of the subjects' age, weight, height and plasma cholesterol

Variable	Mean+SD	Min.	Max.
Age (years)	39.2+7.6	30	56
Weight (kg)	81.7+13.6	57.2	122.6
Height (cm)	180+8	163	193
Cholesterol (mg/dl)	184+40	124	293

Table 2. Independent variables used in regression analysis.

Independent Variable	How Derived
Total Kilocalories	mean intake per day from data base
Protein %	(grams of protein x 4 / total kilocalories) x 100
Carbohydrate %	(grams of carbohydrate x 4 / total kilocalories) x 100
Fat %	(grams of fat x 9 / total kilocalories) x 100
Ethanol %	(grams of ethanol x 7 / total kilocalories) x 100
Caffeine	mean daily intake of caffeine in mg.
PS Ratio	mean linoleic acid intake divided by mean saturated fat intake
Cholesterol	mean daily cholesterol intake
Age	years
Weight	kilograms
Height	centimeters
Weight**.75	weight raised to the .75 power
Body Mass Index	(weight in kilogram) / (height in meters squared)
Kilocalories/Weight**.75	kilocalories / (weight in kilograms raised to the .75 power)

Abbreviations: PS Ratio polyunsaturated to saturated fat ratio

Table 3. Mean, standard deviation, maximum, and minimum plasma values for all subjects

Plasma Value	Mean+SD	Min.	Max.
Simple*			
Cholesterol	185+40	124	293
VLDL-C	8.8+8.3	0	34
LDL-C	112+29	61	184
HDL2-C	17.2+6.9	8.0	42.0
HDL3-C	24.9+5.4	12.0	35.0
ApoA-I	130+15	102	169
ApoA-II	60+8	45	76
ApoB	72+26	20	136
Derived			
VLDL-C+LDL-C*	120+32	63	204
LDL-C/Total-C**	0.603+0.068	0.474	0.719
LDL-C/HDL-C**	2.80+0.91	1.25	5.12
HDL-C*	42+11	25	75
HDL2-C/HDL3-C**	0.70+0.26	0.33	1.50
HDL-C/Total-C**	0.234+0.063	0.140	0.395
ApoB/ApoA-I**	0.558+0.187	0.145	0.880
ApoB/ApoA-II**	1.218+0.450	0.317	2.200
ApoA-II/ApoA-I**	0.456+0.048	0.378	0.613

* Values are in milligrams/deciliter.
** Values are in unitless dimensions.

Table 4. Daily mean, standard deviation, maximum, and minimum for nutrient intake values

Nutrient	Mean+SD	Min.	Max.
Simple			
Total Kcal	2646+785	1346	4438
Protein(gm)	97+36	49	192
Carbohydrate(gm)	303+78	161	520
Fat(gm)	112+44	57	245
Ethanol(gm)	13.3+16.6	0	55.2
Caffeine(mg)	288+251	0	1085
Saturated Fat(gm)	38.4+15.1	20.5	81.3
Linoleic Acid(gm)	19.3+9.4	5.4	51.2
Cholesterol(mg)	494+291	130	1482
Derived			
Protein %	14.3+2.1	10.2	17.9
Carbohydrate %	45.7+5.8	32.9	56.2
Fat %	36.8+5.8	23.9	49.3
PS Ratio	.51+.17	.21	1.02
Ethanol %	3.3+4.2	0	14.5

Table 5. Descriptive statistics for all independent variables

Variable	Mean+Std. Dev.	Min.	Max.
Kilocalories (daily mean)	2646+785	1346	4438
Protein % (daily mean)	14.3+2.1	10.2	17.9
Carbohydrate% (daily mean)	45.7+5.8	32.9	56.2
Fat% (daily mean)	36.8+5.8	23.9	49.3
Ethanol% (daily mean)	3.3+4.2	0	14.5
Caffeine (mg/day)	288+251	0	1085
PS Ratio	0.51+0.17	0.21	1.02
Cholesterol intake (mg/day)	494+291	130	1482
Age (years)	39.2+7.6	30	56
Weight (pounds)	180+30	126	270
Height (cm)	180+8	163	193
Weight**.75	49.1+6.1	37.6	66.6
Body Mass Index	2518+315	2825	3399
Kilocalories/Weight**.75	98.0+28.9	51.9	161.9

Table 6. Complete regression results

Dependent Variable	Independent Variable(s)	Simple F	Simple R	Simple p	Overall F	Multiple R2	Overall p
Cholesterol	1. Age	4.54	.368	.042	4.54	.135	.042
VLDL-C	1. Age	4.86	.379	.036	4.86	.143	.036
LDL-C	1. Tot Kcal	5.88	-.411	.022	5.88	.169	.022
	2. Carbo %	12.26	-.233	.002	10.20	.422	.000*
HDL2-C	1. BMI	6.29	-.423	.018	6.29	.178	.018
	2. P/S Ratio	4.54	.338	.042	5.80	.293	.008
HDL3-C	none in model						
ApoA-I	1. P/S Ratio	4.54	.368	.042	4.54	.135	.042
ApoA-II	1. EtOH %	4.85	.379	.036	4.85	.143	.036
ApoB	1. Age	13.06	.557	.001	13.06	.557	.001
	2. Carbo %	5.08	-.344	.032	9.99	.645	.001
	3. Tot Kcal	13.46	-.377	.001	14.11	.781	.000*
VLDL-C + LDL-C	1. Tot Kcal	6.51	-.428	.016	6.51	.183	.016
	2. Fat %	12.60	.322	.001	10.86	.437	.000*
LDL-C/Total-C	1. Tot Kcal	6.71	-.434	.015	6.71	.188	.015
	2. Fat %	8.56	.258	.006	8.57	.380	.001
LDL-C/HDL-C	1. Kcal/Wt.75	10.22	-.511	.003	10.22	.261	.003
	2. Fat %	8.20	.234	.008	10.48	.428	.000*
Total HDL-C	1. BMI	6.24	-.421	.018	6.24	.177	.018
	2. P/S Ratio	4.05	.322	.054	5.48	.281	.010
HDL2-C/HDL3-C	none in model						
HDL-C/Total-C	1. Kcal/Wt.75	7.98	.464	.008	7.98	.216	.008
	2. Fat %	6.85	-.232	.014	8.22	.370	.002
ApoB/ApoA-I	1. Age	8.69	.480	.006	8.69	.231	.006
ApoB/ApoA-II	1. Age	12.24	.545	.002	12.24	.297	.002
ApoA-II/ApoA-I	1. EtOH %	5.02	.384	.033	5.02	.148	.033
	2. Age	4.29	-.374	.048	4.94	.261	.015

* values less than 0.0005

Table 7. Occurrence of independent variables in regression models.

Independent Variables	# of occurences	Model(s)
Total kilocalories	4	LDL-C, ApoB, VLDL-C+LDL-C, LDL-C/Total cholesterol
Protein %	0	
Carbohydrate %	2	LDL-C, ApoB
Fat %	4	VLDL+LDL, LDL/Total cholesterol, LDL/HDL, HDL/Total cholesterol
EtOH %	2	A-II, A-II/A-I
Caffeine	0	
P/S ratio	3	HDL2, A-I, HDL-C
Cholesterol intake	0	
Age	5	Cholesterol, ApoB, ApoB/A-I, ApoB/A-II, A-II/A-I
Weight	0	
Height	0	
Weight.75	0	
Body Mass Index	3	VLDL, HDL2, HDL
Kcal/weight.75	2	LDL/HDL, HDL/Total cholesterol

Table 8. Occurrence chart for all independent variables

	Independent													
Dependent	KCAL	PROX	CARX	FATX	EtOHX	Caff	P/S	CHOL	Age	Wt	Ht	Wt.75	BMI	K/Wt75
Cholest									p=.042 +					
VLDL-C														p=.036 +
LDL-C	p=.022 −		p=.002 −											
HDL2-C							p=.042 +						p=.018 −	
HDL3-C														
ApoA-I							p=.042 +							
ApoA-II					p=.036 +									
ApoB	p=.001 −		p=.032 −						p=.001 +					
VLDL+LDL	p=.016 −			p=.001 +										
LDL/Total	p=.015 −			p=.006 +										
LDL/HDL				p=.008 +										p=.003 −
HDL-C							p=.054 +						p=.018 −	
HDL2/HDL3														
HDL/Total				p=.014 −										p=.008 +
ApoB/A-I									p=.006 +					
ApoB/A-II									p=.002 +					
ApoAII/AI					p=.033 +				p=.048 −					

Table 9. Independent variables associated with an increase or a decrease in risk for CHD in the present study.

Independent Variable	Risk* Increased	Risk* Decreased	Not in any model
Total kilocalories	0	4	
Protein %			X
Carbohydrate %	0	2	
Fat %	4	0	
EtOH %	2	0	
Caffeine			X
P/S ratio	0	3	
Cholesterol intake			X
Age	4	1	
Weight			X
Height			X
Weight.75			X
Body Mass Index	3	0	
Kcal/weight.75	0	2	

* see Table 11 for risk factor analyis. (These figures refer to the number of models the given variable appeared with the given association with risk for CHD.)

Table 10. Risk factor analysis for CHD*

Risk Factor	Positive		Negative
Plasma cholesterol	yes		
VLDL cholesterol	yes		
LDL cholesterol	yes		
HDL2 cholesterol			yes
HDL3 cholesterol			yes
ApoA-I			yes
ApoA-II		?**	
ApoB	yes		
VLDL-C + LDL-C	yes		
LDL-C / Total cholesterol	yes		
LDL-C / HDL-C	yes		
Total HDL cholesterol			yes
HDL2-C / HDL3-C			yes
HDL-C / Total cholesterol			yes
ApoB / ApoA-I	yes		
ApoB / ApoA-II		?**	
ApoA-II / ApoA-I		?**	

* Positive risk factors are those that are thought to increase risk as the numerical value increases.
Negative risk factors are those that are thought to increase risk as the numerical value decreases.

** The influence of apo A-II on risk has not been determined.

Ref: Avogaro et al. 1979; Castelli 1984; DeBacher et al. 1982.

Table 11. Collinearity of independent variables appearing in the same regression model.

Dependent Variable	Reparameterized correlation matrix
LDL-C	Kcal -.496 Car %
HDL2-C	P/S ratio .007 Body Mass Index
Apo B	Car % -.496 Age -.353 -.034 Kcal Car %
VLDL + LDL	Kcal .350 Fat %
LDL-C/Total-C	Kcal .350 Fat %
LDL-C/HDL-C	Kcal/wt.75 .305 Fat %
HDL-C	Body Mass Index .007 P/S Ratio
HDL-C/Total-C	Kcal/wt.75 .305 Fat %
ApoA-II/ApoA-I	EtOH % -.101 Age

Table 12. Collinearity matrix for entire set of independent variables

	Kcal	Pro %	Car %	Fat %	Caff	P/S	Chol	Age	Weight	Height	EtOH %	Wt.75	BMI
Pro %	.180												
Car %	-.496	-.213											
Fat %	.350	-.048	-.707										
Caff.	.110	.150	-.319	.304									
P/S	.065	-.280	-.101	.224	.005								
Chol	.643	.428	.569	.499	.214	-.114							
Age	-.353	.296	-.034	-.002	-.240	.013	-.063						
Weight	.164	.190	-.251	.105	.073	.026	.212	.159					
Height	.370	.090	-.104	.055	-.021	.036	.300	-.183	.654				
EtOH %	.108	-.153	-.291	-.380	-.056	-.028	-.124	-.101	.103	.022			
Wt.75	.172	.198	-.250	.103	.082	.025	.222	.151	.999	.662	.100		
BMI	-.036	.208	-.239	.085	.125	.001	.082	.328	.854	.169	.105	.849	
KcalWt	.924	.074	-.392	.305	.061	.060	.528	-.409	-.210	.114	.081	-.205	-.353

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APPENDIX

APPENDIX A

History

I. Age _ _ _____ II. Sex_____

III. Past illnesses and present medical problems. Do you have any of the following?

	Yes	No
Diabetes	___	___
Asthma	___	___
High blood pressure	___	___
Thyroid trouble	___	___
Diseases of other endocrine glands? (Cushings disease, Addison's disease, parathyroid or pituitary disease, Stein Leventhal syndrome)	___	___
Coronary heart disease	___	___
High blood lipids? (cholesterol or triglycerides)	___	___
Gallstones or gallbladder disease	___	___
Liver disease	___	___
Cystic fibrosis or other intestinal malabsorption	___	___
Kidney disease	___	___
	___	___
Other	___	___

IV. Personal habits

Do you smoke? not at all /_/ cigarettes /_/ pipe /_/ cigars /_/
How much?_____

Do you get strenuous exercise? yes /_/ no /_/ Type of exercise_____

How often (average number of times per week 15 min. or more)_____

Do you drink alcohol? not at all /_/ rarely, at parties /_/ most weekends /_/
weekends and some week days /_/ most days /_/

Amount of alcohol consumed: 2-3 cups /_/ 4-6 cups /_/ 7 or more /_/

Type of alcohol__ _____

IV. (continued)

Do you take any medicine?	Yes	No
Lipid lowering drugs	____	____
Aspirin or other anti-inflammatory (arthritis) drugs?	___	___
Insulin	___	___
Thyroid	___	___
Diuretics	___	___
Other high blood pressure medicine	___	___
Asthma medicine	___	___
Lecithin, wheat germ, brewers yeast	___	___
Other	___	___

	Yes	No	Amount
Multiple vitamin supplement	___	___	____
Name brand	____		
Vitamin A	___	___	____
Vitamin D	___	___	____
Vitamin E	___	___	____
Vitamin K	___	___	____
Niacin, (Vitamin B_3)	___	___	____
Nicotinic Acid	___	___	____
Food allergies	____	____	____

V. Family history

Do you have any blood relatives with the following?

	Yes	No
Heart attacks before age 60	___	___
Diabetes	___	___
Strokes before age 60	___	___
Kidney failure	___	___
Known high cholesterol or high tryglycerides?	___	___

Physical Examination

Height_____

Weight_____

Blood pressure_____

	normal	abnormal
Eye grounds	____	____
Thyroid	____	____
Heart	____	____
Abdomen	____	____
Xanthoma	____	____

Skin fold thickness: _____Bicep _____Tricep

% body fat _____

APPENDIX B

Participation Consent Form

Project Title: The effects of dietary and certain non-dietary interventions on the individual variability of plasma cholesterol in response to dietary egg.

Objective: The purpose of the present research is to study the interactive effects of dietary cholesterol and dietary or non-dietary intervention on blood cholesterol, lipoprotein lipids and fecal steroid excretion.

Procedures: I will be asked to fill out a medical history or health status form and to have a physical check-up and blood lipid levels. I understand that my participation in the study as a Volunteer subject is subject to my normal health. As a new participant I will be asked to eat lunch which includes three eggs for the first four weeks. I will be also requested to keep daily dietary records and exercise log on provided sheets for computer analysis of nutrient intakes. Once classified as a responder or non-responder according to my blood cholesterol concentration, I am willing to receive a dietary perturbation or non-dietary treatment in addition to the lunch. The dietary intervention might be change in fat intake or switch of dietary fat from butter to margarine. The non-dietary treatment could be change in my daily exercise or no alcoholic beverage. During the entire 10 week study period a total 280 milliliters of blood will be drawn over four times (each time 70 mls). I will receive a fecal marker of F.D.C. Blue No. 1 (50 mg plus 200 mg methylcellulose in a gelatin capsule) and will be asked for 7-day complete fecal collections over three times.

As study progresses I will be informed about the status of my blood lipid levels and may ask any questions and discuss with the investigators in regard to my response to the dietary eggs.

An explanation has been made to me of risk and benefits as follows:

Risk: The dietary eggs will probably change the blood level and composition of lipoproteins which reflect the lipid and apoprotein moities of the plasma lipoproteins. A 5-15% rise of blood cholesterol level can be predicted from the existing information obtained from previous studies while consuming 3-6 eggs per day. The final blood cholesterol level of participants at the end of the experimental period will be determined and an appropriate measure will be taken to lower the elevated blood cholesterol level to the prestudy level by continuous monitoring and under supervision of the principal investigator should the blood cholesterol level be elevated.

Participation Consent Form
page 2

Known Hazards and Discomfort: Possible hematoma formation and psychological disturbance which can cause dizziness and/or fainting. The total volume of blood drawn during the study will not exceed one unit of blood as donated at Red Cross Blood Banks, 450 ml. Any any one blood drawing, no more than 75 ml will be taken. While these amounts are well within safe limits, the subjects will be required to state that they have not donated blood within 3 months prior to the study and will refrain from doing so for 3 months after the study. If there is persistant discomfort or injury upon taking blood samples, a physician (Dr. Gary Wright) at the Student Health Center of the University will be available for treatment.

Benefit: Complete characterization of lipoprotein which will identify propensity for risk of heart disease. Also, participation in this study will clearly show adaptability of blood cholesterol and lipoprotein to dietary eggs. This analysis cannot be obtained in the immediate geographical area (Portland is the closest place) and is very expensive.

Cash reward: I will receive free experimental meals and a cash bonus of $100.00 for satisfactory particpation in the study.

Withdrawal: I undertstand that I am free to withdraw my consent to participate in this study at any time I desire without any further obligations.

Anonimity: All of my records and data will be kept strictly confidential. I will be given an ID number to remain anonymous. The confidentiality of the file record will be carefully guarded and no information by which I could be identified will be released or published.

I have read all of the above, asked questions, received answers concerning areas I did not understand, and willingly give my consent to participate in this program.

Participant:	Principal Investigator:
_____	_____
Name	Name
_____	_____
Signature	Signature
_____	_____
Date	Date

DIL RECORD SHEET DATE / / NAME SUBJECT NO.

Please leave a blank space between each meal. NOTES:
Use a separate sheet for each day.

TIME	LOCATION	FOOD ITEM	BRAND	PREPARATION	AMOUNT	OFFICE USE	

NUTRITIONAL SUPPLEMENTS TODAY? yes/no/vitamins/minerals /protein

TYPE(S)

BRAND(S)

TAKEN OF EACH

Any questions call Terri 758-0046

USEFUL ABBREV:

cup	= C
ounce	= oz
tablespoon	= Tb
teaspoon	= ts
lg, med, sm	

USEFUL MEASUREMENTS:

1 cup = 8fl.oz. = 16 Tb
1 lb. = 454 gr. = 16 oz
3 ts. = 1 Tb. = 1/16 C
1 ts. = approx.5gr.solid

APPENDIX D

GENERAL INSTRUCTIONS FOR DIET RECORDING

1. Please record your complete dietary intake on the record sheets. This means everything you eat and drink with the single exception of water.

2. Try to be as accurate and complete as possible when recording your intake. It helps to record items consumed directly after a meal or snack.

3. If a food is a mixture of several items as in a sandwich or soup, list the major ingredients separately.

4. Please indicate if milk is whole, 2% fat, skim, or dry non-fat milk.

5. Indicate approximate hour of day and AM or PM that food item was eaten.

6. Indicate how food was prepared: raw, broiled, baked, pan-fried, deep-fat fried, steamed, etc.

7. Measure in cups, ounces, milliliters, inches, teaspoons, tablespoons, etc.

   Liquids: cups, ounces, or millilitars
   Fruits/vegetables: number, cups, inches in depth and diameter
   Beans/grains/pasta: cups dry or cups cooked
   Meats/fish/cheese: ounces
   Fats/oils: tablespoons, teaspoons

8. Please specify name brands of foods, if possible.

9. Because this is a study on lipid metabolism, it is extremely important that all items containing fats be recorded and measured accurately. Items such as butter, mayonnaise, sauces and gravies as well as all snacks, chocolates, and such must be documented, along with their brand, if applicable, e.g. - lard, butter, Blue Bonnet margarine, etc.

If you have any questions or problems about recording your diet, please ask me, Terri at 757-3214 or 754-3561, or leave a message.

Individual dietary and plasma data for all subjects

#	Age	Wt kg	Ht cm	Kcal	Pro gm	Car gm	Fat gm	EtOH gm	Caff mg	SFat gm	LA gm	Chol diet	Chol plasma	VLDL	LDL	H2	H3	AI	AII	
														-------		mg/dl	--------			
1	34	67.6	173	2265	61	238	86	47.0	259	26.0	18.4	130	169	4	95	16	28	135	72	77
2	32	75.4	193	2063	66	295	73	0	248	24.8	14.2	210	128	5	66	11	27	143	6	38
3	36	90.3	185	4416	171	368	245	15.4	448	81.3	51.2	1482	158	4	96	15	20	124	56	67
4	52	122.6	190	2289	79	236	105	17.9	14	34.9	17.4	219	143	4	98	10	18	128	55	74
5	46	71.3	170	1811	77	239	68	0	133	26.6	8.9	357	193	22	113	12	20	124	55	91
6	34	90.8	185	1971	59	256	85	0	515	31.8	15.8	419	178	6	128	8	17	107	52	83
7	43	76.2	175	1346	49	161	57	0	590	20.6	7.4	255	189	19	125	18	12	119	45	99
8	44	57.2	165	2380	64	243	119	19.1	176	30.3	25.3	263	248	3	152	42	33	147	75	100
9	32	88.1	188	3122	103	317	171	0	407	62.8	29.0	572	202	10	126	19	28	133	64	80
10	36	73.5	178	2360	87	279	107	0	122	34.8	21.8	567	180	9	125	15	21	125	54	86
11	53	101.2	178	2589	103	334	97	8.0	32	29.1	11.7	474	143	19	77	11	19	117	46	62
12	51	79.0	178	1728	71	195	80	0	530	24.8	16.7	293	293	20	184	17	31	164	70	136
13	35	76.3	178	3518	149	353	171	2.6	1085	58.2	28.2	726	163	5	90	17	29	141	61	59
14	32	97.2	193	2322	100	308	68	18.8	103	20.5	10.6	505	135	10	64	15	31	130	68	59
15	52	80.8	185	2620	103	303	101	20.9	106	34.5	16.5	954	196	0	124	28	31	134	68	79
16	43	61.7	163	1942	75	257	73	0	43	28.5	8.4	265	149	7	83	12	23	118	55	65
17	37	84.9	192	3746	171	391	176	0	0	50.5	32.4	544	148	4	82	22	25	123	53	68
18	34	76.3	178	2290	84	275	62	48.4	288	26.0	5.4	217	165	1	108	15	25	117	57	73
19	34	85.8	180	3147	94	361	154	0.0	462	55.5	25.0	598	213	34	121	18	25	114	53	95
20	33	86.3	183	4438	192	494	173	32.1	454	55.3	31.1	726	124	2	61	22	27	120	61	46
21	56	76.3	170	1887	83	244	69	4.3	276	22.0	16.5	339	242	2	159	20	25	133	59	117
22	37	64.0	173	2030	60	286	79	0	64	30.4	12.3	267	202	2	132	26	35	169	69	70
23	46	86.7	193	2586	87	335	105	0	0	38.2	16.4	565	189	4	119	23	31	130	62	48
24	34	74.5	168	1932	68	235	74	13.6	362	29.2	11.7	242	183	3	125	14	28	124	76	61
25	38	101.2	185	2503	107	254	104	18.2	879	40.6	14.1	791	191	13	131	8	24	117	58	61
26	39	92.2	178	2802	115	254	132	29.8	189	49.0	18.1	812	254	21	149	17	28	138	65	105
27	44	92.2	190	3344	115	319	138	55.2	274	47.0	23.2	595	234	2	144	16	27	156	66	99
28	38	76.3	175	3282	118	394	142	5.1	217	50.4	31.4	567	190	5	101	13	24	129	51	51
29	31	65.8	178	2935	108	335	134	1.2	153	52.5	18.3	855	151	4	106	10	17	102	51	50
30	30	97.2	183	2357	61	309	90	13.9	340	21.7	22.1	156	214	22	118	18	23	138	63	20
31	30	72.6	183	4032	127	520	134	41.1	160	55.3	20.9	335	157	6	76	25	19	125	60	22