THE EFFECT OF FEEDING EITHER EGG WHITE, SOY AND NONFAT DAIRY PROTEIN IN MALE SUBJECTS ON PLASMA LEVELS OF TRIGLYCERIDES AND VERY LOW DENSITY LIPOPROTEINS UNDER CONTROLLED CONDITIONS

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INTRODUCTION

The outlook for better health and longer life for the American population rests in large measure upon elimination of vascular disease as a cause of death and disability. Attention has been focused on various lipid factors in the blood of patients with atherosclerosis of the coronary arteries. Abnormally high concentrations of serum cholesterol have been shown to be present in patients with disease of the coronary arteries. Similarly, other studies have established an association between elevation of serum triglyceride concentrations and coronary artery disease. Advances in laboratory techniques, particularly ultracentrifugation have permitted direct quantification of lipoproteins which have also been tied to the development of atherosclerosis. Moreover, numerous investigations have attempted to demonstrate dietary factors which decrease the serum concentrations of cholesterol, triglycerides and which ultimately could influence the development of cardiovascular disease. Most of the research emphasis to date has been on dietary fat and cholesterol. Some consideration has been given to other dietary constituents such as sugar and fibre, but relatively little attention has been given to dietary protein. This is somewhat surprising since, from the time

of the first successful demonstration of experimental atherosclerosis by dietary means, there have been indications that the process might be influenced by the type of protein in the diet. Moreover, epidemiological data derived from human populations show a positive correlation between animal protein in the diet and mortality from coronary heart disease. Vegetarians are known to have lower plasma lipid levels than other segments of the American population and more recent dietary trials provide evidence that plasma cholesterol can be reduced in humans by substituting plant protein for animal protein in the diet.

Less clear, however, is the association between dietary protein source. Few researchers have reached definitive answers concerning the effect of protein source on serum levels of triglycerides and VLDL.

The present study was designed to investigate the effect of different sources of dietary protein on plasma triglyceride and VLDL levels in young adult male subjects. Males were chosen as the study population because of the higher incidence of coronary heart disease in this sex. Under controlled metabolic conditions, subjects were fed one of three dietary treatments in which the primary source of protein came from either egg white, soy or nonfat dairy products. Dietary treatment was in effect over a four week

period during which time blood lipids were determined. Blood lipid concentrations were also determined at a two week follow-up.

REVIEW OF LITERATURE

I. Plasma Lipids, Lipoproteins, and Coronary Heart Disease

A. Coronary Heart Disease - General Information

Atherosclerosis is by far the leading cause of death in developed countries (Schettler, 1977). The task force on atherosclerosis reports that the United States ranks second highest in the world in mortality attributed to coronary heart disease (Task Force on Atherosclerosis of the National Heart and Lung Institute, 1971). Furthermore, coronary heart disease is the number one cause of disability in the labor force (Social Security Administration, 1971), and sixty percent of the cost of hospital and outpatient treatment are in some way attributed to atherosclerosis (Schettler, 1977).

In effect, the annual costs of the epidemic whether they are derived from direct costs for medical care or indirect costs for losses to production, are estimated to be in the billions (President's Commission on Heart Disease, 1965).

In an effort to approximate the cost of coronary heart disease, Dr. Stamler, chairman of the Department of Community Health and Preventive Medicine at Northwestern Medical

School summarized the scope of the epidemic by stating: ..."the toll in human misery is beyond calculation". (page 9).

B. The Pathogenesis of Coronary Heart Disease.

Even though arterial disease is the chief cause of death in the United States and Western Europe, its cause and pathogenesis remain unresolved (Wissler, 1973).

A major problem is that the disease progresses insidiously for many years before symptoms develop making it difficult to follow early development of the disease in individual patients and to relate causability to the types of lesions found. Consequently, much research in the area has concentrated on risk factors that are present in the affected population. (Stamler et al., 1972). Still, other research has focused on the morphologic and chemical characteristics of the disease observed at autopsy in the human (Bottcher et al., 1960) or in the investigation of animal models of the disease (Kritchevsky, 1969).

Only recently has attention been directed to the pathology of the arterial wall and the key role it plays in the accumulation of smooth muscle cells within the intima (Ross et al., 1975). Normal muscular and elastic arteries consist of three morphologically distinct layers: intima, media and the adventia (Benditt, 1977). The intima is the

cell layer principally involved in atherosclerosis, although secondary changes are occasionally found in the media (Ross, 1975).

Three different types of lesions have been classically recognized. Fatty streaks are characterized by a focal accumulation of relatively small numbers of intimal smooth muscle cells. (McGill, 1965). These fatty streaks cause no obstruction and no clinical symptoms. In addition, they are found in the aorta of every child. Moreover, the number of fatty streaks increases with age (Ross, 1975).

The fibrous plaque is the most characteristic lesion of advancing atherosclerosis (Scanu et al., 1979). It is whitish in appearance and is elevated so that it protrudes into the lumen of the artery. The plaque consists principally of an accumulation of intimal lipid laden smooth muscle cells; the lipids being primarily cholesterol, cholesterol esters, and triglycerides. The cells are also surrounded by lipid, collegen, elastic fibers and mucopolysaccrides. Together, these cells and the extracellular components form a fibrous cap. The fibrous plaque stiffens the wall and reduces the caliber of the arterial lumen.

The third lesion appears to be a fibrous plaque that has become altered as a result of hemorrhage, calcification, cell neurosis and thrombosis (Scanu et al., 1979).

The major current theories of the genesis of atherosclerosis share the belief that the lesions begin as localized excessive accumulations of smooth muscle cells in the intima (Benditt, 1977; Ross et al., 1975; Scanu et al., 1979). The current debate, then, is what initiates the proliferation of smooth muscle cells. Low density lipoproteins and insulin have demonstrated a supportive role in the proliferation process; however, further research is needed in determining the exact cause of cell proliferation (Yasugi, 1977).

In summary, clinical manifestations of coronary heart disease include myocardial infarction and angina pectoris. However, the pathogenesis common to all is atherosclerosis of the coronary arteries (Hurt, 1975). Scientists believe that atherosclerosis originates in the intima by proliferation of the smooth muscle cells. The thickening of the arterial wall narrows the arterial passageway and in advanced stages the plaque can become obstructive and may eventually cause arterial occlusion (Ross et al., 1975).

II. The Role of triglycerides and very low density lipoproteins in the development of ischemic heart disease.

A. Prospective Epidemiological Studies.

1. Evidence for the independent role of serum triglycerides in the development of ischemic

heart disease.

No single factor is an absolute cause of either atherosclerosis or of coronary disease. Many factors are interrelated and to the extent that they are present in any one individual, they increase the risk of the disease (Smith, 1978). Primary risk factors include elevated serum cholesterol, hypertension, and cigarette smoking. Secondary risk factors include familial history of coronary heart disease, glucose intolerance, obesity, stress, personality patterns, lack of physical exercise and lipid abnormalities (Inter Society Commission for Heart Disease Resources, 1970). The relationship between serum total cholesterol and atherosclerosis is well established (Blankethorn et al., 1978; Conner, 1968; Goldstein et al., 1972). However, some controversy still exists concerning the relationship of elevated serum concentration of triglycerides and the development of atherosclerosis. Most available data on serum triglyceride levels indicate an association between elevated concentrations of this lipid and ischemic heart disease (Carlson et al., 1979). Differences of opinion exist, however, over whether serum triglycerides exist as a risk factor in and of themselves; independent of serum cholesterol (Brown et al., 1965; Kannel et al., 1979).

The controversy surrounding elevated serum triglyceride levels as an independent risk factor plagues researchers even today. Prospective epidemiological studies have demonstrated conflicting results. Researchers from Stockholm, Sweden, support the contention that plasma triglyceride levels act as a risk factor independent of plasma cholesterol. In a nine year follow-up study, Carlson and co-workers (1972) attempted to relate plasma cholesterol and triglyceride levels to the rate of new events of ischemic heart disease. Subjects were participants in a previous study and were men who had suffered myocardial infarctions during the previous nine years. Statistical analysis indicated a linear increase in the incidence of ischemic heart disease with increasing concentration of triglycerides and cholesterol. To study the role of high levels of only one or both plasma lipid levels, the men were placed into four groups: (1) men with normal levels of both cholesterol and triglycerides; (2) men with high cholesterol levels only; (3) men with high triglyceride levels only, and (4) men with elevation of both lipid components. The rate of new events of ischemic heart disease was highest in the group with high values for both types of plasma lipids followed by the group with elevated triglycerides only (Carlson et al., 1972).

Results of the fourteen year follow-up of the Stockholm Prospective study were recently reported (Carlson et al., 1979). Using more complex statistical analysis, the authors reached essentially the same conclusions reported earlier. Multiple logistic analysis which included eight independent variables showed that both serum cholesterol and triglycerides were independent risk factors with a similar degree of statistical significance. However, when only age, blood pressure, smoking, serum triglyceride and cholesterol were introduced into the multiple logistic analysis, triglyceride appeared more strongly as a risk factor than did cholesterol. From the pure statistical point of view, this analysis suggests that the role of cholesterol as a risk factor found in single factor analysis was not due to cholesterol itself, but rather to the association between cholesterol and some of the other independent risk factors.

Carlson and Bottiger caution against assuming causation when interpreting multivariate regression analysis, particularly when two or more variables are intercorrelated. Their contention is particularly appropriate for the two variables - plasma triglyceride and cholesterol which occur together combined in fixed proportions in the plasma lipoprotein particles (Carlson et al., 1979).

2. Additive risk of associated serum cholesterol and triglycerides.

In a four year prospective study, Brown and co-workers (1965) were able to relate triglycerides and cholesterol levels to both the incidence and prevalence of ischemic heart disease. In addition they were able to assess the asociation between the two lipid components and atherosclerosis. At the time of the initial determinations, 140 of the 1851 sample subjects were known to have ischemic heart disease. Comparison of those with and without the disease was made initially. No significant differences in serum triglyceride values were evident in normal and diagnosed patients. However, significant differences were found in serum cholesterol levels. When groups were divided into thirds based on determination of cholesterol values (lowest third, medium third and highest third) and triglyceride values, ischemic heart disease was more prevalent in individuals with increasing levels of either lipid. Since the mean cholesterol level rises in each triglyceride range, the researchers postulated that the lipids are correlated and the increased prevalence apparently associated with either lipid could be attributed to an interaction between the two. Furthermore, they found that triglyceride concentrations played a determinant role in the prevalence of ischemic heart disease when cholesterol concentrations were below 274 mg/100 ml. However, when cholesterol levels were above 274

mg/100 ml, elevated triglyceride levels had little influence on the prevalence of the disease. Fifty-six subjects acquired ischemic heart disease during the four year study period. The disease occurred more frequently in association with increasing levels of either cholesterol or triglyceride. The authors concluded that it was impossible, based on the small number of subjects, to confirm that elevated triglycerides had an independent effect on the incidence of ischemic heart disease. However, a stronger prevalence was observed when both serum cholesterol and triglyceride levels were elevated (Brown et al., 1965).

Similar associations were found between serum triglycerides and serum cholesterol based on angiographic findings of myocardial infarct patients (Page et al., 1970). Incidence equasions were derived for predicting coronary heart disease. Age and total cholesterol were found to be highly discriminating in predicting risk. Serum triglyceride levels were found to be a fair discriminator of risk. Triglyceride levels, however, were not linearily related to increased risk. That is, risk was shown to increase up to 220 mg/liter, but at higher levels the discriminating power was lost. But, the combination of age, elevated total serum cholesterol, and elevated serum triglycerides demonstrated the greatest probability of having obstructive lesions.

The risk of coronary heart disease was examined prospectively in 2,282 men and 2,845 women according to antecedent lipoprotein and cholesterol status in the Framingham study (Kannel et al., 1971). An increased risk proportional to the antecedent serum cholesterol was found whether or not it was associated with elevated triglyceride levels. When adjustment was made for the concomitant triglyceride concentration and other factors related to coronary heart disease, a residual gradient of coronary heart disease risk proportional to the serum cholesterol was still evident. On the other hand, when risk of C.H.D. was examined according to the triglyceride concentration adjusting for cholesterol, no residual risk gradient remained in men. However, in women, over 50, serum triglyceride level was superior to serum cholesterol level in discriminating potential among heart disease cases.

A subsample (2815 men and women) of the Framingham cohorts was followed between 1971-1975 (Gordon et al., 1977). Blood profiles included total cholesterol, total triglyceride, low density lipoproteins and high density lipoproteins. One hundred and fourty-two individuals developed coronary heart disease during the four year study period. Multivariate and univariate analysis revealed a strong negative correlation between serum HDL level and ischemic

heart disease. Serum LDL level showed a direct positive relationship to the development of coronary heart disease. However, the statistical association was much weaker. Univariate analysis for women over age 50 identified triglycerides as a statistically significant risk factor in the development of ischemic heart disease. However, discriminating power of serum triglycerides was lost in multivariate analysis. On the basis of their analysis, the authors concluded that the most indicative lipid profile for predicting ischemic heart disease was a combination of total serum cholesterol, serum HDL and serum triglycerides (Gordan et al., 1977).

3. Lack of evidence of involvement of serum triglycerides in the development of coronary heart disease.

Several prospective studies have attempted to investigate various risk factors and the role they play in the development of ischemic heart disease. For the most part these investigations have demonstrated a lack of the role of elevated triglycerides in coronary heart disease development even in the presence of associated serum cholesterol (Castelli et al., 1977; Heyden et al., 1979; Hulley et al., 1980; Rosenman et al., 1976; Wilhelmsen et al., 1973).

Wilhelmsen and co-workers (1973) used multivariate analysis to assess the association between coronary heart disease and nine associated risk factors. They demonstrated that triglycerides had little predictive validity in evaluating the risk of coronary heart disease. The highest predictive risk factors found in the sample population of 834 men ages 50 followed for nine years were serum cholesterol levels, smoking and systolic blood pressure.

Multiple logistic risk analysis, used to assess the direct predictive strength associated with risk factors was utilized in the Western Collaborative Group Study (Rosenman et al., 1976). The Western Collaborative Group Study was a prospective epidemiological study of 3,154 healthy men aged 39-59. Subjects were followed over an 8 year period. Statistically significant relationships were found between ischemic heart disease, serum cholesterol, behavior pattern (type A) and cigarette smoking. Serum triglycerides in statistical competition with other lipids demonstrated a negligible association with incidence of coronary heart disease in all age groups.

Researchers in the Cooperative Lipoprotein Phenotyping Study found that Puerto Rican men had exceptionally higher triglyceride levels than did other comparative groups in the presence of normal cholesterol (Castelli et al., 1977).

Furthermore the incidence of coronary heart disease was lower in this population. In short, the researchers found a lower prevalence of coronary heart disease in the presence of elevated serum triglycerides without the normal association of elevated serum cholesterol that often accompanies this lipid elevation. This observation raises doubts about the independent role of elevated triglycerides in the development of ischemic heart disease.

Serum triglyceride levels were found to be of predictive validity for ischemic heart disease in a sample population of Evans County, Georgia, only in white females, age 50 and older (Heyden et al., 1979). However, in white men and all blacks no association was found between triglyceride levels and mortality from coronary heart disease. Statistical adjustments were made to account for the effects of the confounding risk variables in order to isolate the effect of serum triglycerides on CHD mortality. The authors conclude from their follow-up study of 4 1/2 years that:

> The evidence so far in systematic follow-up studies is that serum triglycerides or the triglyceride rich lipoproteins are relatively weak contributors to simple CHD risk prediction and are essentially non-contributory after adjustment is made analytically for the associated serum cholesterol level (Heyden et al.,1979, p. 282).

A recent expose written by Hulley and colleagues (1980) the investigators in the Western Collaborative Group Study

prompted much response within the medical community (Hulley et al., 1980). Hulley's treatise dealt with the lack of association between triglyceride levels and coronary heart disease. Furthermore, the author questioned the efficacy of clinical management of elevated triglycerides due to the deficiency in experimental evidence of the role of triglyceride in coronary heart disease. Hulley's contention is that serum cholesterol is more indicative of risk than are serum triglycerides. The evidence supporting the role of serum cholesterol has unequivocally been demonstrated by epidemiological studies (Brunner et al., 1977; Cramer et al., 1966; Cohn et al., 1976; Heinle et al., 1969), pathological observations that atheromas contain cholesterol (Zilversmit, 1968), biochemical studies (Small, 1977) and animal studies (Armstrong et al., 1970).

In contrast, the spectrum of evidence implicating triglyceride as a causual risk factor is narrower and subject to much debate and controversy. In effect, most of the investigators to date on triglycerides have been of the epidemiological type (Hulley et al., 1980). Hulley contends that Carlson and Bottinger's conclusions in the Stockholm Prospective Study implicating triglycerides as an independent risk factor is based on their failure to include HDL in their multivariate analysis as well as population dif-

fernces, technical differences and chance. Some of the debate surrounding the influence of serum triglycerides on the development of coronary heart disease can be traced to the statistical models used and interpretation of the findings (Hulley et al., 1980). The Western Collaborative Group Study used multivariate logistic analysis with several models (Rosenman et al., 1976).

Hulley (1980) explains that in single factor analysis, triglycerides are indeed implicated in the development of coronary heart disease. When both serum triglycerides and cholesterol are entered into the analysis, both lipid factors reach statistical significance. However, when the strength of each lipid variable is held constant, serum triglycerides were no longer a significant factor. Cholesterol, however, remained a strong predictor after adjustment for triglycerides, suggesting that the apparent association between triglyceride operates largely through the correlation between triglyceride and cholesterol. Subsequent statistical analysis indicated that the association between serum triglyceride and coronary heart disease was attributed to HDL and body mass factors (Hulley et al., 1980). In addition, triglycerides and cholesterol share mutual transport mechanisms in circulation (Fredrickson et al., 1967). Moreover, the triglyceride rich very low density lipopro-

teins (VLDL) contain small amounts of cholesterol and are metabolic precursors of the cholesterol rich low density lipoproteins (Grundy, 1978). The situation becomes complex specifically if such interrelated factors are involved (Hulley et al., 1980).

In response to Hulley's critical evaluation of the literature with reference to triglycerides, Bailar (1980) offers several observations. The author is in agreement with Hulley's criticism concerning weaknesses present in epidemiological studies. Clearly, epidemiologic studies are not worthless, rather they are limited in their conclusions. For example, statistical associations are subject to many interpretations. This ambiguity exists because of the difficulty in sorting out causes, effects, concomitant variables and random flucuations when the causes are multiple and diffuse. In this instance there is much room for individual judgement. Furthermore, epidemiologic studies do not lend themselves to experimental manipulations. In conclusion, associations derived from epidemiologic data should be supplemented by relevant data of other types (Bailar, 1980). In a rebuttal to Hulley's article, Brunzell (1980) critisized Hulley's suggestion of eliminating the control of serum triglycerides in clinical management and the use of serum triglyceride levels in screening for coronary heart disease.

Brunzell conceded that Hulley's analysis applied only to healthy middle aged men. His analysis failed to examine selected subgroups of patients, specifically diabetics, familial combined hyperlipidemics, and renal dialysis patients. The coronary disease in diabetics could be explained by the associated decrease in HDL, obesity, hypertension or the diabetes itself. Further, the coronary heart disease in familial combined hyperlipidemics may be associated with an increase in serum low density lipoproteins. However, serum triglyceride levels in hemodialysis patients have been demonstrated to be an appropriate measure for predicting coronary heart disease (Brunzell, 1980). In a preliminary report on the incidence of coronary heart disease in 322 long term dialysis patients, those who had the disease had significantly higher serum triglyceride levels than did patients without evidence of coronary heart disease (Brunzell, 1977). HDL cholesterol was similar in all groups. Thus, triglycerides and not HDL appeared to be a better predictor of coronary heart disease in this group of patients. In a rebuttal article, Carlson and Bottiger (1981) discuss three concepts that may explain the differences found between the Stockholm Prospective Study and the Western Collaborative Group Study. The study designs as well as the population samples were very similar. Carlson

and Bottiger propose that although obvious ethnic, geographic and environmental differences exist between the two population samples, this consideration is often overlooked by researchers. Secondly, the choice of endpoints used in both studies may explain the contradictory results. The Stockholm Prospective Study used only two endpoints: proven myocardial infarction and death from ischemic vascular disease.

In the Western Collaborative Group Study several endpoints were used including angina pectoris, symptomatic myocardial infarction and silent myocardial infarction (Rosenman et al., 1975). In effect, investigators from the Western Collaborative Group Study were working with a less defined and most likely a less advanced disease population than were the Stockholm Prospective Study. Furthermore, it may well be that there are significant differences between these categories (angina, myocardial infarction, silent myocardial infarction) of ischemic heart disease in relation to risk factor dependence (Carlson et al., 1981).

There is indeed an indication of such difference in the Western Collaborative Group Study (Rosenman et al., 1975). It is stated that while for the subgroup with symptomatic myocardial infarctions there were significant associations with both serum cholesterol and triglycerides. Whereas the

diagnosis angina was only associated with serum cholesterol and the category silent myocardial infarction was not associated with any serum lipid. Thirdly, the authors emphasize the fact that although the logistic model does not recognize serum cholesterol as an independent risk factor in the Stockholm Prospective Study, this does not imply the hypercholesterolemia does not carry a high risk for coronary heart disease in Stockholm. The authors conclude that it is important to differentiate between the clinical conception hypercholesterolemia and the notion of high cholesterol used in epidemiological studies. While hypercholesterolemia may imply a serum cholesterol level that exceeds 308 mg/100 ml, high cholesterol may be as low as 193 mg/100 ml (Carlson et al., 1980).

B. Studies of Serum Lipids in Survivors of Myocardial Infarction and Controls

1. Evidence for the independent role of serum triglyceride.

Several studies have examined the blood lipid profiles of survivors of myocardial infarctions in comparison with those of matched control subjects. In effect, this particular type of study attempts to examine the differences in lipid levels between normal subjects and those with ischemic heart disease in order to isolate risk factors (Albrink et

al., 1959; Albrink et al., 1961; Billimoria et al., 1979; Dolder et al., 1975; Rhoads et al., 1976; Valek et al., 1974). Early studies of Albrink and co-workers (1959, 1961) have clearly demonstrated elevated serum triglyceride levels as the most common lipid metabolic error in patients with coronary heart disease.

Albrink and Mann (1959) demonstrated a strong relationship between elevated serum triglyceride concentration and the development of coronary artery disease. These investigators looked at concentration of serum cholesterol and triglyceride levels in diagnosed myocardial infarct patients compared to controls. Normal ranges for cholesterol and triglycerides were established as two standard deviations above and below their mean concentrations in serum based on data obtained from ninety-two normal men and women aged 20-29. When cholesterol concentrations were graphed and a horizontal line drawn at the upper limit of normal, only 18% of coronary patients had cholesterol concentrations exceeding this figure. Triglyceride concentrations were also expressed on the horizontal line and 175 mg % was established as the upper limit. Most controls fell below the line. In contrast, 70% of male coronary patients had triglyceride concentrations above this level. Further analysis of the data indicated that the segregating power of trigly-

ceride concentration decreases with age. That is, as age increases in the normal individual, so do triglyceride levels. However, serum concentrations of triglyceride levels in the coronary population remained high. This was constant in all ages. The authors suggest, based on their findings, that an error in the metabolism of triglycerides is the lipid abnormality operative in coronary artery disease. Further, they contend that increased cholesterol and increased low density lipoprotein are secondary to decreased efficiency of triglyceride utilization with resulting accumulation of triglyceride in the plasma (Albrink et al., 1959).

Several years later, the same investigators did a similar study in which serum lipids were measured in 115 patients who had suffered a myocardial infarction and healthy men of various ages (Albrink et al., 1961). Serum triglyceride concentrations were increased about 5.4 milliequivalents per liter in 5% of normal men 20-29 years of age, 32% of normal men aged 30 and over, and in 82% of all patients with coronary artery disease. High serum cholesterol concentrations or hypertension appeared to increase the risk of coronary disease in persons with high serum triglycerides but by themselves seemed to carry little risk. An exception was the occurrence of high serum cholesterol without high serum triglycerides in a small number of patients.

Similar results were obtained but to a lesser degree when proportional distributions of serum cholesterol and triglycerides were analyzed in 240 survivors of myocardial infarction from nine countries (Dolder et al., 1975). Cut off points of 280 mg/100 ml for cholesterol and 200 mg/100 ml for triglycerides were established. Twenty-five percent of patients had cholesterol levels above the cut off point. Thirty-five percent of patients had triglycerides above the cut off point. Using the same criteria, twenty percent of all 240 patients showed hypertriglyceridemia without associated hypercholesterolemia. Fifteen percent showed both hypertriglyceridemia and hypercholesterolemia. Ten percent showed hypercholesterolemia without associated hypertriglyceridemia. Although differences between serum triglycerides and cholesterol are small, elevated serum triglycerides seem to occur more predominantly in survivors of myocardial infarction.

Similar results were obtained when proportional distribution was used to determine the main lipid abnormality found in subjects with coronary heart disease (Billimoria et al., 1979). Patients were grouped according to their class of hyperlipoproteinemia based on serum triglyceride, cholesterol and lipoprotein analysis. The highest percent distribution (fifty-six percent) of hyperlipoproteinemia

occured in the coronary heart disease patients was type IV with only serum triglycerides elevated above normal. Twenty-seven percent of all CHD patients had a mixed hyperlipoproteinemia. The majority of these showed elevations in the VLDL fraction.

In an attempt to explain their results, the authors suggested that in studies relating angiography and lipoprotein abnormalities, patients with hypertriglyceridemia have distel vessel blockage. Those with hypercholesterolemia suffer from blockage of the main vessels (Billimoria, 1979). Therefore, coronary patients that manifest elevated triglyceride levels are better able to survive an infarct than those that manifest elevated serum cholesterol.

2. Lack of evidence of the involvement of serum triglycerides in the development of coronary heart disease.

In other studies, arteriographic findings and serum lipids and lipoproteins have demonstrated a lack of association between triglyceride levels and extent of lesions (Rhoads et al., 1976) (Valek et al., 1974). Valek and coworkers (1974) performed coronary angiography in ninety patients with coronary artery disease to assess the extent of lesions present in the disease state. The results of the angiography were compared to fasting levels of serum cho-

lesterol, triglycerides and weight index. A significant correlation was found between serum cholesterol and coronary atherosclerotic level. However, triglycerides and weight failed to show a relationship to the increment of atherosclerotic lesions. Similar conclusions were demonstrated when a subsample of the Honolulu Heart Study was used to ascertain the frequency of defined hyperlipoproteinemia and to investigate the relationship between lipoprotein fractions and coronary heart disease (Rhoads et al., 1976). Males with coronary heart disease were compared to a 30% probability sample of normal men from the Honolulu Heart Study. Height, weight, skinfold, blood pressure, serum total cholesterol, LDL, HDL, VLDL and triglycerides were determined for each control. Calculated prevalence rates of coronary heart disease rose with increasing levels of total cholesterol and LDL. A highly significant difference in serum HDL levels was also evident between cases and controls. HDL levels tended to fall with increasing weight as determined by skinfold. No significant differences in triglycerides and VLDL (very low density lipoproteins) were found between groups. When the distribution of lipoprotein phenotypes were investigated for the population, twenty-six percent were found to have typs IV hyperlipoproteinemia (cholesterol less than 210 mg/liter, triglycerides greater than 190 mg/liter). The

prevalence rate of type IV hyperlipoproteinemia showed no correlation with the incidence of coronary heart disease (Rhoads, 1976).

C. Arteriographic findings and composition of lesions

Prospective epidemiological studies and comparisons of survivors of coronary heart disease with controls can show associations between risk factors and the development of coronary artery disease. More direct examination of the extent of atherosclerotic lesions can be performed by arteriography and histochemical estimation.

In a report that examined triglycerides in tissue cultures, Adams (1967) found that triglycerides exhibited little sclerogenic activity. In contrast, when cholesterol was implanted in tissue cultures, it was found that this lipid displayed sclerogenic activity. Nevertheless, saturated and monosaturated triglycerides promoted platelet aggregation and experimental thrombosis as well as accelerated platelet coagulation. On the basis of the results, Adams (1967) concluded that cholesterol is partly responsible for atheromatous thickening of the arteries, whereas triglycerides may initiate some part of the thrombastic occulsive episode. Together, these two processes lead to the onset of coronary heart disease (Adams, 1967). The composition of the atherosclerotic lesion lends supporting evidence implicating

plasma cholesterol concentration in the development of coronary artery disease. Triglycerides have also been found in the lesions (Bottcher et al., 1960), but there is some speculation concerning their presence and the development of coronary heart disease (Abdulla et al., 1969). Data on coronary arteries based on six human autopsies reported negligible amounts of triglycerides found in the arteries; whereas, cholesterol and cholesterol esters were the predominant lipids found in the histochemical examination. The researchers stipulate that previous reports demonstrating triglycerides present in the coronary lesions were due to contaminating advential adipose tissue which is extremely hard to remove from the coronary arteries at autospy. In effect, triglycerides account for very little of the lipid in the intima of the coronary artery (Abdulla et al., 1969).

Block and co-workers (1976) performed coronary arteriography in fourty-six patients to assess the relationship of type II and type IV hyperlipoproteinemia and the extent of damage to the coronary arteries. Hyperlipoproteinemia is found to be a risk factor in the development of atherosclerosis (Grundy, 1978). Furthermore, type II and type IV are the predominant lipoprotein phenotypes found in coronary heart disease (Witztum, 1978). Type II is characterized by serum triglycerides exceeding 150 mg/100 ml and serum cho-

lesterol exceeding 220 mg/100 ml. Type IV is characterized by elevations in triglycerides (above 150 mg/100 ml) but normal serum cholesterol concentrations (National Heart and Lung Institute, 1978). Analysis revealed that distinct differences in atherosclerotic lesions were found between the two subgroups (Bioch et al., 1976). Type II patients had a high prevalence of main left coronary disease and of distal coronary atherosclerosis. Type IV patients usually had disease localized in the proximal coronary artery. The authors contend that these differences may be related to the differing plasma lipoprotein patterns in the two syndromes. Damage to the main left coronary artery (found in type II) has been demonstrated to be more lethal than damage to the proximal artery (type IV). The results of this study implicate both serum cholesterol and serum triglyceride in damage to the coronary arteries; however, serum cholesterol has demonstrated to have a more lethal role in the development of coronary heart disease.

D. Triglyceride and HDL relationship mechanisms

Some researchers claim that the elevation of serum triglycerides and its effect on the development of coronary heart disease is attributed to the relationship between serum triglycerides and HDL (Chan et al., 1979; Kaukola et al., 1980; Miller et al., 1977; Sauar et al., 1980; Schaefer et al., 1978).

Miller and co-workers (1977) found that survivors of myocardial infarction had lower levels of HDL than did matched controls. Furthermore, higher levels of low density lipoproteins were observed in myocardial survivors. However, this relationship was not as strong. No statistically significant differences were found between patients and controls with reference to serum triglyceride concentrations. It is difficult to assess whether there was any correlation between HDL triglyceride levels since analysis did not include this correlation.

Sauar et al. (1980) and Chan and co-workers (1979) offer an alternative explanation based on lipoprotein lipase activity to clarify the HDL/ triglyceride relationship. They suggest that the inverse correlation reflects the reduced ability of HDL in hypertriglyceridmia states to activate lipoprotein lipase, thereby affecting peripheral removal of triglycerides.

Schaefer and his co-workers have suggested a hypothesis that might explain the inverse relationship of high density lipoproteins and serum triglycerides (Schaefer et al., 1978). They contend that the protein constituents of HDL (apo-protein A-I and apo-protein A-II) are found in chylomicrons. Individuals with defective chylomicron catabolism, particularly in the hypertriglyceridemic state lack the constituents and precursors needed to form HDL.

In a more recent study, serum cholesterol, triglycerides and high density lipoproteins were determined in 56 male survivors of myocardial infarction and 82 matched controls (Kaukola et al., 1980). Little difference in total serum cholesterol existed between the two groups. However, HDL cholesterol as well as triglyceride levels were statistically different between myocardial survivors and controls. In addition, serum HDL and triglyceride levels were strongly negatively correlated. That is, high levels of HDL (recognized to be a protective factor against coronary heart disease) exist simultaneously with low levels of triglycerides. Because of this relationship, the authors contend that triglycerides are implicated as an independent risk factor in the development of ischemic heart disease. Furthermore, Kaukola and co-workers suggest the following mechanism that may explain the inverse correlation between triglycerides and high density lipoproteins: HDL cholesterol originates as a result of lipoprotein lipase activity from very low density lipoproteins (VLDL). Consequently, the ability of HDL to activate lipoprotein lipase is reduced in hypertriglyceridemic states (Kaukola et al., 1980).

E. Summary of the Independent Role of Triglyceride in the Development of Coronary Heart Disease

In summary, a great deal of controversy exists concerning the effect of elevated serum triglycerides and its independent role in the development of ischemic heart disease. Conclusions from prospective epidemiological studies as well as research from survivors of myocardial infarction and controls show conflicting results. More direct methods such as angiographic findings and histochemical estimations have also demonstrated controversial results. Much of the dispute surrounding elevated serum triglycerides is due to it's association with other known risk factors. Elevated serum cholesterol, obesity, abnormal glucose tolerance and elevated blood pressure may exist simultaneously with elevated serum triglycerides. When confounding variables are controlled for, elevated serum triglycerides seem to lose their discriminating power. Nevertheless, an increased risk of developing ischemic heart disease is associated when elevated serum triglycerides are considered with each of the risk factors.

Theoretically, the controversy may hinge largely on whether an elevated triglyceride level is defined as a primary or secondary risk factor.

III. The Plasma Lipoproteins

A. General Lipoprotein Information

Plasma lipoproteins are lipid protein complexes that transport lipids in the circulation and regulate lipid synthesis and catabolism (Miller et al., 1979) (Smith et al., 1978). The major functions of the plasma lipoproteins include transporting endogenous and exogenous triglyceride to sites of utilization and storage and to transport cholesterol between sites of absorption, synthesis, catabolism, and excretion (Morrisett et al., 1975).

The classification and nomenclature of the plasma lipoproteins have been based primarily on operational definitions as determined by their electrophoretic mobility or by their rate of ultracentrifugal flotation in salt solutions (Skipski, 1972). Based on these criteria, human plasma lipoproteins have been divided into four classes: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (Morrisett et al., 1975).

Each lipoprotein class has a characteristic composition but the amounts of lipid and protein constituents do not occur in fixed ratios within each class. In effect, each lipoprotein group is heterogenous with respect to lipid and protein components (Shore et al., 1973). All plasma lipoproteins are implicated in atherogenesis either as carriers of excessive lipid to the vessel wall or indirectly as pro-

genitors of the lipoproteins interacting with the vessel wall. In addition lipoproteins, specifically HDL, serve as protective agents that limit the accumulation of lipid within the vascular intima (Getz, 1979).

Triglycerides can gain entry into the circulation in two primary ways; as chylomicrons from dietary lipids and as very low density lipoprotein made in the liver using stored fatty acid from adipose tissue or fatty acid newly made from carbonydrate (Smith, 1978).

B. Chylomicron Production and Metabolism

Chylomicrons are the largest lipoproteins ranging in diameter from 750-1200A (Jackson et al., 1976). They have flotation values (S) in the range of 300-10000 and densities of less than 0.95 g/ml (Herbert et al., 1978). Chylomicrons are formed in the small intestine (Roheim et al., 1966) and consist largely of triglyceride with relatively small amounts of cholesterol, cholesterol ester, phospholipid and protein in the form of apoprotein (Morrisett et al., 1975). Average values for the composition of chylomicrons by weight are: 80-95% triglycerides, 1-3% unesterified cholesterol, 2-4% esterified cholesterol, 3-6% phospholipids, and 1-2% protein (Herbert et al., 1978). Findings from several reports implicate apoprotein C and apoprotein B as major protein components of chylomicrons and apoprotein A as

a minor component of the chylomicron (Getz et al., 1979; Miller, 1979; Morrisett et al., 1975; Steinberg, 1979). Apoproteins A and B originate in the liver (Arickman et al, 1976) and apoprotein C is acquired by net transfer from HDL during metabolism of the chylomicron (Havel et al., 1973). Apoprotein C-II is necessary for optimal activation of lipoprotein lipase at the peripheral tissues. The synthesis of the mature caylomicron is then hydrolized by lipoprotein lipase which results in a reduction in the chylomicron core and concomitant reduction of the surface area mainly through the loss of surface constituents - apoprotein A and C phospholipid and free cholesterol, with HDL probably serving as a recipient of these elements. The resulting particle is the chylomicron remnant, containing cholesterol ester in its central core and apoprotein B and phospholipid on its surface (Getz, 1979). The triglyceride poor, cholesterol rich chylomicron remnant is then available for further catabolism in the liver (Gotto et al., 1977).

C. VLDL Production and Metabolism

The very low density lipoproteins (VLDL) are particles which vary from 280-750 A in diameter. Their flotation values (S) range from 20-400 and float at densities between 0.95-1.006 g/ml (Herbert et al., 1978). The size of the VLDL particle is directly proportional to its triglyceride

content and inversely proportional to the protein and phospholipid conten (Eisenberg, 1975). Very low density lipoproteins contain an average of 45-64% triglycerides, 15-20% unesterified cholesterol, 16-22% esterified cholesterol, 15-20% phospholipids and 6-10% protein by weight (Herbert et al., 1978).

The major apoprotein constituents are apoprotein B, apoprotein C and apoprotein E. Apoprotein A exists as a minor component of the VLDL particle (Fredrickson et al., 1972; Shelburne et al., 1974). The primary function of VLDL is to transport endogenously synthesized triglycerides from the liver and intestine to adipose tissue for storage (Jackson et al., 1976). After secretion by the liver or intravascular maturation of intestinal VLDL, the VLDL are transported to adipose tissue for the lipoprotein lipase mediated hydrolysis of its triglyceride content (Goldstein et al., 1977).

Following hydrolysis from lipoprotein lipase and the loss of the triglyceride derivative of VLDL, the triglyceride depleated particle (VLDL) may be further catabolized to LDL. The VLDL derivative can also be removed and degraded by the liver (Jeng et al., 1980). The conversion of the LDL particle involves the loss of triglyceride as well as a loss of the associated VLDL apoproteins. However, the newly

formed particle acquires an abundance of cholesterol ester (Getz, 1979). The particle remaining in this case is referred to as IDL (intermediate density protein) which is subsequently converted to LDL at a site and by a mechanism yet to be clarified (Goldstein et al., 1977). The transformation of VLDL to LDL involves a loss of many of the constituents of the large particle VLDL, but the quantitative retention of apoprotein B (75-90%) may suggest a direct precursor-product relationship between VLDL and LDL (Getz, 1979). In addition, very low density lipoproteins can also be removed and degraded by the liver after hydrolysis of lipoprotein lipase (Jeng et al., 1980).

IV. The Effect of Dietary Protein on Plasma Lipids and Lipoproteins

A. Historical overview

The prevalence of and disability associated with atherosclerosis have led researchers to search for those factors which may influence the incidence of the disease. In the quest for associated factors, diet has become one of the focal points of inquiry. The first clear demonstration that diet plays a role in the development of atherosclerosis was reported as early as 1908 by Ignatowski. He investigated the effects of feeding animal products such as meat, milk and eggs to rabbits and observed that some of the animals

developed lesions in the aorta resembling those seen in atherosclerosis. However, since animal products fed in these early studies contain cholesterol and after it was shown that atherosclerosis could be produced in rabbits by feeding cholesterol (Anitschkaw, 1933), subsequent work concentrated on cholesterol feeding as a method of producing experimental atherosclerosis and possible effects of other dietary components were largely ignored. Subsequent experiments by Meeker and Kesten (1940,1941) showed that rabbits fed a high protein, cholesterol-free diet with casein as the source of protein became hypercholesterolemic and developed atherosclerosis. These effects were not observed when soy bean flour replaced the casein in the diet. The soy bean flour also appeared to have an inhibitory effect on atherosclerosis produced by the addition of cholesterol into the diet. However, these studies on dietary protein made no lasting impression and after the discovery in the early 1950's that feeding polyunsaturated fats caused a lowering of serum cholesterol levels in humans, most of the emphasis in atherosclerosis research was concentrated on the effects of dietary fat (Carroll, 1975).

B. Dietary Intake Data and the Association of Animal and Vegetable Protein in the Development of Coronary Heart Disease

Evidence suggesting a protective role for vegetable protein and an atherogenic role for animal protein comes from dietary intake data (Conner et al., 1972; Moore et al., 1976). Using product moment correlation coefficients between coronary heart disease mortality and dietary intake from various nutrients, Connor and Conner (1972) were able to demonstrate that intake of animal protein was strongly associated with death from coronary heart disease. Dietary intake of vegetable protein showed a weak negative correlation.

Moore and co-workers (1976) showed a strong negative correlation between intake of vegetable protein and the extent of atherscerotic lesions; however, no association was found between extent of damage and intake of animal protein. Dietary histories were obtained retrospectively by interviewing spouses of 253 New Orleans men who had died of CHD. This information was then used to calculate average daily intake of selected dietary nutrients and determine any possible association between nutrient intakes and the extent of raised lesions found at autopsy. In addition to the relationship found between vegetable protein and sceroyenic damage, crude fiber and total carbohydrate were associated with less atheroscerotic involvement. Moreover, there was no indication that intake of total calories, total fat, satu-

rated fat, unsaturated fat, sugar or cholesterol were related to the extent of lesions found.

C. Coronary Heart Disease Morbidity and Mortality in Seventh Day Adventists

In addition to dietary intake data correlating protein source to the incidence and prevalence of coronary heart disease, vegetarians particularly Seventh Day Adventists have demonstrated a substantially lower mortality rate from coronary heart disease in comparison to their nonvegetarian counterparts. This may in fact be attributed not only to protein source, but lifestyle as well as lower intakes of saturated fatty acids and higher intakes of fibrious material (Phillips et al., 1978). Similarly, Wyner and co-workers (1959) reported that hospital admissions of patients with coronary heart disease were 40% fewer for the Seventh Day Aventists men and 15% fewer for the Seventh Day Aventists women, thus indicating that adherence to a vegetarian lifestyle has a protective role for subsequent development of coronary heart disease.

D. Serum Lipids of Vegetarians and Non-Vegetarians

From the early 1950's to the present, researchers have suspected that the type of dietary protein might play a contributing role in the development of coronary heart disease (Burslem et al, 1978; Conner et al., 1972; Hardinge et al., 1954; Ruys et al., 1976; Sacks et al., 1975; Walden et al.,

1964; West et al., 1968). Much of the early information implicating dietary protein in the development of atherosclerosis came from comparisons between vegetarians and non-vegetarians. Cumulative evidence indicates that vegetarians have lower serum lipids than their non-vegetarian counterparts. Whether the protein moiety in the diet is responsible for this lipid lowering effect is subject to some speculation. Vegetarians are know to ingest lower intakes of saturated fatty acids and cholesterol. This too could be a contributing factor in lowering of serum lipids. Vegetarians also have higher intakes of fiber which have also demonstrated a lipid lowering effect (Burslem et al., 1978). Researchers do believe however, that protein from vegetable sources is in part responsible for lower serum lipids.

Early studies of Hardinge and Stare (1954) demonstrated that vegetarians had lower serum cholesterol levels than non-vegetarians. Dietary analysis and serum cholesterol levels were investigated in eighty-six lacto-ovo vegetarians and twenty-six pure vegetarians. These results were compared to values for a control group of eighty-eight non-vegetarians. Analysis of dietary intake demonstrated that vegetarians (pure, lacto-ovo) had lower intakes of calories furnished by fat, particularly animal fat. In addition, the non-vegetarians had significantly higher dietary intakes of

cholesterol than did either type of vegetarian. Although Hardinge and Stare (1954) suspected that the protein source might be the reason for the lower serum cholesterol concentrations observed in the vegetarian group, they attributed the lower concentration to dietary intakes of cholesterol. In short, the authors believed that higher dietary intakes of cholesterol lead to a concomitant elevation of serum cholesterol.

Walden and co-workers (1964) investigated serum cholesterol as well as serum triglyceride concentrations in a New York City based population sample of Seventh Day Aventists. One hundred and fourty-five Seventh Day Aventists ranging in age from 20-91, participated in the study. Data was compared to that for a normal New York City population sample matched for age and sex. Dietary intake data indicated that the contribution of calories from carbonydrate was higher in the vegetarian group than in controls. Furthermore, calorie intake from fat was lower in the vegetarian sample. Likewise, vegetarians were consuming fats higher in polyunsaturated fatty acids than non-vegetarians. Protein intake as a percent of total calories was comparable in both diets. The median levels for serum total cholesterol for both Seventh Day Aventists (SDA) men and women were lower at all ages than those of the men and women in the New York

City population sample. Furthermore, the median serum total cholesterol level of the SDA men reaches its maximal value at age 54, some ten years later than the peak level found among the New York City men. However, no differences were noted in the slope and the shape of the curve representing age serum cholesterol concentrations when women were compared. When serum triglycerides were examined, differences with respect to age were noted. Median values for serum triglycerides in vegetarians were lower than in matched controls to age 39 and somewhat higher thereafter. The SDA women experienced a similar pattern. Serum triglyceride concentrations were lower than those of the normal sample up to age 47 and slightly higher values were noted thereafter. When age change curves were plotted in the two population samples, with regard to serum triglyceride concentrations, little differences between the two groups were noted; that is, the age change curves between the vegetarians and controls were similar.

When the serum lipid values for subjects in the coronary prone age range (men over 32; women over 34 years) were tabulated, a comparison between the two groups was more easily made. Median serum total cholesterol levels of SDA men 33 years of age were 13 percent less than those of the New York City population sample. However, the median serum

triglyceride value for male SDA of these ages is 19 percent higher than that of New York City men. Seventh Day Aventist women over 35 had median serum total cholesterol values 21 percent lower than aged matched controls. Median serum triglyceride levels in this age range were the same among the women of both population samples. In summary, Walden and co-workers (1964) found that serum cholesterol levels were lower in the SDA population than matched controls; this relationship persisted at all ages. In contrast, serum triglyceride levels were lower in the vegetarian population up to age thirty-five and were slightly elevated thereafter when compared to the non-vegetarian counterpart.

In a later study, Seventh Day Aventists were also used as the sample population when investigating the serum cholesterol levels in vegetarians (West and Hayes, 1968). West and Hayes found results compatable with earlier work (Hardinge and Stare, 1954; Walden et al., 1964) done with vegetarians. That is, serum cholesterol levels were lower in vegetarians than matched controls. Evaluation of nutrient intake of non-vegetarians indicated a significantly higher intake of total fat, saturated fatty acids, protein and cholesterol than the vegetarian study population.

Sacks and co-workers (1975) found similar results with regard to cholesterol concentrations when serum lipids of

vegetarian and non-vegetarians were analyzed. However, in contrast to the results of Walden et al., 1964, Sack and co-workers found that serum triglyceride concentrations of vegetarians were lower at all ages when compared to their non-vegetarian counterparts. The sample consisted of vegetarians adhering to a microbiotic diet and a random group of offspring from the original Framingham Study who were non-vegetarians. The microbiotic diet is essentially vegetarian and includes dietary staples such as whole grains, beans, fresh vegetables and soy products. Quantification of serum lipids and lipoproteins included: total cholesterol, triglycerides, HDL, LDL, and VLDL. Significant differences in all plasma lipids and lipoproteins were noted when vegetarians and controls were compared. Serum triglycerides in controls and vegetarians were 86 mg/100 ml and 59 mg/100 ml respectively. Similar differences were found in the VLDL fraction (7.2 mg/100 ml in controls; 11.8 mg/100 ml vegetarians).

Further evidence indicating the beneficial role of the vegetarian diet came from an isolated case of a young male vegetarian (Sacks et al., 1975). During adherence to the microbiotic diet, his VLDL fraction was elevated to 52 mg/100 ml and he was classified as having type III hyperlipoproteinemia. He withdrew from the vegetarian diet and

subsequently upon further examination, it was found that his VLDL had markedly increased to 78 mg/100 ml and triglycerides were elevated to 199 mg/100 ml. He resumed the vegetarian diet and his VLDL as well as serum triglycerides fell substantially. The authors concluded on the basis of the results from the study and this isolated case that adherence to a vegetarian diet had beneficial effects with regard to plasma lipids and lipoproteins (Sacks et al., 1975).

Blood lipid levels in one hundred and eighty-three SDA adolescents were examined and compared to a free living population of adolescents from the general Australian population of Sidney (Ruys et al., 1976). Significant differences in serum cholesterol concentrations existed between the two groups. Vegetarians exhibited lower serum cholesterol concentrations than did controls. In addition vegetarians exhibited lower but not significantly lower serum triglyceride concentrations than controls.

Burslem and co-workers (1978) investigated the serum lipid, lipoproteins and apoprotein levels of sixty-eight vegetarians living on a farm in Tennessee and compared the findings to a matched set of controls. Mean total cholesterol, HDL and LDL levels were lower in vegetarians than in controls. Although vegetarians had lower triglyceride concentrations (82 mg/100 ml) than controls (95 mg/100 ml),

this did not reach statistical significance. The very low density lipoprotein fraction was lower in vegetarians, but again this did not reach statistical significance. Moreover, apoprotein A and apoprotein B were also lower in vegetarians. Although HDL concentrations were lower in vegetarians, the HDL to apoprotein A ratio was high reflecting an enhanced binding of cholesterol by the HDL apoproteins apparent in vegetarian plasma. In addition, the researchers found a strong negative relationship between the HDL and triglyceride concentrations. The authors concluded on the basis of these results that vegetarians are in a lower risk category for developing coronary heart disease (Bursiem et al., 1978).

In summary, researchers have continually found that vegetarians have lower serum cholesterol concentrations than their non-vegetarian counterparts. However, conflicting results have been observed when plasma triglyceride concentrations have been compared between vegetarians and meat eaters. Whether the lower concentration of plasma cholesterol and triglycerides are attributed to lower intakes of saturated fat and cholesterol as opposed to the type of protein is still subject to much debate.

E. Effects of Animal and Vegetable Protein on Serum Lipids

Based on the evidence that vegetarians have lower serum lipids than non- vegetarians, researchers began to suspect that the lipid lowering effect might be attributed to the protein in the diet. Based on this premise, several animal and human controlled experiments were initiated that compared the effects of feeding animal versus vegetable protein on serum lipid levels (Anderson et al., 1971; Campbell et al., 1965; Hamilton et al., 1974; Neves et al., 1980; Walker et al., 1960).

Hamilton and Caroll (1974) investigated the effects of feeding proteins from various plant and animal sources to rabbits fed a low-fat, cholesterol- free, semi-synthetic diet. They consistently found that dietary proteins from animal sources tended to be more hypercholesterolemic than those from plant sources. In contrast, Neves et al., 1980 failed to demonstrate any difference in serum cholesterol concentrations with rats fed either pure or crude plant proteins compared with pure and crude animal proteins. Triglyceride levels varied independently of the dietary source.

Human studies that involve comparison of plant and animal protein have shown conflicting results. Walker and coworkers (1960) were able to demonstrate a significant lowering of plasma cholesterol when plant protein replaced animal protein in the diets of 12 young women. The study was

designed to test the difference in serum lipids between individuals fed two dietary protein sources (animal and vegetable). Sources of vegetable proteins included rice macaroni, wheat cereals, oat cereals, legumes and soy powder. Animal proteins included uncreamed cottage cheese, skim milk, veal, turkey and fish. The protein content of the diet averaged 45-50 grams or 8 percent of calories. The diets were identical in all dietary components except for the source of protein. Covariate analysis showed at the end of two weeks and five weeks serum cholesterol of the subjects receiving the vegetable protein diet was significantly lower than in those eating the animal protein diet. No significant differences were observed between the two groups with regard to serum triglyceride levels. Thus, the researchers concluded that replacement of vegetable protein for animal protein in the diet of healthy young women showed a lowering of serum cholesterol concentrations.

Campbell and co-workers (1965) studied the effect of the kind of proteins on serum lipids by comparing a diet containing wheat gluten as the chief source of nitrogen with a diet containing an isonitrogenous amount of a mixture containing casein and lactalsumin as a replacement for the wheat gluten. The vegetable and animal proteins were each tested in diets with an assortment of fats having a 12 per-

cent linoleic acid content for one period of the study and a 40 percent linoleic acid content for another period. A cross-over design was utilized and each dietary treatment lasted twenty-five days. The results indicate that no differences existed in serum cholesterol or triglycerides between individuals on the two dietary treatments. This situation persisted whether the diet contained 12 percent of linoleic acid or 40 percent linoleic acid.

Anderson et al. 1971, investigated the effect of feeding a 120 gram protein diet to eleven male volunteers. Sixty grams of the protein came from either wheat gluten serving as the vegetable source or egg white serving as the animal source. Diets were identical in all respects except for the type of protein. Blood lipids were analyzed the last day of treatment. The mean serum cholesterol level of individuals fed the gluten diet was higher by 4 mg/100 ml than in those fed egg white diet but this difference was not statistically significant. Similarly, mean serum triglyceride level was 92 mg/100 ml for egg white treatment and 84 mg/100 ml for the wheat gluten diet. Thus, mean serum triglyceride level was higher by 8 mg/100 ml when the men were eating the egg white diet but like serum cholesterol, this difference did not reach statistical significance. The researchers concluded that changes in the protein content of

the diet are of no particular value in designing diets for the reduction of serum cholesterol.

F. The Effect of Feeding Soy Protein on Plasma Lipid Levels

1. Animal Experiments

The hypocholesterolemic effect of soy has aroused the interest of many researchers. Interest began to grow as a result of the early work of Howard and co-workers (1965). They observed a hypercholesterolemic effect in rabbits fed a low-fat, low-cholesterol diet. However, when soy protein replaced casein in the diet, serum cholesterol decreased significantly. In addition, examination of the aortas demonstrated sudanophilia with the casein diet. However, no gross sudanophilia was observed in rabbits fed the corresponding diet containing soy protein.

Since the discovery by Howard and co-workers in the 1960's, researchers have sought to investigate the effects of feeding soy under several experimental conditions (Carroll et al., 1979; Fumagalli et al., 1978; Howard et al., 1965; Huff et al., 1977; Kim et al., 1978; Nagata et al., 1980; Nagata el al., 1981). Investigations by Huff et al. (1977) demonstrated results similar to those of Howard et al. (1965). Huff et al. (1977) found the same hypocholesterolemic effect in rabbits fed soy diets. In addition,

other plant proteins demonstrated the same cholesterol lowering effect. Moreover, when a 1:1 casein:soy diet was fed it also lowered serum cholesterol. A 3:1 casein:soy diet demonstrated a smaller lowering effect. Upon further analysis the researchers hypothesized that the differing effects of casein and soy protein isolate could be due to differences in their amino acid composition (Carroll et al., 1979). In an attempt to resolve this question, feeding trials were carried out either with enzymatic digests of the proteins or mixtures of the amino acids simulating the amino acid composition of the protein. Enzyme hydrolysates gave similar results to those obtained with the intact protein. Similarly the mixture of the amino acid corresponding to intact casein demonstrated identical results to those obtained with the intact protein. However the feeding of a mixture of amino acids corresponding to soy protein isolate resulted in a somewhat higher level of plasma cholesterol then did the intact protein (Carroll et al., 1979; Huff et al., 1977).

Punagalli (1978), also using rabbits, demonstrated similar results. Six rabbits were fed a sequence of these diets: laboratory stock diet, a semi-purified diet containing 25 percent casein, and a similar diet in which soya bean meal replaced casein. On changing from the stock chow diet

to the casein, the plasma cholesterol rose four fold after sixteen weeks, but fell 50 percent after twelve weeks on the soy diet. Balance studies showed that replacement of casein with soya meal in the semipurified diet caused an increased fecal excretion of sterols; however, bile acids were unaffected by the three dietary treatments.

Kim et al. (1978), working with swine, were able to demonstrate a hypocholesterolemic response of soy as compared to casein even in the presence of a high fat and high cholesterol intake. A series of three experiments were undertaken. In all instances swine fed a mash diet served as controls. In the first experiment swine were fed a high-fat, high-cholesterol diet with protein coming either from soy or casein. Serum cholesterol levels were significantly higher in animals fed casein than in those fed the soy diet or lab mash diet. In the second experiment, swine were fed a high-fat, high-cholesterol diet with a 1:1 mixture of casein to soy. Although fluctuation and more variation were observed in serum cholesterol levels, mean concentration of serum cholesterol was lower when 1:1 casein soy diet was fed in comparison to the casein fed group. The third experiment incorporated the addition of methionine to the soy diet. This was done because some scientists attribute the hypocholesterolemic effect of soy diets to methio-

nine deficiency. Again, soy diets whether supplemented with methionine or not, resulted in a significantly lower serum cholesterol concentration than did the casein (Kim et al., 1978). Throughout the experiment, cholesterol balance was examined. The researchers wished to investigate which parameters of cholesterol balance were altered by the soy protein product to account for the altered effect on serum cholesterol. Whole body cholesterol synthesis, cholesterol absorption, and bile acid excretion demonstrated no significant differences between groups. The authors hypothesize that the differences in serum cholesterol may be attributable to the amino acid composition, dietary fiber or the saponin content present in soy (Kim et al., 1978).

Nagata et al. (1980) examined the influence of soy and casein diets in rats in the presence of different amounts of fats. When rats were given diets containing maize oil at 50 grams per kilogram of body weight, the concentration of serum cholesterol was the same for both dietary treatment groups. However, when the dietary fat level was reduced to 10 grams per kilogram of body weight, soy protein produced a significantly lower serum cholesterol level than the casein. Triglyceride changes were independent of dietary treatment. Later, Nagata and co-workers (1981) examined the effect of an amino acid mixture that simulated soya bean protein or

casein on serum cholesterol, LDL, HDL fractions as well as serum triglycerides. The amino acid mixtures were supplemented with lysine or arginine in order to make the arginine:lysine value in the nitrogen sources identical. Differences in the nature of dietary nitrogen sources (either as intact proteins or amino acid mixtures) did not cause changes in the concentration of serum triglycerides. The addition of specific amino acids to the diets displayed no additional effect on these lipid components except for the increase in serum triglycerides of rats given diets containing soya bean protein or a simulated amino acid mixture supplemented with lysine. Both soya bean protein and its simulated amino acid mixture exhibited a hypocholesterolemic effect by comparison with the corresponding casein diet.

In summary, studies with animals have consistently demonstrated that feeding soy protein lowers cholesterol; this effect may be attributed to the increased fecal excretion of sterol. Some investigators believe that the hypocholesterolemic effect of soy may be due to other dietary components contained within the soy product which include fiber or saponin. Still, others contend that the amino acid composition may be responsible for the effect. Enzyme hydrolysates of soy displayed identical results to that of the intact protein; however, the serum cholesterol lowering

effect was decreased when an amino acid mixture corresponding to the amino acid pattern of soy was fed. In addition, methionine deficiency has been proposed by some to explain the hypocholesterolemic effect of soy. However, in feeding trials with swine, methionine supplemented soy had no effect on the cholesterol lowering properties. In the presence of other nutrients such as fat, soy loses its cholesterol lowering properties suggesting some interaction between the protein moiety and fat.

Although soy protein exerts a cholesterol lowering response in animals, investigations have demonstrated that serum triglyceride concentrations are unaffected when soy replaces casein in the diet.

2. Human Experiments with Soy Protein

Researchers hae sought to study the effect of feeding soy in humans based on the results obtained with animal experiments. Studies involved normal healthy individuals (Carroll et al., 1978; Hodges et al., 1967; Van Raaj et al., 1981), individuals with mildly elevated cholesterol (Shorey et al., 1981) and those that have been classified as having type II hyperlipoproteinemia (Descoulch et al., 1980; Sirtori et al., 1977; Sirtori et al., 1979). Hodges and co-workers (1967) were able to demonstrate a cholesterol and triglyceride lowering effect when vegetable protein, primar-

ily from soy, was fed to six prison inmates. The investigation consisted of four experimental periods. Throughout all treatment periods, vegetable protein replaced animal protein in the diet and the P/S ratio remained constant at 1.0. However, carbohydrate source varied (simple, or complex) as well as level of fat (15% or 45%) during the treatment period. Significant changes were observed in the serum cholesteral concentrations. These changes seemed to persist regardless of fat level or source of carbohydrate. However, serum triglyceride levels were more responsive to changes in dietary source of carbohydrate and fat. In the presence of a low fat diet (15%) with starch as the carbohydrate source, serum triglycerides fell significantly below baseline levels (160 mg/ 100 to 133 mg/100). When sugar replaced starch as the carbohydrate source in the low fat diet, serum triglyceride rose to 208 mg/100 ml. When fat was increased to 45 percent of calorie intake and starch was the carbohydrate source, serum triglycerides fell to 88 mg/100 ml; however, when sugar replaced starch in the presence of a high fat diet, triglycerides significantly increased to 211 mg/100 ml.

In summary, Hodges and co-workers (1967) found that as soon as vegetable protein replaced animal protein, serum cholesterol levels decreased markedly and remained low regardless of source of carbohydrate or level of fat. Serum

triglycerides were more responsive to dietary source of carbohydrate, rising with sucrose and falling with starch. Although the level of fat did affect triglyceride levels, the source of carbohydrate was the dominant factor.

Based in Italy, Sirtori and co-workers (1977) were able to demonstrate a significant decrease in serum cholesterol concentration in patients diagnosed as having type II hyperlipoproteinemia when soy replaced animal protein in the diet. Twenty patients participated in the study; all were diagnosed as having type II hyperlipoproteinemia and were admitted to the metabolic ward for study. A crossover design was utilized in which eleven of the twenty patients consumed a lipid lowering diet first (low fat, low cholesterol) and the soybean diet second. The other ten patients received the soybean diet first and the lipid lowering diet second. Diets were identical with respect to carbohydrate, fat and P/S ratio. Sixty-two percent of protein came from either animal or soy products. Each dietary treatment lasted three weeks. Results indicate that soybean diets given before or after the low fat diet significantly decreased serum cholesterol. The mean decrease in serum cholesterol in the presence of a soybean diet was 21 percent. Plasma triglycerides were significantly decreased by both diets. However, the difference was greater when the

soybean diet was given first (217 mg/100 ml to 180 mg/100 ml). Overall, plasma triglycerides were slightly decreased by both diets, during the first dietary period and tended to stabilize during the second. In all instances plasma triglycerides were decreased more on the soybean diet than on the lipid lowering diet, although this difference was not significant. In a second part of the experiment, eight subjects classified as having type II hyperlipoproteinemia were recruited to investigate the effects of adding 500 mg of cystalline cholesterol to the soy diet. In effect, the researchers wanted to verify that the lipid lowering properties of the soy diet could not be attributed to the low cholesterol content of the diet. Here again, a crossover design was utilized and soy diets were identical in fat, carbohydrate, and protein. Five hundred mg of cholesterol were added as one of the dietary treatments. The addition of cholesterol did not influence either the rate of decrease or the serum cholesterol concentration. The findings of the second experiment support the contention that the cholesterol lowering effect of soy protein is independent of the lipid composition of the diet. The authors conclude that replacement of soy protein for animal protein has a beneficial effect in the treatment of type II hyperlipoproteinemia (Sirtori et al., 1977).

Carroll et al. (1978) found similar results in serum cholesterol concentration, but to a lesser extent, when soy protein replaced animal protein in the diets of healthy young women (9% decrease in plasma cholesterol concentrations). The study ran for seventy-three days, during which a mixed diet containing 70 percent animal protein was fed for twenty-four days. Soy products replaced animal products during the second phase of the experiment which lasted thirty-six days. Subjects returned to a mixed protein diet for the concluding thirteen days of the experiment. The plasma cholesterol level declined during period 1, remained relatively low during period 2, and then showed a definite increase during the second week of period 3. Proximate analysis revealed that polyunsatured fatty acids were high in the soy based diets and cholesterol was 50 mg higher in the mixed protein diets. Consequently any change in serum cholesterol concentration could have been due to a higher P/S ratio and lower dietary cholesterol content in the soy diet. However, the authors contend that the difference would not be predicted to raise the level of plasma cholesterol by more than 4 mg/100. The results, therefore, did not rule out the possibility that dietary protein may have been partially responsible for the lower average plasma cholesterol. Subsequently, a second experiment was designed to correct

for the dietary differences found between the two groups. That is, soy diets were supplemented with cystalline cholesterol so that cholesterol content of both dietary treatments was identical. In addition, the P/S ratio was corrected in the soy diet, so that each diet was equivalent in polyunsaturated fat to saturated fat ratio. A crossover design was used for the second experiment and diets were identical to the first with the corrections made. Analysis of variance showed that the level of plasma cholesterol was significantly higher on the animal protein diet compared to the soy protein diet. Plasma triglyceride concentrations were unaffected by the dietary changes ranging from individual values of 47 mg/100 ml - 95 mg/100 ml in the first study and 67 mg/100 ml - 106 mg/100 ml in the second study (Carroll et al., 1978). The researchers contend that the relatively small response to changes in dietary protein and its subsequent effect on serum cholesterol is not incompatible with the larger changes reported by Sirtori et al. (1977). That is, hypercholesterolemic individuals may show a greater response to changes in dietary proteins. In addition, the authors offer a possible explanation that may help to clarify the observed differences between the two dietary treatments; contending that the rates of cholesterol oxidation and turnover are faster when soy protein diets are fed (Carroll et al., 1978).

In a 1979 report, Sirtori summarized data from a previous investigation (Sirtori et al., 1977) and compared results with results obtained from a more recent study. Previous investigations from type II hyperlipoproteinemic patients showed that feeding soybean diets lowered serum cholesterol and that this relationship persisted even with the addition of 500 mg of cholesterol (Sirtori et al., 1977). Of the seven patients fed the high P/S diet first, total cholesterol decreased by 21.4% and LDL cholesterol by 25.5%. A small decrease in triglyceride levels and an increase in VLDL levels were noted but these changes did not reach statistical significance.

Switching from the high to the low P/S regimen caused cholesterol to increase with concomitant increases in the LDL fraction. VLDL also increased (by 17 mg/100 ml), significantly above pre-treatment levels. The overall results of the inpatient studies (Sirtori et al., 1977; Sirtori et al., 1979) demonstrated that the soybean diet exerted a hypocholesterolemic effect in most patients whatever protocol was followed.

The overall data from the forty-two patients studied indicate that three weeks of soybean protein diet gave a mean total cholesterol decrease of 19.4 percent. Low density lipoprotein cholesterol decreased by 20.9 percent and

VLDL decreased by 9.3 percent. Changes in serum triglycerides of the three protocols demonstrated modest changes.

When the data was collapsed and analyzed according to phenotype of hyperlipoproteinemia, type II B (serum triglycerides elevated above 180 mg/100 ml and VLDL exceeds 40 mg/100 ml) showed a significant decrease in the VLDL fraction. Although triglycerides decreased 25 mg/100 ml when patients were on the soybean diets, this did not reach statistical significance. In contrast type II-BIII (concomitant elevations in VLDL and LDL) showed significant decreases in serum cholesterol concentrations while on the soybean diets, but no significant changes were observed in the VLDL fraction. The authors conclude that treatment with the soybean diet is an effective regimen for inducing a significant cholesterol reduction in type II patients refractory to standard low lipids regimens (Sirtori et al., 1979).

Further evidence indicating a possible role for soy in the treatment of hyperlipoproteinemia comes from outpatient studies in Switzerland (Descovich et al., 1980). Animal products in the diet were replaced by a textured vegetable protein product. Before the initiation of treatment, patients were fed a low lipid diet. Baseline values of serum cholesterol and triglyceride were compared to treatment levels. Serum cholesterol showed a 19 percent drop

from initial values. Reintroduction of the animal protein, low-fat diet resulted in a progressive rise in plasma total cholesterol. Serum triglycerides decreased during treatment. However, this was not statistically significant. Regression analysis comparing pretreatment plasma cholesterol levels with cholesterol reductions suggest that patients with a moderate degree of hypercholesterolemia respond well to soy protein diets.

Shorey et al. (1981) working with mildly hypercholesterolemic subjects were unable to demonstrate a unique hypocholesterolemic effect of substitution of soy for animal protein. The diets were identical in cholesterol content (200 mg cholesterol), fat, and carbohydrate, P/S ratio was maintained at 0.4. Sixty-five percent of protein in the diet came either from animal products or soy products. Initial plasma cholesterol and triglyceride concentrations were compared to treatment levels. Subjects consuming an animal protein diet exhibited a 16 percent decrease in serum cholesterol, whereas those subjects who consumed the soybean diet showed a 13 percent decrease. Plasma triglycerides increased significantly on the soy diet from 80 mg/100 ml to 145 mg/100 ml. Partial correlation coefficients for the difference in blood values between initial and experimental diets revealed that changes in plasma cholesterol were most

strongly associated with dietary fat and cholesterol and changes in triglyceride were affected by change in dietary carbohydrate. The authors conclude that the hypocholesterolemic response to both animal and soy proteins suggests that dietary factors other than source of protein were operating. In explaining the conflicting results compared to those of Sirtori et al. (1979), Shorey and co-workers (1981) postulated that Sirtori et al. (1979) used severely hypercholesterolemic individuals which would demonstrate a greater response to dietary changes than mildly hypercholesterolemic individuals. Secondly, much of Sirtori et al. (1979) data were obtained using diets with high P/S ratios. Shorey and co-workers (1981) used P/S ratios in the experimental diets that were not significantly different from those in the normal diet. It is possible that changes in the P/S ratio and protein interact.

Van Raaij et al. (1981) were not able to demonstrate any appreciable change in total serum cholesterol concentration when soy and casein diets were compared, but changes did exist in the lipoprotein fractions. Seventy-six subjects participated in the thirty-eight day study. All subjects consumed a control diet consisting of a 1:1 mixture of casein and soy for ten days. During the test period of twenty-eight days, subjects were divided into three groups matched for sex and initial serum cholesterol concentration.

Group I continued to receive the 1:1 casein:soy mixture (Cassoy). Group II received a diet in which sixty-five percent of the protein came from casein. Group III received a diet in which soy replaced the casein protein. Food records and chemical analysis indicated no differences between the experimental diet with respect to carbohydrate, fat, cholesterol or fiber. During the cassoy control period of ten days, serum total cholesterol concentration decreased slightly in all groups. No appreciable change in serum cholesterol was revealed in subjects on any of the diets during the experimental period. Likewise, the casein group nor the cassoy group showed no change in lipoprotein fractions. However, LDL decreased and HDL increased in the soy group. Very low density lipoproteins showed no significant changes during the test period (Van Raaij et al., 1981). However, when duplicate portions of the same diets were fed to twelve New Zealand white rabbits the casein diet resulted in much higher serum cholesterol concentrations than did the soy diet. The authors offer several explainations as to the differences obtained in the results.

Significant differences were noted in the rabbit response to the soy diet in comparison to the human response. Van Raaij et al. (1981) contends that humans are less sensitive to changes in dietary proteins than are rabbits, thus in part accounting for the different responses.

Commenting on Sirtori et al. (1979, 1981) results, Van Raaij et al. (1981) believed that the differing conclusions could be explained by the lipid status of the two population samples. Sirtori et al. (1977, 1979) used hypercholesterolemic subjects whereas Van Raaij used normal individuals. It could be that normal cholesterolemic subjects are less sensitive to changes in dietary protein. Van Raaij concluded that although total serum cholesterol did not change when soy protein diets were fed, significant differences were obtained in the LDL and HDL fractions suggesting the possibility that soy protein facilitates beneficial changes in cholesterol lipoprotein fractions even in the presence of a constant total serum cholesterol concentration (Van Raaij et al., 1981).

In summary, soy protein diets have demonstrated a lowering of serum cholesterol. Marked decreases have been observed in individuals who are already hypercholesterolemic, and small but significant differences have been noted in normal healthy individuals. One study revealed that although total serum cholesterol did not significantly change during the treatment period, alterations in the lipoprotein fractions (LDL, HDL) were evident. On the whole, serum triglycerides and VLDL have shown small and insignificant changes in the presence of soy protein diets, and seem more responsive to the carbohydrate content of the diet.

Researchers have attempted to explain the factors responsible for the serum cholesterol lowering properties of soy. These include amino acid composition, methionine content, glutamic acid, fiber and saponin content. Further research is needed to explain the exact role soy has in cholesterol metabolism.

G. The Effect of Feeding Milk Protein on Plasma Lipid Levels

Initially prompted by investigations dealing with oral calcium supplementation, milk and yogurt have also been isolated as factors which reduce serum cholesterol.

Bierbaum and associates (1972) found that the ingestion of two grams of supplemental dietary calcium carbonate daily over a period of one year by ten hyperlipidemic patients caused a significant decrease in serum cholesterol after subjects had shown stable levels for the previous year. A decrease in triglycerides also accompanied the fall in cholesterol, but this was not statistically significant.

During feeding trials with Maasai warriors, Mann and co-investigators (1974) observed that large intakes of fermented cow's milk caused low levels of serum cholesterol to go even lower. This occurred despite weight gain and intake of 960 miligrams of cholesterol in the eight liters of yogurt the men consumed daily. Serum triglycerides concentrations were not measured in the study.

Howard (1977) and co-workers supplemented diets of sixteen volunteers with milk. Half of the group consumed supplemental amounts of whole milk and the other group consumed supplemental amounts of skim milk. Serum triglycerides and cholesterol values were taken at baseline, week one, week two and follow-up.

At the end of three weeks, there was a fall in serum cholesterol in both groups. However, the skim milk group demonstrated a greater decrease in concentrations of serum cholesterol (15% fall in skim milk, 5% fall in whole milk). Triglyceride values did not show any reductions in either experimental diet.

Later, the same investigators examined the effects of skim milk powder, yogurt, lactose, leicestershire cheese, cream and butterfat on serum cholesterol (1979). The greatest decrease in serum cholesterol was found in those individuals fed skim milk. The decrease was related to the amount fed. Yogurt produced a similar change. Lactose and cheese showed no significant hypocholesterolemic effect. Butterfat and cream increased serum cholesterol.

Hepner (1979) studied the effect of milk products on serum cholesterol and triglycerides using supplemental amounts of pasteurized yogurt, non-pasteurized yogurt and 2% butterfat milk. Serum cholesterol was significantly reduced

by 5% to 10% after one week of supplementation with either type of yogurt. The two percent butterfat milk reduced serum cholesterol to a smaller less significant degree. Serum triglycerides were unaffected by the diet.

More recently, Rossauru and co-workers (1981) were able to demonstrate significant differences in serum cholesterol and triglyceride concentrations in young men fed either cream, yogurt or skim milk. Subjects maintained their normal eating patterns during the treatment period except that they consumed supplemental amounts (two liters) of either full cream, skim milk or yogurt. Serum total cholesterol fell throughout the experimental period only in subjects fed the skim milk. The yogurt and full cream groups demonstrated an initial rise in cholesterol levels during the first two weeks. Values fell to baseline levels at the end of three weeks. These changes in serum cholesterol could be correlated with appropriate changes in dietary total fat and cholesterol intake accounted for by the differing lipid composition of milk products. Low density lipoproteins fell in the skim milk group below baseline levels. Low density lipoproteins increased in the yogurt and full cream groups, but like total cholesterol, LDL returned to baseline levels at the end of three weeks in the yogurt and cream groups. In the yogurt and full cream milk groups, changes in HDL

cholesterol generally paralleled total cholesterol and could have accounted for a large proportion of the variation in total cholesterol. This however was not apparent in the skim milk group where the fall in total cholesterol was accompanied by a rise in HDL.

Serum triglycerides dropped significantly from baseline levels of 131 mg/100 ml to 99 mg/100 ml at the end of treatment for the skim milk group. Individuals in the full cream group demonstrated equivalent effects in serum triglyceride levels. Values in this group decreased from 123 mg/100 ml at baseline to 100 mg/100 ml at treatent termination. In the yogurt group a transcient significant rise in triglyceride levels (from 96 mg/100 ml to 142 mg/100 ml) was observed after one week. These levels fell below treatment levels at the end of the experimental period. The researchers postulated that the elevation at week one of treatment may have been due to the increased consumption of refined carbohydrate.

The authors contend that the general trend toward lowered serum triglycerides in the skim milk and full cream group as well as the yogurt group may be due to the same spontaneous dietary adaptations responsible for the fall in serum lipids during the baseline week. If is difficult to ascribe it to any property of the milk products used since

the fall in triglycerides continued after stopping milk supplementation.

An interesting finding was the apparent lability of HDL cholesterol which fell during the baseline weeks and rose transiently on all milk products, but more so in the full cream milk group. This rise could be attributed to the total serum cholesterol variation of the diets as well as the differing P/S ratios. Even so, milk irrespective of fat content may promote an increase in HDL concentrations.

In summary, there have been few human studies that have investigated the effects of feeding milk protein under controlled metabolic conditions. Most controlled studies have dealt with calcium supplementation or milk supplementation of diets in an uncontrolled situation. On the whole, milk proteins have demonstrated a cholesterol lowering effect. This effect seems to parallel the fat content of the milk product. That is, skim milk, as opposed to whole milk products, has resulted in larger decreases in serum cholesterol. Serum triglycerides have demonstrated little change in the presence of milk proteins. Any differences in serum triglyceride concentrations seem to be attributed to the carbohydrate content of the diet.

H. Lack of Studies on Egg White Feeding and Serum Lipids

The research focusing on egg white protein and its subsequent effect on serum lipids is nonexistent.

MATERIALS AND METHODS

I. Experimental Design

Twenty-four healthy males between the ages of 18-28 were assigned to one of three treatment groups. The subjects were fed diets of similar nutrient composition; however, dietary protein source was varied between groups. All groups received a vegetarian diet as the basal diet providing 100 grams of protein. Group A received 75 grams of protein from soy products and 25 grams from non-treatment sources. Group B received 75 grams of protein from nonfat dairy products; the remaining 25 grams came from non-treatment sources. The third group (Group C) received 75 grams of protein from egg white and 25 grams from non-treatment sources.

The subjects were assigned to groups based on plasma total cholesterol values and body weight measured prior to the study. This was done by first ranking all cholesterol values from highest to lowest and dividing them into eight groups of three. Using a randomized block design, subjects were assigned to a treatment group, so that average cholesterol level and weight were initially the same in each treatment group.

The study ran for six consecutive weeks. The first four weeks involved feeding under controlled dietary conditions. During this period all subjects were required to eat three meals per day, seven days per week at the metabolic unit of the Department of Human Nutrition and Foods. Only food and drink prepared and served at the unit were permitted. Coffee, tea and non-nutritive beverages were allowed ad libitum. Occasionally, when possible, bag lunches were carried out for the convenience of both the subjects and staff. During the fifth and sixth weeks (follow-up) the subjects were allowed to resume their individual normal dietary habits. Subjects were requested to adhere to their normal patterns of physical activity throughout the study.

Two 60 ml and five 45 ml blood samples were drawn from all subjects during the course of the study. An initial 45 ml blood sample was required for screening determinations. A 60 ml sample was taken the morning of the first experimental breakfast. The other 45 ml samples were taken at weekly intervals for four consecutive weeks. The final blood sample of 60 ml was drawn 2 weeks after termination of the experimental period.

Body weights were recorded weekly. Losses in body weight during the experimental period were adjusted for by adding extra calories as combinations of bread and margarine

to the diets (see Table 1). Weekly determinations of plasma total triglycerides and VLDL were performed. A schematic diagram of the experimental design is shown in Figure 1.

II. Recruitment of the Subjects

Posters and flyers providing basic information about the study were placed throughout academic and recreational buildings on the Campus of Virginia Polytechnic Institute and State University (Appendix A). Persons wishing to learn more about the study were requested to call the Department of Human Nutrition and Foods for further details. Information concerning the study was given during the initial telephone contact. In addition, information concerning exercise level, height, weight and smoking habits was elicited during the conversation. Due to the large influx of calls and the need to eliminate subjects, it was necessary to obtain this information in the early stages of recruitment. Indeed, smoking and high activity levels would introduce extraneous variables into the study that would not be controlled for. Furthermore, normal weight individuals were needed for the subject population. Therefore, height and weight data were necessary to select a homogenous group. The Metropolitan Life Insurance tables were used as a reference in determining normal body weight for height (Appendix B).

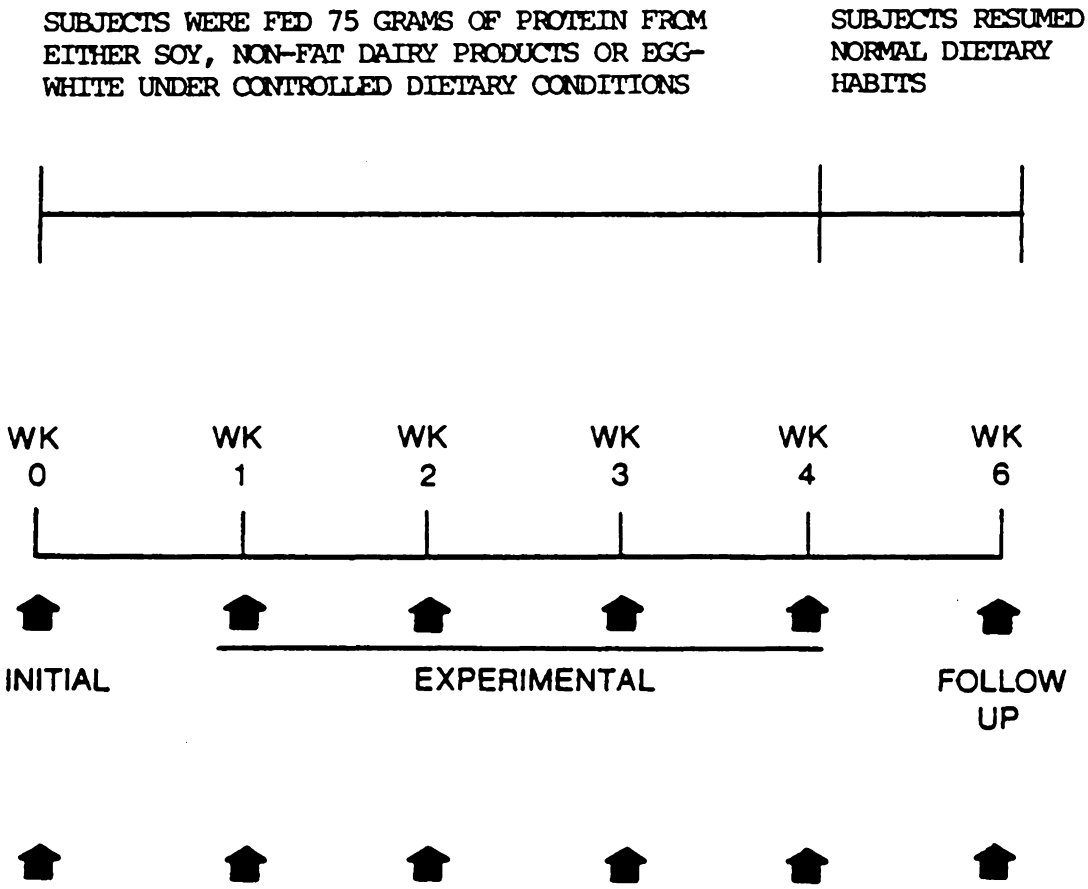

FIGURE 1. A schematic diagram of the experimental design.

III. Screening of Subjects

Based on the initial telephone contact, individuals were requested to come to an orientation meeting. A detailed written explanation of the study was given to each subject (Appendix C). Participant's responsibilities concerning adherence to the dietary regimen and scheduling of blood samples were also discussed. Subjects were aware of obligations and accountability in being study participants.

Several different questionnaires were disseminated at the orientation meeting which served as screening devices for determining eligible candidates for the study. The Pre-Experimental Questionnaire (Appendix D) elicted information concerning familial history of cardiovascular disease as well as hyperlipoproteinemia. Questions pertaining to diagnois of hyperglycemia and diabetes mellitus were also included. Use of medication or supplemental vitamins was determined. Smoking and drug habits were assessed. Furthermore, information regarding food allergies was requested from subjects inasmuch as allergies might have interfered with the dietary regime. The experimental design necessitated that subjects be in town for the duration of the study. Therefore, a question concerning travel plans was also included in the pre-experimental questionnaire.

Secondly, a Food Frequency record (Appendix E) was given to each potential subject in order to obtain information regarding eating habits as well as food likes and dislikes. Consumption of a "normal American diet" was a criteria for eligible candidates. In addition, a twenty-four hour food recall was used to more closely examine daily food intake (Appendix F).

An exercise and activity level questionnaire (Appendix G) was used to assess level of physical activity and aerobic exercising. The questionnaire determined intensity, duration and times per week the subject engaged in different activities. Three specific activities were used to classify each subject based on exercise level:

1) running or jogging less than 15 miles per week
2) swimming less than 2 hours per week
3) cycling (bike) less than 30 miles per week.

Based on the three questionnaires, individuals were eliminated as potential subjects if they: (1) indicated a family history of heart disease, (2) had hypertension or diabetes, (3) indicated a weekly routine of strenuous exercise (jogging, biking, or swimming in amounts greater than those described above), (4) had body weights under or above 10% of the ideal weights for their sex, age, height according to standard weight/height tables, (5) were cigarette

smokers, (6) habitually consumed alcoholic beverages and/or recreational drugs, (7) indicated a dislike or allergy for food that constituted part of the study menu, (8) planned or anticipated being out of town for more than 24 hours during the study.

Those subjects who were eligible received a physical examination by a physician employed by the Student Health Service of Virginia Polytechnic Institute and State University (Appendix H). Hematocrit and hemoglobin levels were determined by finger prick (Appendix I, Appendix J). Normal values for hematocrit and hemoglobin were taken to be 40%-54% and 14-17 gm/100 ml respectively (Sauberlich et al., 1974). Presence of glucose in the urine were determined by colormetric grid using a dip and read test.[1] Finally, serum total cholesterol values were determined. Those individuals who had values no less than 120 mg/100 ml and no more than 220 mg/100 ml were eligible. Triglyceride determinations were not performed during screening and, therefore, did not serve as eligibility criteria.

IV. Description of Study Participants

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[1]Combistix by Ames Laboratory, Division of Miles Laboratory, Indiana.

Forty-four males participated in all of the screening procedures. Eighteen were eliminated as potential subjects. Of those persons eligible, 24 volunteered to participate in the study. All were required to provide signed consent prior to participating in the study (Appendix K).

Twenty-four normal-weight, non-smoking males between the ages of 18-28 were selected to participate in the metabolic study. None indicated a familial history of cardiovascular disease, with the exception of one subject who had an uncle who died of cardiovascular disease. The study population was considered to be "sedentary", but in good physical health. Blood analysis revealed normal hemoglobin and hematocrit levels (Appendix L) and cholesterol values between 120-220 mg/100 ml (Appendix M). Furthermore, diet histories indicated that study participants regularly consumed a normal American diet.

V. Composition and Feeding of Diets

Three sets of diets (A,B, and C) each consisting of four daily menus were prepared to meet the objectives of this study (Appendix N). The diets were similar in fat, protein, carbohydrate, cholesterol and polyunsaturated to saturated fat ratio. The only difference between the diets was in the protein source. Menus 1, 2, 3, and 4 (A,B,C,)

were served consecutively every four days so that the 4 menu cycles were repeated seven times throughout the study. All treatment groups received the same proportional distribution of 15% protein, 50% CHO, and 35% fat. Cholesterol content was kept constant for all treatment groups at 500 mg per day. The cholesterol source was egg yolk. Each day, all subjects consumed two egg yolks providing approximately 500 mg of cholesterol.

The polyunsaturated to saturated fatty acid ratio (P/S) was maintained at .4 daily. The P/S ratio was obtained by dividing the grams of saturated fatty acids into the grams of linoleic acid (Guthrie, 1975).

Calories to maintain body weight were kept at approximately 2800 kcal, although adjustments in intake were made if subjects demonstrated a significant change in body weight. That is if subjects began to lose or gain weight consistently, additional calories (276 kcal or 552 kcal) were provided.

A 100 gram protein diet was used for all treatment groups; 75 grams of protein came from the treatment source and 25 grams came from non-treatment sources. Group A received a vegetarian diet consisting of 100 grams of protein providing 75 grams of protein from soy products (soymilk, soy granules, soybean curd, textured vegetable pro-

tein) and 25 grams of protein from non-treatment sources. Likewise, Group B received a vegetarian diet containing 100 grams of protein; 75 grams of protein came from nonfat dairy products (yogurt, skim milk, low-fat cottage cheese, low-fat cheddar cheese) and 25 grams came from non-treatment sources. Group C also received a vegetarian diet containing 100 grams of protein; 75 grams of protein came from egg white (liquid, fresh or powder) and 25 grams came from the non-treatment sources.

All the diets were nutritionally complete; that is, they met or exceeded the subjects' requirements for calories and essential nutrients as established by the Food and Nutrition Board, National Academy of Sciences - National Research Council (1974). The nutrient composition of the diets were calculated according to values listed in the Food and Agriculture Handbook 456 (1975). A partial list is shown in Appendix O.

After the second week of the experimental period, either 276 or 552 calories as combinations of Roman Meal bread and Parkay margarine (Appendix P) were added to the diets of eleven subjects because of body weight loss. These food items were chosen to add extra calories without significantly altering the caloric distribution among protein, carbohydrate and fat and P/S ratio (Appendix Q). Appendix R

indicates the average daily nutrients consumed per subject according to treatment groups. All foods were weighed to the nearest tenth of a gram to insure adequate control. The food items served during the 4 day cycle menu were kept as nearly identical as possible between treatment groups. That is, similar non-treatment sources of protein were served and entree items containing a large proportion of the treatment sources of protein were similar in composition, except for the treatment source itself. Food items and the quantities (in grams) served in all diets are shown in Appendix N.

All treatment diets throughout the 28-day study were prepared, weighed and served in the metabolic kitchen of the Department of Human Nutrition and Foods. Regular meal times were established for breakfast, lunch and dinner. All subjects consumed their food at the Metabolic unit except for snacks provided to them or take-out lunches. Coffee, tea, water and non-nutritive beverages were allowed ad libitum. Food from each days menu's was homogenized daily and aliquots from each treatment diet were taken for proximate analysis. Percent moisture, percent ash, percent fat and grams of protein were determined for 1 four day cycle.

VI. Collection and Preparation of Blood Samples

Subjects were informed of the importance of fasting 12-14 hours prior to all blood sampling. Blood samples were drawn by a licensed medical technologist between 7:00 and 8:30 a.m. Multiple sample needles (21 gauge, 1 inch) and 15 ml vacutainers containing solid disodium ethylenediaminetetracetic acid (EDTA) were used at all times. Once the vacutainers were filled, blood was promptly and thoroughly mixed by gentle inversion of the vacutainer. Vacutainers were labeled and placed in wet ice.

Within one hour after collection, plasma was separated from cells by low speed centrifugation at room temperature for 30 minutes. Plasma was removed by pipetting and stored in 7 ml storage vials. After an aliquot of plasma was recovered for total cholesterol and triglyceride determinations and for separation of HDL, the remaining plasma was refrigerated at 4° C and prepared for ultracentrifugation within 30 minutes.

VII. Plasma Total Triglyceride Determination

Triglyceride determination was performed using a colorimetric procedure in which the sample is partitioned between acidified isopropanol and n-heptane. The triglycerides are selectively extracted into the heptane layer; thus, leaving the more polar phospholipids in the isopropanol

layer. Hydrolysis of the triglycerides from an aliquot of the heptane layer forms free fatty acids and glycerol. The glycerol is oxidized to formaldehyde and formic acid. The formaldehyde is condensed with acetylacetone in the presence of ammonium ions to give a yellow dihydrolutidine derivative which is then measured spectrophotometrically.

Reagents for the assay were prepared by STANBIO[2] laboratory and detailed elsewhere (Appendix S). Laboratory protocol set forth by STANBIO was followed.

A. Preparation of working standards:

Four standards, for triglyceride determination were prepared using 10 ml volumetric flasks and total delivery glass pipets (1ml, 2ml, 3ml, 4ml). Standards were prepared by adding the stock triglyeride standard to each of the four volumetric flasks. One, 2, 3, and 4 ml of stock triglyceride standard was delivered to each appropriately labeled volumetric flask, each flask was then brought up to volume with isopropyl alcohol. The flasks were gently inverted to mix contents and refrigerated at 4° C when not in use.

B. Extraction of non-polar lipids

With a calibrated Eppendorf pipet, 2.0 ml of extraction reagent and 4.0 ml of acid alcohol reagent were delivered into clean 16 x 100 mm screw-cap tubes. Exactly 0.5 ml of

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[2]STANBIO Laboratory ESC Triglyceride Test Kit, Texas.

water was delivered into the blank tubes and 0.5 ml of each of the triglyceride working standards was added into the standard tubes. Then .5 ml of sample was delivered into the appropriately labeled sample tubes. Water (0.5 ml) was added to the blank tube and each of the sample tubes. They were capped tightly, vortexed for 15 seconds and centrifuged at half speed for 3 minutes. Water (0.5 ml) was added to the standard tubes. The standard tubes were then capped, vortexed for 15 seconds and centrifuged at half speed for three minutes.

C. Saponification of Extracted Triglyceride

Two tenths of a ml of the upper heptane layer was transferred from the extraction tubes to a second set of tubes. Then 2.0 ml of working saponification reagent were added to each tube. The contents of the tubes were mixed well with a vortex and allowed to stand for 5 minutes at room temperature.

D. Oxidation and Color Development

One ml of oxidizing reagent was added to each tube. One ml of the color reagent was then added to each tube. The tube contents were mixed by vortex for 15 seconds and allowed to incubate at 70° C for 10 minutes. The tubes were allowed to cool for 3-4 minutes and standards and samples were read against the blank at 425 nm in 12/115 mm cuvettes in a spectrophotometer.

E. Computation of Results

The absorbance values from the spectrophotometer were converted to triglyceride concentration values (mg/100 ml) from linear regression curves using the four standard solution absorbance values.

VIII. Quantification of VLDL Triglyceride

A. Separation of lipoproteins by ultracentrifugation

Plasma fractions with densities less than 1.006 g/ml containing VLDL or greater than 1.006 g/ml containing LDL and HDL were separated by a single ultracentrifugal spin in a Beckman preparative ultracentrifuge (model L5-75B)[3] according to the LRC procedure (1974).

B. Preparation of samples for ultracentrifugation

The samples were allowed to warm to room temperature (23 C). Using class A pipets, 5 ml of plasma were delivered into the cellulose nitrate tubes specifically used for ultracentrifugation spins. Saline (0.15 ml) (.02% EDTA, pH=7) was delivered into the centrifuge tubes on top of the plasma layer. The tubes were capped and placed in a precooled rotor (50.3 TI) and the samples were centrifuged for 18 hours at 10° C at 40,000 RPM.

C. Preparation of the Ultracentrafuge Fraction

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[3]Beckman Instruments, California

Following the 18-hour spin, the rotor was allowed to stop or slow down for 30 minutes without using the brake. Each tube was slowly and gently removed from the rotor using the extraction tool. The caps were removed using a cap wrench instrument. Tube caps were gently removed by sliding along the edge of the tube to remove any lipoprotein adhering to the underside of the cap. While holding the cap over a small beaker, approximately 1 ml of saline was used to wash the cap of any remaining lipoprotein. Cap washings were added to 5 ml volumetric flasks. A 5 ml syringe was used to remove approximately 3 ml of the supernatant from the original centrifuge tubes and dispensed into a 5 ml volumetric flask. Volume was brought to 3 ml with saline. During week 1 and week 5, a 60 ml blood sample was taken for each subject. Ultracentrigation protocol was identical during this spin; however, 3 ml of the supernatant was used in addition to approximately 2 ml of the clear zone. In effect total volume for this spin was 5 ml. This is the zone beneath the VLDL fraction. On all other weeks, that is, week 2, 3, 4 and follow up, the clear zone was discarded.

The volumetric flasks were stoppered and contents mixed by gently inverting the flasks. The VLDL fluid was transfered to labeled 7 ml storage vials and frozen at -20° C until further analysis.

D. Determination of Triglycerides in VLDL

The triglycerides present in the VLDL fraction were determined by the same procedure used for determining triglycerides in serum described previously.

IX. Statistical Analysis

Mean plasma total triglycerides and very low density lipoprotein triglyceride values were expressed as mg/100 ml concentrations. A two way analysis of variance model was used to determine if differences existed in triglyceride and VLDL values between treatments and within treatments across weeks (Harvey, 1976). The level of significance was set, a priori, at 0.05. When significant differences were found, Dunn's test for multiple comparison (Roscoe, 1969) was used to determine the exact location of differences.

RESULTS

I. Subjects

General physical characteristics of the individual subjects are listed in Table 1. Subjects ranged in age from 19 to 28 years old with a mean of 23.5 ± 3 years. The average weight of the study participants was 72.7 ± 9 kg. Mean height of the subjects was 177 ± 6 cm.

None of the subjects were cigarette smokers. Two had indicated that they had smoked previously; however, these two subjects stopped smoking at least one year before the study period.

Information on the pre-experimental questionnaire indicated that all subjects were in excellent health. Only one reported a family history of heart disease (uncle died of heart attack). None of the subjects were taking prescribed medication prior to or during the experimental period. Those who were taking vitamin/mineral supplements prior to the study were asked to discontinue their use during the experimental period.

Throughout the study, the subjects were requested to maintain their usual pattern of physical activity. For all subjects this involved some type of moderate exercise such as bicycle riding, walking, swimming or hiking. None could

TABLE 1

General Subject Information

Subject Number	Age	Height (cm)	Weight (kg)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	Treatment Assignment	Additional Kcal Intake
1	19	184.5	71.5	201.2	92.6	Soy	+552
2	21	179	74.7	164.5	97.5	Soy	
3	26	173.8	71.6	157.1	67.8	Soy	
4	23	172	63.0	185.2	92.6	Soy	
5	21	179.5	82.5	154.1	41.3	Soy	+552
6	24	194.4	80.6	156.7	27.3	Soy	+276
7	24	182.5	80.8	180.0		Soy	+276
8	27	165.0	59.3	164.7	44.6	Soy	
9	24	172.3	60.6	151.2	27.3	Non-fat Dairy	
10	21	191.0	87.6	146.4	58.7	Non-fat Dairy	+552
11	24	182.3	68.8	206.7	54.6	Non-fat Dairy	+276
12	28	170.2	71.0	193.4	54.6	Non-fat Dairy	+552
13	25	178.5	78.6	167.5	52.1	Non-fat Dairy	
14	22	176.0	74.7	162.1	85.9	Non-fat Dairy	
15	28	180.8	66.7	162.9	43.0	Non-fat Dairy	
16	24	179	76.4	159.4	54.6	Non-fat Dairy	+276
17	26	166	66.3	179.3	58.7	Egg White	
18	21	169.3	55.8	188.0	67.8	Egg White	
19	22	183.5	74.8	161.9	85.1	Egg White	-552
20	21	173.0	64.1	156.6	52.1	Egg White	
21	28	178.3	90.3	193.4	76.0	Egg White	+552
22	22	177.0	80.2	148.6	59.5	Egg White	+276
23	22	176.0	76.4	169.8	43.8	Egg White	
24	22	174.1	72.3	178.7	105.8	Egg White	
X ± SEM	23.5±1	177 ± 1	72.7 ± 2	170.6 ± 3	65.7 ± 5		

be categorized as being in a strenuous exercise group. Strenuous exercise was considered to be jogging more than 15 miles per week or equivalent activity. Subject participation throughout the study was excellent. There were no drop-outs during the experimental period nor at follow-up. Study participants received a monetary compensation of $90 for completing the study.

The mean initial and weekly body weights of subjects are listed in Table 2. Mean body weight loss from initial baseline to the last day of the experiment were similar for all treatment groups (-1.4 kg soy; -1.3 kg non-fat dairy; -1.4 kg egg-white). No significant differences ($P>0.05$) in body weight existed between groups. Body weights did not change significantly throughout the experimental period ($P>0.05$). During the experimental period, eleven subjects began to lose weight. If a consistent decrease in body weight was noted (±1.3 kg or 3 pounds), 276 kcal in the form of bread and margarine were added to the diets of those subjects. If subjects did not return to initial body weight within 3 to 4 days, a further increase in intake of 276 kcal was made. Individual body weight data are listed in Appendix U.

II. Dietary Intake

TABLE 2

Body Weights (kg) of Subjects Receiving 75 gm of Protein Per Day From Soy, Non-fat Diary Products or Egg White

Treatment	Body Weights (kg)[a]					
	Week					
	Initial	Experimental				Follow-up[b]
	0	1	2	3	4	6
Soy (n=8)	72.9± 3	72.7 ± 3	72.1± 3	71.9± 3	71.5 ± 3	
Non-fat Dairy (n=8)	73.0 ± 3	72.8 ± 3	72.3 ± 3	71.9 ± 3	71.7 ± 3	
Egg White (n=8)	72.2 ± 3	71.7 ± 3	71.3 ± 3	71.2 ± 3	70.8 ± 3	

No Significant differences were found in body weights between dietary treatments throughout the study ($P>0.05$).

[a]Values are means ± SEM.

[b]Data unavailable.

Daily nutrient consumption of subjects in the three treatment groups is shown in Appendix R. Minimal differences existed in the total amounts of calories, protein, carbohydrate and fat consumed daily in all treatment groups. The fatty acid composition as reflected by the P/S ratio was also similar for the three treatment diets. Cholesterol was fed to all treatment groups in the form of 2 egg yolks per day equivalent to 504 mg of exogenous cholesterol. On the average, subjects consuming the nonfat dairy protein diet had cholesterol intakes 76 mg higher than those consuming either the soy diet or the egg white diet.

In order to maintain body weight throughout the study, eleven subjects received additional calories (Table 1) in the form of bread and margarine. These foods were added as supplements to increase calories without significantly altering the fatty acid composition of the treatment diets. Differences in total calories, protein, carbohydrate and fat were negligible between adjusted and unadjusted diets. The average daily nutrient consumption of subjects receiving the adjusted diets is shown in Appendix Q.

Proximate composition of the treatment diets including percent ash, percent moisture, percent fat and grams of protein is shown in Appendix T. Aliquots were analyzed from food composites which represented a four hour food intake

for subjects receiving each treatment diet. One cycle of menus (four days) for each treatment group was analyzed. Values for each of the four days were combined and presented as mean values of percent ash, percent moisture, percent fat and grams protein (Table 3).

The laboratory values obtained for grams of protein are in general agreement with the calculated values for protein. Only slight differences were noted in the percent protein content of the three diets. In addition, percent protein was determined for certain products contained in the experimental diets (Table 4). Protein values for these products were not available in Food and Agricultural Handbook 456(1975) and initial calculations were based on nutrition labeling provided by the manufacturers. Thus, in order to verify values indicated on the label, percent protein was determined using the Kjeldall method (AOAC, 1975). As indicated, laboratory values were in close agreement with nutrition labeling values.

III. Plasma Triglyceride Concentrations

Mean plasma total triglyceride concentrations are shown in Table 5 and Figure 2.

The combined least-squares analysis of variance for plasma triglycerides is presented in Table 6. As indicated,

TABLE 3

Percent Ash, Moisture, Fat and Protein Content of Experimental Diets (Days 1-4)

Treatment	Percent[a] Ash	Percent Moisture	Percent[a] Fat	Grams of Protein
Soy	2.6 ± .4	69.6 ± 4	6.9 ± .5	108.5 ± 12
Non-fat Dairy	2.3 ± .2	70.1 ± 5	7.0 ± 1	110.2 ± 8
Egg White	2.3 ± .07	71.5 ± 3	6.6 ± 1	114.3 ± 5

[a]Mean ±

[b]Percent ash and fat on a wet sample basis.

TABLE 4

Comparison of Protein Content of Specific Products Determined in the Laboratory to Protein Values Provided by Manufacturer

Product	Kjeldahl % Protein	Nutrition Labeling % Protein
Textured Vegetable Protein	49.6	52.0
Tofu (soybean curd)	11.2	8.0
Egg White	80.0	81.0
Vegetarian Ham Chunks	47.1	52.0
Soy Granules	48.0	50.0
Soy Powder	36.0	40.0

TABLE 5

Plasma Total Triglyceride Concentrations (mg/100 ml) in Subjects Receiving 75 Grams of Protein per Day from Soy, Non-fat Dairy Products and Egg White

Group	Total Plasma Triglycerides[a]					
	Week					
	Initial	Experimental				Follow-up
	0	1	2	3	4	6
Soy (n=8)	90.4 ± 10[b]	86.2 ± 10[c]	66.8 ± 10[d]	91.8 ± 10[e]	81.3 ± 10[f]	96.6 ± 10[g]
Non-fat Dairy (n=8)	76.1 ± 10[b]	91.2 ± 10[c]	65.4 ± 10[d]	82.3 ± 10[e]	64.8 ± 10[f]	83.0 ± 10[g]
Egg White (n=8)	70.9 ± 10[b]	70.4 ± 10[c]	62.4 ± 10[d]	80.6 ± 10[e]	51.1 ± 10[f]	70.6 ± 10[g]

[a]Mean ± SEM

[b,c,d,e,f,g]Values with the same superscript are not significantly different (P 0.05) between dietary treatments.

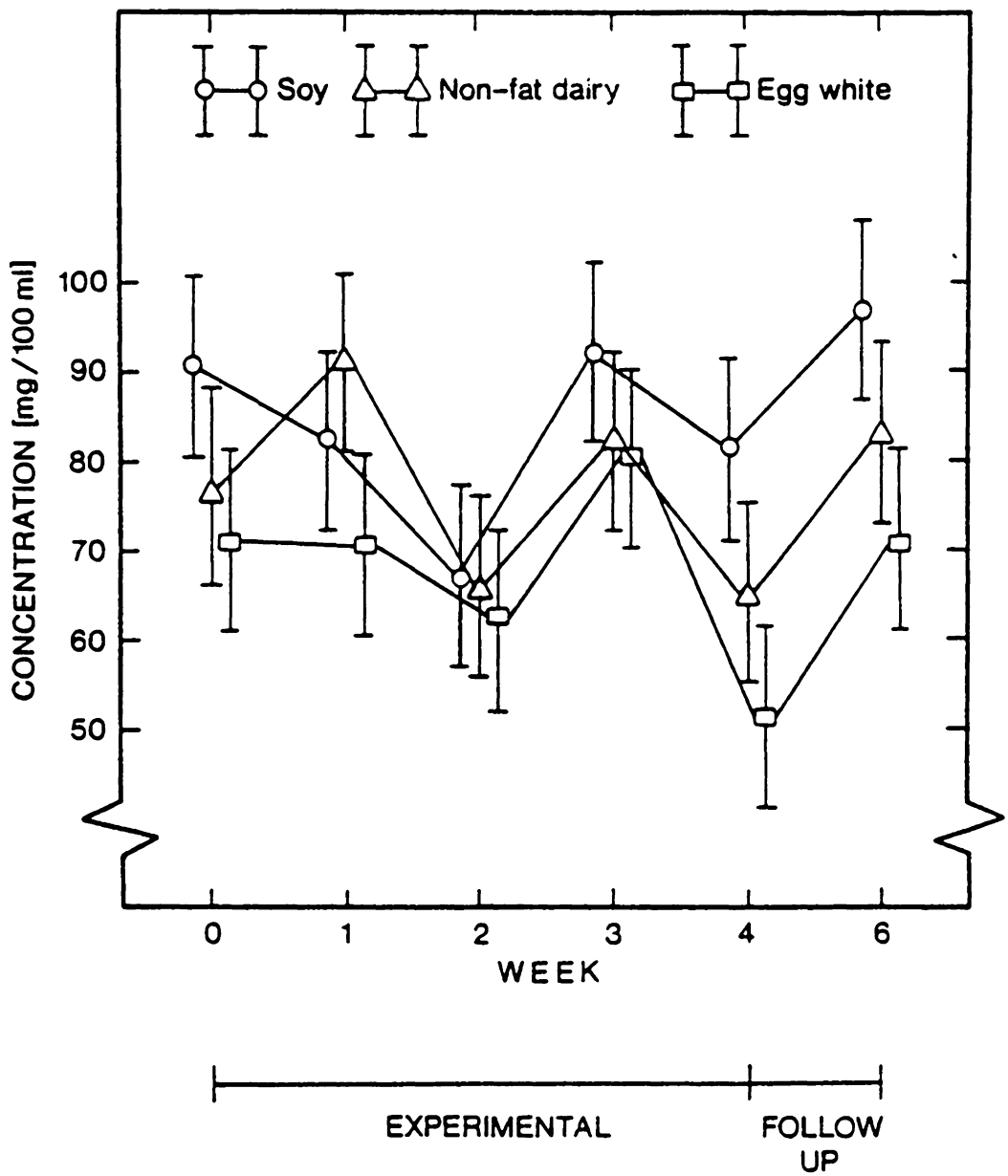

FIGURE 2. Plasma total triglyceride concentrations (means ± SEM) in subjects consuming 75 grams of protein per day from either soy, non-fat dairy products or egg white.

the F value for treatment effects (1.185) was not significant ($P>0.05$), nor was the F value for the interaction between treatment and diet (1.126). An F value of 5.585 indicated a significant week effect ($P<0.05$). Because no significant interaction effect between treatment and weeks had been determined, no rationale existed to test individual group means from week to week using one way ANOVA procedures. Although significant differences in values from week to week may have been indicated by such a test, no differences in trends for the three dietary treatments would have been indicated.

An F value of 5.585 attested to the fact that significant differences in values did exist between weeks. Subsequently Dunn's test for multiple comparisons (1969) was performed on combined treatment means to locate individual significant week effects. Results of the multiple comparison test established that significant differences existed between plasma triglyceride values obtained at week one and week two, week three and week four, week four and week five. In effect, all treatments operated in exactly the same fashion throughout the study. Therefore individual means for all treatment groups were combined in order to demonstrate the similar trends occurring within all treatment groups over the four week experimental period. These values are

TABLE 6

Combined Least-Squares Analysis of Variance

for Plasma Triglycerides

Source of Variation	Degrees of Freedom	Mean Squares	F Value
Between Treatment Diets	2	7265	1.185
Interaction Between Treatment and Weeks	12	410	1.126
Between Weeks	6	2033	5.585*

*Significant week effect (P>0.05).

shown in Table 7 and Figure 3. Individual values for each week within each treatment group are discussed descriptively in the following paragraphs.

During the experimental period, similar trends in changes in triglyceride values from week to week were noted in all treatment diets. In subjects consuming the soy diets, a small decrease of 4 mg/100 ml was noted from the beginning of dietary treatment to the end of week one. In contrast, those receiving the non-fat dairy protein experienced an opposite trend. That is, their triglyceride concentrations increased 15 mg/100 ml from the initial baseline value to the end of the first week on the experimental diet. Triglyceride concentrations at week 0 were 76 mg/100 ml and at the end of week 1 were 91 mg/100 ml. Plasma triglyceride values for subjects receiving the egg white diet remained stable from initial baseline values to the end of the first experimental week. Serum triglyceride concentrations were 70 mg/100 ml in both instances.

During the second week on the experimental diets, similar trends were noted between all treatment groups. The largest decrease in serum triglyceride concentrations was exhibited by those subjects consuming the non-fat dairy protein. A 26 mg/100 ml change was noted. Serum triglyceride concentrations were 91 mg/100 ml at the end of week one and

TABLE 7

Mean Plasma Total Triglyceride Concentrations of All Subjects Combined Over Experimental Diets

Week			Triglyceride Concentration (mg/100 ml)[a]
0	(initial)	n=24	79.1 ± 6[b]
1	(experimental)	n=24	82.6 ± 6[b]
2	(experimental)	n=24	64.8 ± 6[c]
3	(experimental)	n=24	84.9 ± 6[d]
4	(experimental)	n=24	65.7 ± 6[e]
6	(follow-up)	n=24	83.4 ± 6[f]

[a]Mean ± SEM

[b,c,d,e,f]Means with different superscripts are significantly different from one another (P>0.05) (Dunn's Test for Multiple Comparisons).

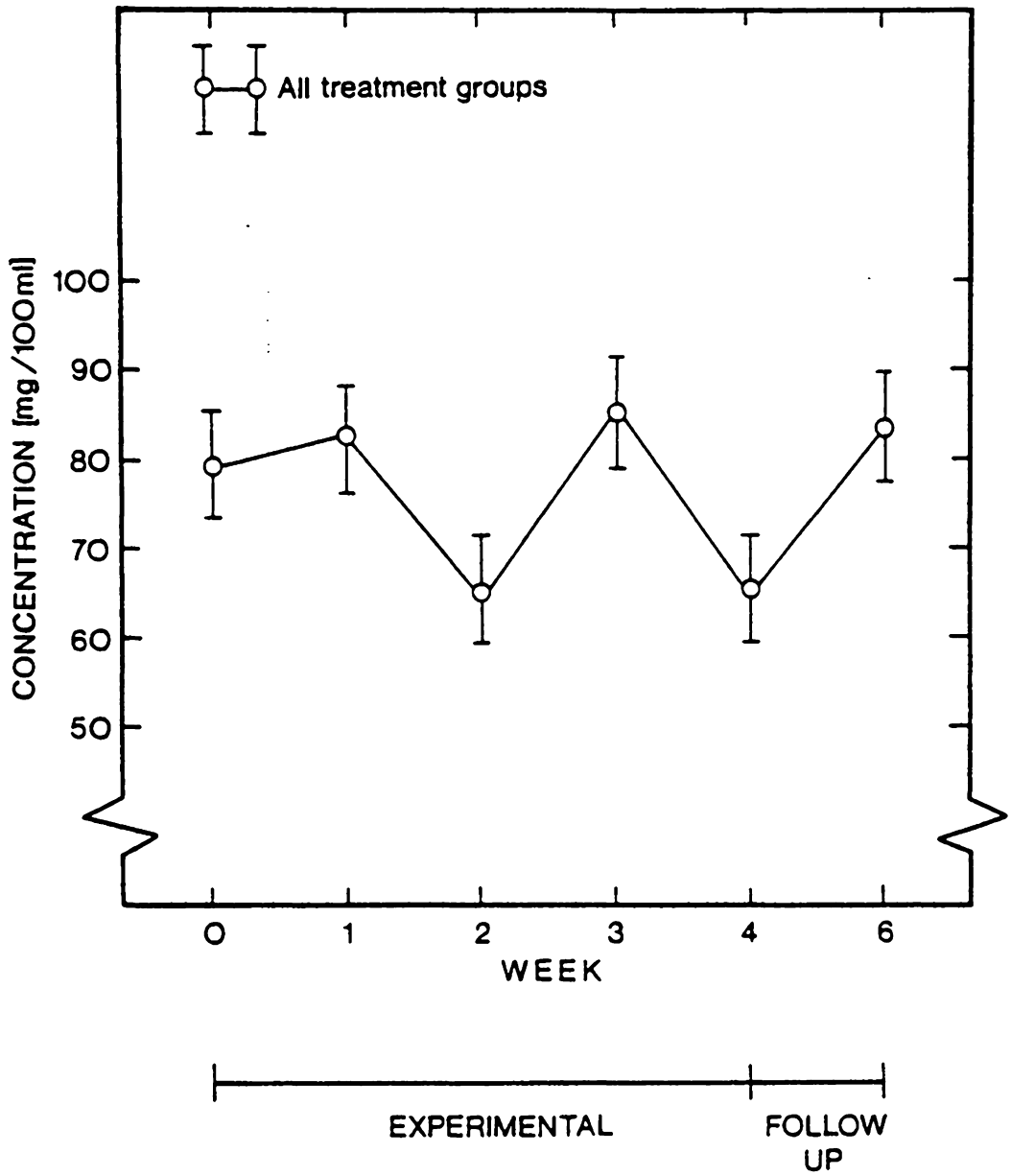

FIGURE 3. Plasma triglyceride concentrations (means ± SEM) of combined experimental groups across time.

decreased to 65 mg/100 ml at the end of week two. A similar decrease was noted in subjects consuming the soy diets. Serum triglyceride concentrations at the end of week one were 86 mg/100 ml and decreased to 66 mg/100 ml. Likewise, subjects receiving 75 grams of protein from egg white experienced a fall in serum triglyceride concentrations from week one to week two but to a lesser extent. Serum triglyceride concentrations in this group decreased 8 mg/100 ml. At the end of week one serum triglyceride concentrations were 70 mg/100 ml and decreased to 62 mg/100 ml at the end of week two.

When comparing serum triglyceride concentrations between week two and week three, a similar trend in all treatments is again noted. However, in this instance, all treatment groups experienced a rise in serum triglyceride concentrations rather than a fall as observed in week 2. Subjects receiving the soy diets displayed an increase of 25 mg/100 ml (66 mg to 91 mg/100 ml). Likewise, subjects receiving the non-fat dairy protein diets experienced an increase in serum triglyceride concentrations, but to a lesser extent. Subjects in this group had serum triglyceride concentrations of 65 mg/100 ml at the end of week 2 and 82 mg/100 ml at the end of week 3, an increase of 17 mg/100 ml. An 18 mg/100 ml increase was noted from week two to week

three in subjects consuming the egg white diet. Their plasma triglyceride concentrations rose from 62 mg/100 ml to 80 mg/100 ml from the end of week two to the end of week three.

Serum triglyceride concentrations during week four showed similar trends in all treatments. A decrease was noted for all experimental diets. The largest decrease in serum triglyceride concentrations (29 mg/100 ml) was observed in those individuals consuming 75 grams of protein per day from egg white. Their serum triglyceride values fell from 80 mg/100 ml at week 3 to 51 mg/100 ml at week 4. Similar decreases were noted in those subjects consuming 75 grams of protein per day from non-fat dairy products. Their serum triglyceride concentrations fell from 82 mg/100 ml at week 3 to 64 mg/100 ml at week 4, a difference of 17 mg/100 ml. A smaller decrease (10 mg/100 ml) in serum triglyceride concentration was observed in those subjects consuming 75 grams of protein per day from soy. Triglyceride concentrations fell in this group from 91 mg/100 ml at week 3 to 81 mg/100 ml at week 4.

Comparison of serum triglyceride values during the last week of dietary treatment to follow-up values showed an expected increase in all groups. Subjects consuming egg white and non-fat dairy protein diets demonstrated a 19

mg/100 ml increase. Serum triglyceride concentration at week four was 64 mg/100 ml and increased to 83 mg/100 ml at follow-up for those in the non-fat diary protein group. Those subjects receiving the egg white protein had serum triglyceride concentrations at week 4 of 51 mg/100 ml increasing to 70 mg/100 ml at follow-up. Similar increases were noted in subjects consuming 75 grams of protein per day from soy. Their serum triglyceride concentrations increased from 81 mg/100 ml at week four to 96 mg/100 ml at follow-up, a difference of 15 mg/100 ml. When baseline serum triglyceride concentrations were compared to follow-up values, no differences were found.

Subjects receiving 75 grams of protein from soy had initial serum triglyceride concentrations of 90 mg/100 ml; at follow-up, triglyceride values showed little change (96 mg/100 ml) with a difference of 6 mg/100 ml. A similar trend was noted in subjects receiving 75 grams per day of non-fat dairy protein. Baseline values of serum triglyceride in this group were 76 mg/100 ml. Follow-up values increased slightly to 83 mg/100 ml, a difference of 7 mg/100 ml. Likewise, subjects receiving 75 grams of protein from egg white showed no change from baseline to follow-up with serum triglyceride concentrations of 70 mg/100 ml in both instances. Serum triglyceride concentrations for individual subjects are shown in Appendix V.

IV. Plasma VLDL-Triglyceride Concentrations

Mean plasma VLDL-triglyceride concentrations are shown in Figure 4 and Table 8. The combined least-squares analysis of variance for plasma triglycerides is presented in Table 9. As indicated the F value for treatment effects (1.138) was not significant ($P>0.05$) nor was the F value for the interaction between treatment and diet (.517). Because no significant interaction effect between treatment and weeks had been determined, no rational existed to test individual group means from week to week using one way ANOVA procedures. Although significant differences in values from week to week may have been indicated by such a test, no differences in trends for the three dietary treatments would have been noted. An F value of 8.682 gave credence to the fact that significant differences ($P<0.05$) in values did exist between weeks. Accordingly, Dunn's test for multiple comparison (1969) was performed on the combined treatment means to locate individual significant week effects. Results of the multiple comparison test established that significant differences existed between the initial baseline value and week one, week one and week two, week three and week four. There was however, no significant difference between week two and week three. For all practical purposes, all dietary treatments operated in the same fashion

throughout the study. Therefore individual means for all treatment groups were combined in order to demonstrate the similar trends occurring within all treatment groups over the four week experimental period. These values are shown in Figure 5 and Table 10.

Individual values for each week with each treatment group are discussed descriptively in the following paragraphs.

During the experimental period, similar trends in changes in plasma VLDL-triglyceride concentrations were noted in all treatment diets. From the beginning of dietary treatment to the end of week one. All groups experienced a decrease in plasma VLDL-triglyceride concentrations. The largest decreases (20 mg/100 ml) were observed in those subjects fed the soy diets and in those receiving the egg white diets. Plasma VLDL-triglyceride concentration at the initiation of dietary treatment was 55 mg/100 ml and decreased to 35 mg/100 ml at the end of week one in those individuals fed the soy diet. Subjects fed 75 grams of egg white per day showed a VLDL-triglyceride concentration of 49 mg/100 ml at initiation of treatment. This value decreased to 29 mg/100 ml at the end of week one. A similar effect was observed in those subjects receiving the non-fat dairy protein. VLDL-triglyceride concentration in this group at initiation of treatment was 40 mg/100 ml and decreased to 21 mg/100 ml at the end of week 1, a difference of 19 mg/100 ml.

TABLE 8

Plasma VLDL-Triglyceride Concentrations (mg/100 ml) in Subjects Receiving 75 Grams of Protein Per Day from Soy, Non-fat Dairy Products and Egg White

Group	VLDL-Triglyceride Concentration[a] (mg/100 ml)					
	Week					
	Initial	Experimental				Follow-up
	0	1	2	3	4	6
Soy (n=8)	55.0 ± 6[b]	35.5 ± 6[c]	42.9 ± 6[d]	45.0 ± 6[e]	32.8 ± 6[f]	41.8 ± 6[g]
Non-fat Dairy (n=8)	40.9 ± 6[b]	21.6 ± 6[c]	38.8 ± 6[d]	34.4 ± 6[e]	22.5 ± 7[f]	40.1 ± 6[g]
Egg White (n=8)	49.9 ± 6[b]	29.2 ± 7[c]	38.5 ± 6[d]	41.7 ± 6[e]	23.8 ± 6[f]	34.0 ± 6[g]

[a]Mean ± SEM

[b,c,d,e,f,g]Values with the same superscript are not significantly different between dietary treatments (P 0.05).

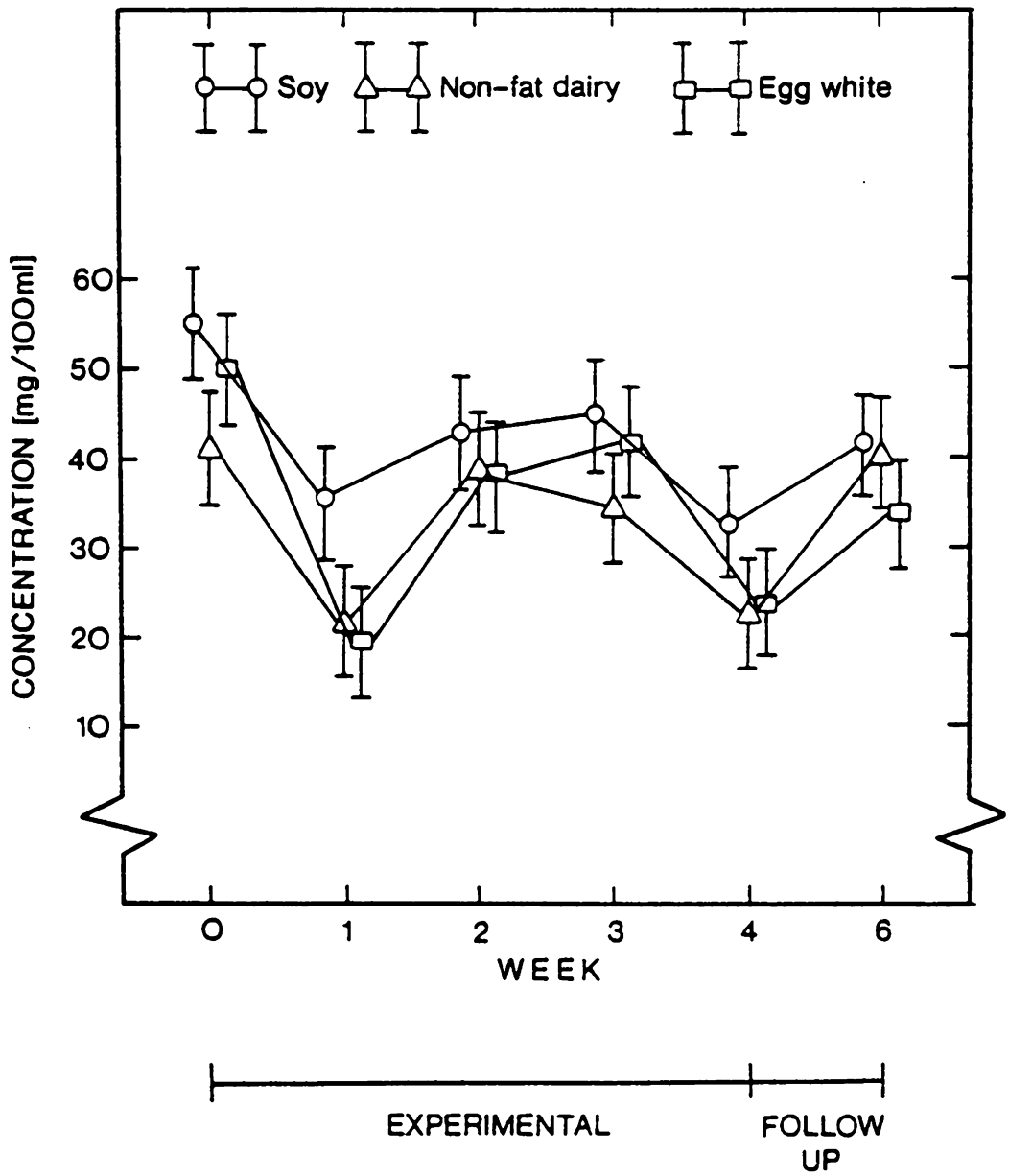

FIGURE 4. Plasma VLDL-triglyceride concentrations (means ± SEM) in subjects consuming 75 grams of protein per day from soy, non-fat dairy products or eggwhite.

TABLE 9

Combined Least-Squares Analysis of Variance

for Plasma VLDL-Triglycerides

Source of Variation	Degrees of Freedom	Mean Squares	F Value
Between Treatment Diets	2	989	1.138
Interaction Between Treatment and Weeks	10	88	.517
Between Weeks	5	1469	8.682*

*Significant week effect (P>0.05).

TABLE 10

Mean Plasma VLDL-Triglyceride Concentrations of All Subjects Combined Over Experimental Diets

Week			VLDL-Triglyceride Concentrations (mg/100 ml)[a]
0	(initial)	n=24	46.8 ± 3[b]
1	(experimental)	n=21	28.7 ± 3[c]
2	(experimental)	n=24	40.1 ± 3[d]
3	(experimental)	n=24	40.4 ± 3[d]
4	(experimental)	n=21	26.4 ± 3[e]
6	(follow-up)	n=23	38.6 ± 3[f]

[a]Mean ± SEM

[b,c,d,e,f]Means with different superscripts are significantly different from one another (P>0.05) (Dunn's Test for Multiple Comparisons).

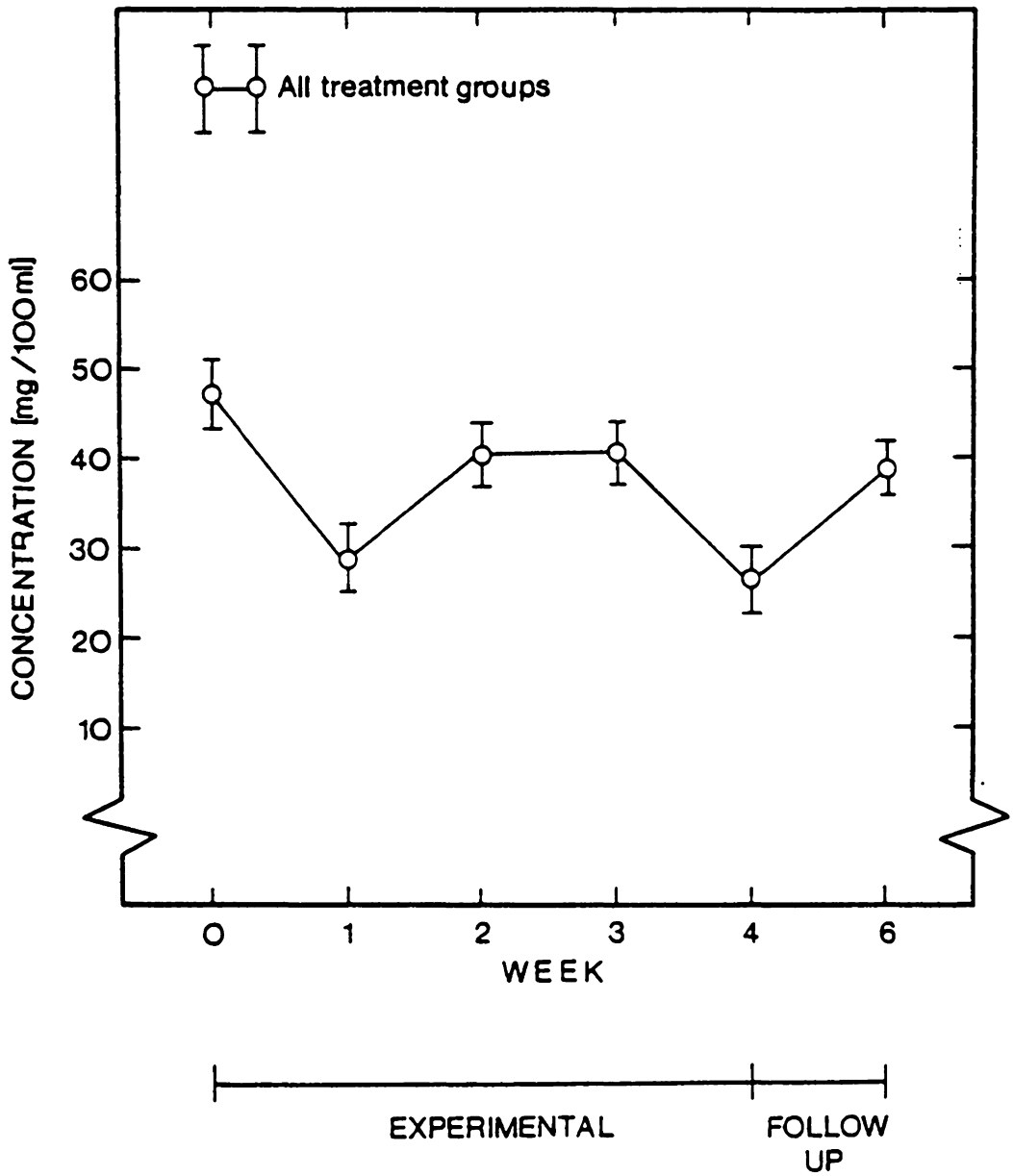

FIGURE 5. Plasma VLDL-triglyceride concentrations (means ± SEM) of all subjects combined over experimental diets.

During the second week of dietary treatment, all treatment groups experienced a similar increase in plasma VLDL-triglyceride concentrations. In subjects fed soy protein, plasma VLDL-triglycerides increased from 35 mg/100 ml at week one to 42 mg/100 ml at week two, a difference of 7 mg/100 ml. A larger decrease (17 mg/100 ml) was obersved from week one to week two in subjects fed 75 grams of non-fat dairy protein. Concentrations of VLDL-triglycerides in this group were 21 mg/100 ml at week one and 38 mg/100 ml at week two. Subjects fed the egg white diet experienced a similar increase but to a lesser extent. Plasma VLDL-triglyceride concentrations at week one were 29 mg/100 ml and 38 mg/100 ml at week two, a difference of 9 mg/100 ml.

Plasma VLDL-triglyceride concentrations from week 2 to week 3 showed different trends between treatments. That is, both groups of subjects fed the soy and the egg white diets experienced small increases in plasma VLDL-triglyceride concentration, whereas those fed the non-fat dairy protein displayed a small decrease in plasma VLDL concentrations. In subjects fed 75 grams of soy protein per day, plasma VLDL-triglyceride concentrations increased from 42 mg/100 ml at week 2 to 45 mg/100 ml at week 3. A similar increase was observed from week three (38 mg/100 ml) to week 4 (41 mg/100 ml) in subjects fed 75 grams of egg white protein per day.

A decrease in serum VLDL-triglyceride concentration was noted in subjects consuming the non-fat dairy protein. Plasma VLDL-triglyceride concentrations fell from 38 mg/100 ml at week two to 34 mg/100 ml at week three, a difference of 4 mg/100 ml.

From week 3 to week 4 of dietary treatment all treatment groups displayed similar decreases in plasma VLDL-triglyceride concentrations. Plasma VLDL-triglyceride values in subjects consuming the soy protein diet fell from 45 mg/100 ml at week three to 32 mg/100 ml at week four. Values for those subjects consuming 75 grams of protein from non-fat dairy products fell from 34 mg/100 ml at week three to 22 mg/100 ml at week four. Larger decreases in plasma VLDL-triglyceride concentrations were noted from week three to week four in those subjects consuming the egg white protein. Serum VLDL-triglyceride concentrations at week three were 41 mg/100 ml and fell to 23 mg/100 ml at week four, a difference of 18 mg/100 ml.

From the last week of dietary treatment to follow-up, all groups exhibited increases in plasma VLDL-triglyceride concentrations. Subjects fed 75 grams of protein per day from soy showed an increase in their plasma VLDL-triglyceride concentrations from 32 mg/100 ml to 41 mg/100 ml, a difference of 9 mg/100 ml. Similarly, those subjects consuming

the egg white diet showed an increase in their plasma VLDL-triglyceride concentrations from 23 mg/100 ml to 34 mg/100 ml, a difference of 11 mg/100 ml. A larger increase in plasma VLDL-triglyceride concentration (18 mg/100 ml) was observed in subjects consuming the non-fat dairy protein. Mean plasma VLDL-triglyceride concentration for the non-fat dairy group was 22 mg/100 ml at week 4 and increased to 40 mg/100 ml at follow-up. Comparison of values from baseline to follow-up indicate that little change occurred in those subjects fed 75 grams of protein per day from non-fat dairy products. Initial baseline and follow-up values were 40 mg/100 ml in both instances. In subjects fed soy protein, follow-up values were 14 mg/100 ml less than initial baseline values. A similar trend was observed in the subjects fed egg white protein when baseline and follow-up values were compared. Baseline values for subjects fed egg white protein were 49 mg/100 ml and 34 mg/100 ml at follow-up.

DISCUSSION

I. Introduction

The present study examined the effect of feeding either soy, non-fat, dairy products or egg white protein on plasma triglyceride and VLDL-triglyceride levels in young men. Analysis of variance indicated that no significant differences in serum lipid levels due to dietary treatment was obtained, nor was there any significant diet and week interaction effect. All subjects, regardless of dietary treatment, responded in the same way from week to week.

II. Effect of Dietary Protein on Serum Triglycerides and Very Low Density-Triglycerides

The lack of any significant effect due to dietary treatment is not surprizing in light of results obtained in other similar studies (Walker et al., 1960; Campbell et al., 1965; Carroll et al., 1978; Anderson el al., 1970). When plant protein replaced animal protein in the diet of healthy young women, no significant differences were observed between the two groups in serum triglyceride levels (Walker et al., 1960). Similar results were obtained in a later study (Carroll et al., 1978) when soy replaced animal protein in the diets of healthy young women. A mixed protein diet was

fed initially for twenty four days. The next phase of the dietary period involved replacing the animal protein contained in the mixed diets with soy protein. Analysis of variance indicated that no significant differences in serum triglyceride concentrations were observed between the two dietary treatments thus indicating that soy protein did not lower serum triglyceride levels.

Campbell and co-workers (1965) compared diets containing wheat gluten to diets containing an isonitrogenous amount of casein-lactalbumin. No differences existed in serum triglycerides between individuals on the two dietary treatments. This situation persisted whether the diet contained 12 percent linoleic acid or 40 percent linoleic acid.

When egg white and wheat gluten diets were compared (Anderson et al., 1971), no statistically significant differences existed in serum triglyceride concentrations; although subjects consuming the egg white diet had serum triglyceride concentrations of 92 ±13 mg/100 ml and those consuming the wheat gluten diets had serum triglyceride concentrations of 84±9 mg/100 ml, a difference of 8 mg/100 ml.

In summary, when the effect of plant and animal protein diets on serum lipids are compared, no significant differences in serum triglycerides between dietary treatments is observed (Walker et al., 1960; Campbell et al., 1965; Car-

roil et al., 1978; Anderson et al., 1971). Evidence from published reports indicates that serum triglycerides are more responsive to changes in dietary carbohydrate and fat (Anderson, 1967; McDonald, 1967; Nestel et al., 1970) rather than to cnanges in the protein moiety of the diet.

In 1967, Hodges and co-workers replaced animal protein in the diet of six prison inmates with soy protein. In addition, carbonydrate source as well as fat level was varied during the experimental period. Serum cholesterol decreased throughout the study period regardless of source of carbohydrate or level of fat. Serum triglycerides, however, were more responsive to dietary carbohydrate and fat influences. In the presence of a low-fat diet (15% of caloric intake) with starch as the carbohydrate source, serum triglycerides fell significantly below baseline levels (166 mg/100 ml to 133 mg/100 ml). When sugar replaced starch as the carbohydrate source in the low-fat diet, serum triglycerides rose to 208 mg/100 ml. When fat was increased to 45 percent of caloric intake and starch was the carbohydrate source, serum triglycerides fell to 88 mg/100 ml; however, when sugar replace starch in the presence of a high fat diet, triglycerides significantly increased to 211 mg/100 ml. In effect, serum triglycerides were more responsive to changes in dietary carbohydrate and fat rather than to changes in protein.

Shorey and co-workers (1981) found that serum triglycerides significantly increased from baseline levels when soy diets were fed. In contrast, the serum triglyceride levels of subjects consuming animal protein diets remained stable throughout the study. When treatment diets were compared to previous self selected diets, it was found that dietary carbohydrate was significantly higher on the treatment diets. Thus, the change in triglyceride levels during the experimental period was attributed to the change in dietary carbohydrate.

Hyperlipidemic subjects experienced a significant decrease in plasma triglyceride concentrations when a low-fat diet (consisting of 60 percent of protein from animal sources, cholesterol intake of less than 100 mg/day and a P/S ratio of 2.7) was fed. Similar decreases were noted when soy replaced animal protein in the diets of the same subjects (Sirtori et al., 1979). These results seem to indicate that the lowering of serum triglyceride can not be attributed to the protein moiety of the diet alone. In all likelihood, the triglyceride lowering effect is the result of some dietary component(s) present in both diets. The soy protein and the animal protein diet had similar fatty acid compositions (P/S=2.7). Evidence indicates that polyunsaturated fatty acid content of the diet influences serum tri-

glyceride concentrations (Nestel et al., 1970; McDonald, 1967). No data is available on the source of carbohydrate fed by Sirtori et al. However, both groups were consuming comparable amounts of carbohydrates as a percentage of total caloric intake.

In addition, blood lipid values of subjects prior to any dietary treatment are an important factor determining response to dietary manipulation (Van Raaij et al., 1979). Sirtori el al. were working with hyperlipidemic subjects whose serum cholesterol and/or triglycerides were elevated. This may explain why both groups experienced similar decreases in triglycerides, that is subjects with elevated serum lipid concentrations are more sensitive to dietary manipulation designed to lower these serum lipids.

The effects of ingesting supplemental amounts of milk on serum lipids has been investigated (Howard et al., 1977; Howard et al., 1979; Hepner, 1979). These reports indicate that serum triglyceride levels are unaffected by milk protein. However, Rossauru and co-workers (1981) demonstrated a significant triglyceride lowering effect when supplemental amounts of either skim milk or cream were fed to subjects. Serum triglycerides dropped from baseline levels of 131 mg/100 ml to 99 mg/100 ml at the end of treatment for subjects consuming supplemental amounts of skim milk. Similar

decreases were observed in those subjects consuming full cream. Values in this group decreased from 123 mg/100 ml at baseline to 100 mg/100 ml at treatment termination. In a third group, fed supplemental amounts of yogurt, a transcient significant rise in triglyceride levels (from 96 mg/100 ml to 142 mg/100 ml) was obtained after one week of treatment. These levels fell below baseline levels at the end of the experimental period. The researchers postulate that the elevation at week one of treatment may have been due to the increased consumption of refined carbohydrate. In effect, all groups experienced a lowering of serum triglyceride levels. The authors contend that "spontaneous dietary adaptations" were responsible for the fall in serum triglycerides and that it is difficult to ascribe the fall in serum triglycerides to any property of the milk products used since the fall in serum triglycerides continued after supplementation with milk products ceased.

Only limited data is available concerning the effect of feeding different protein sources on the very low density lipoprotein fraction in serum. Lack of sufficient research on the VLDL fraction stems in part from the time consuming method of separating lipoproteins and the equipment needed for ultracentrifugation. In addition, researchers have preferred to look at the cholesterol carrying lipoproteins

(LDL, HDL) which are more likely to change in response to dietary manipulation than is the VLDL fraction. The very low density lipoprotein is responsible for carrying a large portion of the triglyceride molecule (Herbert et al., 1978). In fact, VLDL contains 45-65% triglyceride (Herbert et al., 1978). Because of this relationship, any changes in serum triglyceride would be reflected by concomitant changes in the VLDL fraction. Furthermore, VLDL would be expected to respond to dietary components in much the same way that triglycerides do.

Van Raaij et al (1981) found no appreciable change in serum very low density lipoproteins when casein and soy diets were compared. No significant differences existed between the two groups with regard to VLDL levels.

In summary, studies comparing animal and vegetable protein diets have found no significant differences in serum triglyceride or VLDL levels between treatments. Differences reported in serum triglyceride levels can possibly be attributed to the carbohydrate content of the diet. In addition, the fatty acid composition of the diet exerts an influence on serum triglyceride levels. Sirtori et al (1979) found that both a lipid lowering diet (low fat, low cholesterol) and a soy-based diet lowered serum triglycerides. However, the population sample was hyperlipidemic and significant

changes in serum lipids may have been a result of increased sensitivity to dietary manipulation.

Studies which do report significant differences in serum triglyceride VLDL levels between groups fed differing sources of dietary protein may have resulted in large changes in source and amount of dietary carbohydrate between self selected diets previously consumed and experimental diets.

The results found in the present study are in agreement with results obtained from similar studies. That is, no significant differences in serum triglycerides were found between treatment diets differing in protein source only. In the present study, all subjects were consuming approximately a 1:1 ratio of simple to complex carbohydrate. Carbohydrates were provided as a constant percentage of caloric intake across all diets. In effect, any blood lipid responses would not be expected to be the result of different carbohydrate sources or amounts in the present study. Similarly, because fat content and compositions of all diets were constant in the present study, these factors were not expected to influence serum triglyceride or VLDL concentrations.

III. Variability in Serum Lipid Concentrations

Although no significant differences in serum lipid levels were found between treatment groups, a significant week effect was observed ($P<0.05$). From week to week serum triglycerides increased or decreased reaching a peak value of 84 mg/100 ml at week three. In a previous study in this laboratory, examining the effect of daily cholesterol intake (400 mg vs 1400 mg) on serum triglyceride concentrations, Flaim (1979) found similar trends in changes in serum triglyceride concentrations (Figure 6). No significant differences in serum triglyceride values due to dietary treatment were found ($P>0.05$). Both groups regardless of cholesterol intake experienced a decrease in serum triglyceride levels at week two. Serum triglycerides from week one to week two fell from 62 mg/100 ml to 58 mg/100 ml in subjects fed 400 mg of cholesterol. A similar small change was noted in serum triglycerides of those subjects consuming the 1400 mg cholesterol diet. Serum triglyceride concentrations at week one were 56 mg/100 ml and decreased to 55 mg/100 ml, a difference of 1 mg/100 ml. From week two to week three an increase in serum triglyceride concentrations was experienced by both groups. However, the magnitude of change was much larger from week two to week three. Subjects fed 400 mg of cholesterol exhibited serum triglyceride concentrations of 58 mg/100 ml at week two rising to 71 mg/100 ml at

week three, a difference of 13 mg/100 ml. Similar increases were noted in subjects consuming 1400 mg of cholesterol per day. Serum triglyceride concentrations at week 2 were 55 mg/100 ml and increased to 73 mg/100 ml, a difference of 18 mg/100 ml. From week three to week four both groups experienced decreases in serum triglyceride concentrations. Serum triglyceride concentrations at week 4 were 60 mg/100 ml for the subjects consuming 400 mg of cholesterol. This was a decrease of 9 mg/100 ml from the preceding week. Similar decreases were noted in subjects fed 1400 mg of cholesterol. These subjects also experienced a decrease of 9 mg/100 ml in serum triglyceride values. From week 4 to follow-up, both groups experienced increases in serum triglyceride concentrations. Serum triglycerides of subjects consuming 400 mg/100 ml increased from 60 mg/100 ml at week 4 to 76 mg/100 ml at follow-up, a difference of 16 mg/100 ml. Subjects consuming 1400 mg of cholesterol displayed large increases in serum triglyceride determinations. At week 4 serum triglycerides were 64 mg/100 ml and increased to 93 mg/100 ml. (Flaim, 1979).

The response pattern of serum triglyceride levels observed in Flaim's study is in agreement with that observed in the present study suggesting that factors other than a response to dietary components are responsible for the

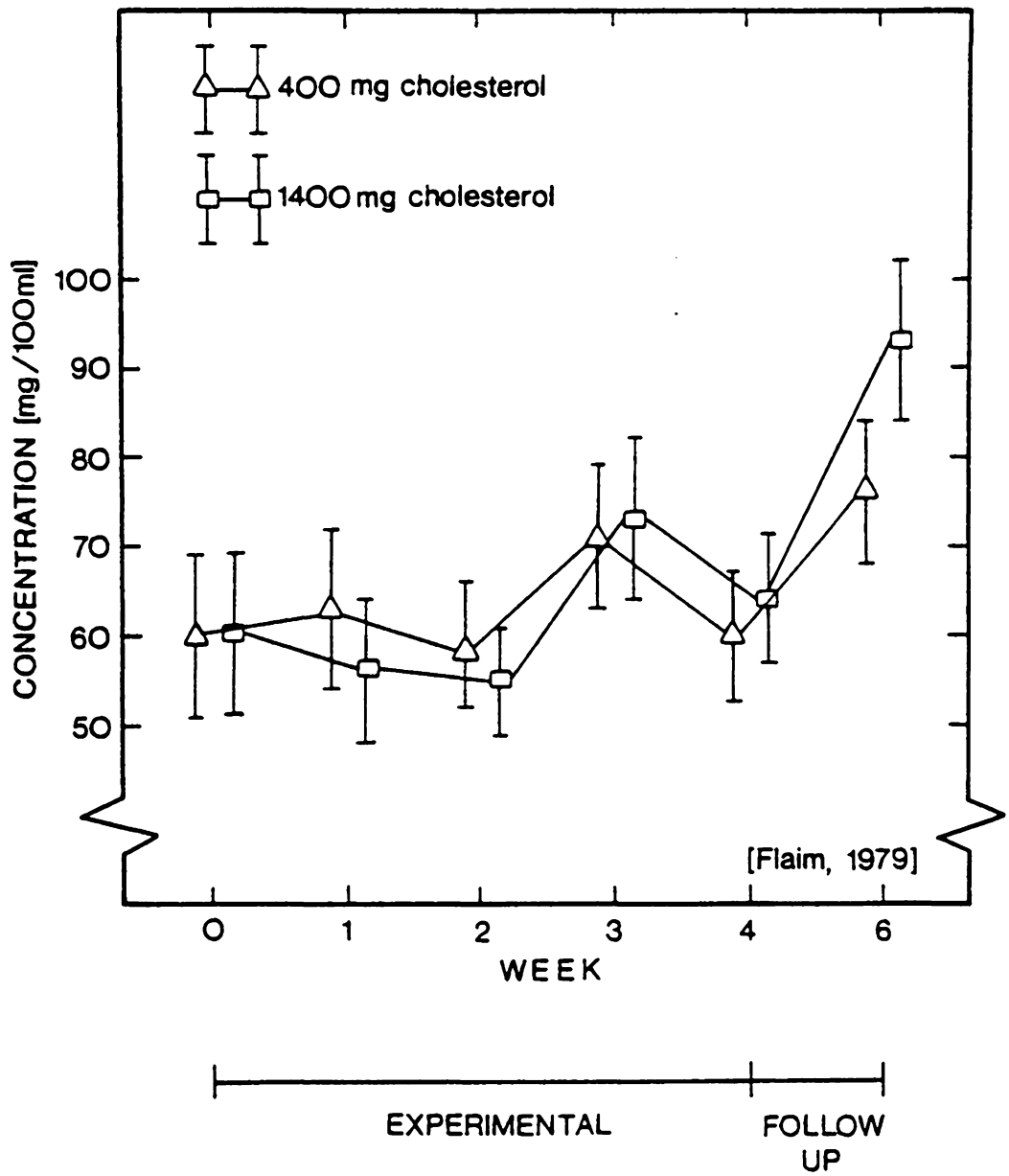

FIGURE 6. Plasma total triglyceride concentrations (means ± SEM) in subjects consuming 400 mg or 1400 mg of cholesterol per day (Flaim, 1979).

observed effect. These factors may include limitations in current available methodology, normal biological variation in triglyceride concentrations, and individual variability.

Chromie et al (1963) measured serum triglyceride levels in eighty-eight subjects. Blood sampling for triglyceride determination was repeated five times in order to determine the extent of variability within subjects. Chromie et al (1963) found spontaneous variability within the same individuals when triglyceride determinations were repeated on five different occasions. The mean serum triglyceride concentrations obtained in repeated sampling of the same subjects on different days is plotted in Figure 7. As can be seen the same type of random fluctuation occurred in the study of Chromie et al (1963) as in the present study.

From sample number one to the second sample, serum triglycerides decreased from 120 mg/100 ml to 81 mg/100 ml. An increase in serum triglyceride levels was noted from sample two to sample 3. Serum triglyceride levels increased from 81 mg/100 ml in sample two to 118 mg/100 ml in sample 3. A mean decrease of 13 mg/100 ml was observed from sampling number three to sample number four. (118 mg/100 ml to 105 mg/100 ml). When sample number 4 was compared to sample number five, a mean increase of 10 mg/100 ml was observed. Mean serum triglyceride concentrations were 105 mg/100 ml at

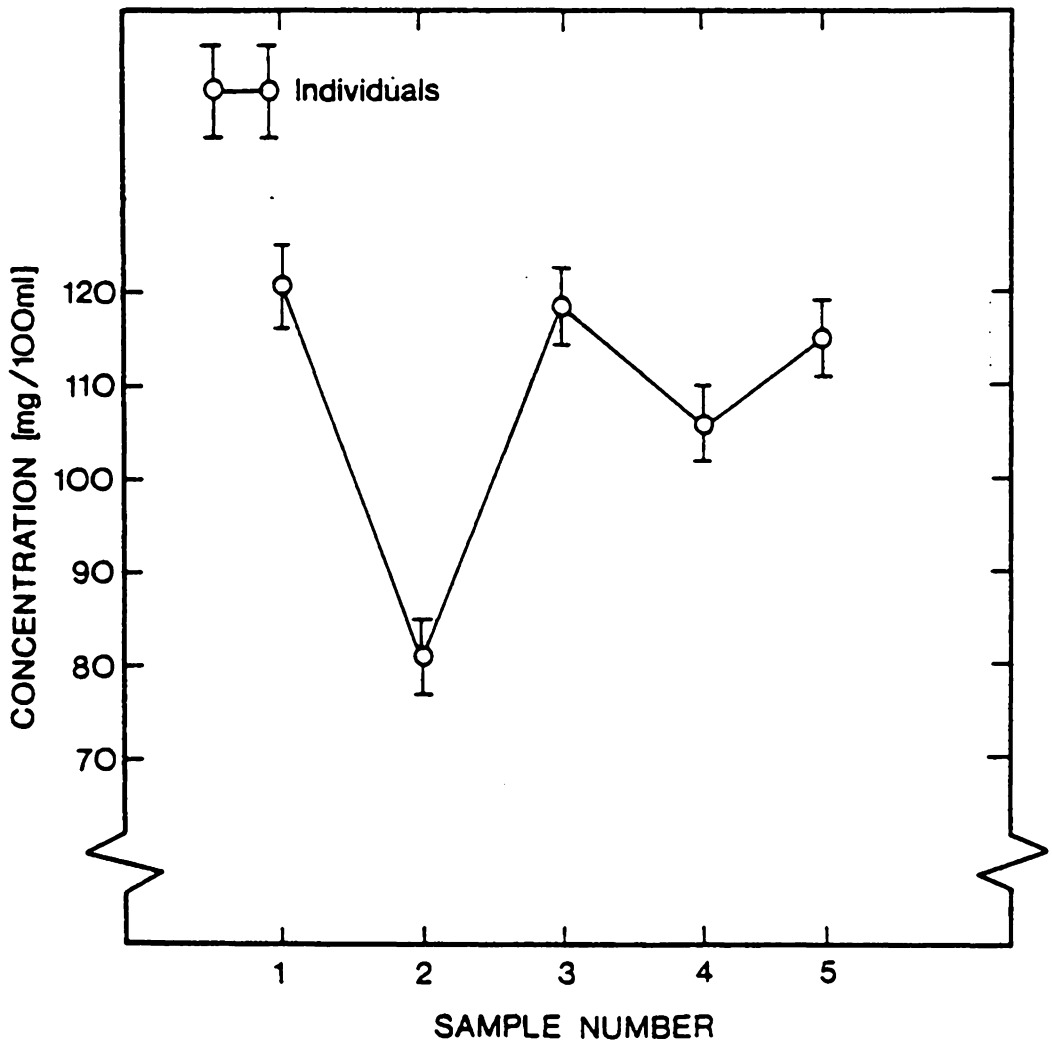

FIGURE 7. Plasma triglyceride concentration (mean ± SEM) on repeated sampling of the same five subjects on different days (Chromie, 1963).

the fourth sample and increased to 115 mg/100 ml at the fifth sampling.

Similar individual variation was observed when twice weekly determinations of serum triglycerides were made in 28 men over a 12 week period (Hollister et al., 1964). Coefficients of variation for serum triglycerides within individuals ranged from 7.1% to 34.8% with a mean of 16%, suggesting that random fluctuation as high as 35% occurred within the same individual. This variation seen in the studies of Chromie et al (1963) and Hollister et al (1964) parallels changes observed in the present study. A pattern of variation in serum triglyceride levels is evident in the present study when individual concentrations are averaged over weeks (Table 7). In addition, examination of individual data listed in Appendix V gives further testimony to the existence of spontaneous variation in serum triglyceride concentrations.

Published reports indicate that previous diet can influence serum triglyceride response to dietary manipulation (Shoney et al., 1981; McDonald, 1967; Nestel et al., 1970). Although a 24-hour recall and a food frequency record were administered prior to the present study in an effort to determine and minimize differences in previous dietary intake of subjects, the type of responses elicited

from these tools do not necesarily allow for complete assurance in selection of a homogenous population with regard to dietary habits. This situation could have been rectified if all subjects consumed a controlled house diet prior to the experimental study.

IV. The Use of Serum Triglyceride Values in Clinical Medicine

Current literature dealing with serum triglycerides has been conflicting. There is some indication that serum triglyceride levels are not associated with an increased risk for developing coronary heart disease. Rather, the best prognostic indicators of risk seem to include total serum cholesterol and high density lipoproteins (Castelli et al., 1977). In most situations, persons at increased risk have elevated levels of serum cholesterol and this is followed by concomitant elevations in serum triglycerides (Hulley et al., 1981). In effect elevated serum triglycerides seem to be secondary to elevations of serum cholesterol concentrations (Page et al., 1970; Gorden et al., 1977; Brown et al., 1965).

Some doubt concerning the efficacy of using serum triglyceride measures in clinical medicine has been expressed within the medical community. The hypothesis that elevated

triglyceride levels may be a cause of coronary heart disease although never universally accepted, has strongly influenced the practice of preventive medicine. Most of the evidence implicating serum triglycerides in the development of coronary heart disease comes from epidemiological studies. These types of studies may infer an association between the two but by no means indicate a casusal relationship between elevated serum triglycerides and coronary heart disease. Information from other types of studies (histochemial and arterigraphy) is also meager, contrasting with the spectrum of evidence supporting the hypothesis that elevated cholesterol levels are a causative factor in coronary heart disease. Some practitioners believe that the widespread use of identifying and treating hypertriglyceridemia in apparently healthy persons for the purpose of preventing coronary heart disease is inappropriate unless more persuasive evidence becomes available (Hulley et al., 1981).

The difficulty in obtaining conclusive evidence and reaching a consensus concerning the relationship of serum triglyceride concentrations to coronary heart disease may be due to spontaneous variation that is present within individuals. Individual variability has been observed in subjects in controlled diets (Plain, 1979) in normal populations consuming self selected diets (Hollister et al., 1964; Caronie

et al., 1963) and in patients with coronary heart disease (Chromie et al., 1963).

In addition, it is difficult to ascribe changes in serum triglyceride levels to changes in a single dietary component as dietary factors including caloric intake, carbohydrate level, carbohydrate source and fatty acid composition all appear to exert an influence on serum triglyceride levels (Nestel et al., 1970). The problem of lack of control for unrecognized influences (such as previous diet) can be critical in studies using small experimental populations. The subjects in the present study were not on a controlled experimental diet prior to the initiation of treatment diets. It was assumed that dietary patterns were similar between subjects. The instruments used in the present study to determine eating habits of subjects (24-hour food recall and food frequency) are crude measures at best (Guthrie, 1975). The type of responses elicited from those totals do not allow for complete assurance in selection of a homogenous population with regard to dietary habits. This situation could have been rectified if all subjects consumed a controlled house diet prior to the experimental study.

In the present study, plasma triglycerides and VLDL-triglycerides were investigated in healthy adult males fed diets containing 75 grams of protein per day either from

soy, non-fat dairy products or egg white under controlled dietary conditions. Differences in total caloric intake, carbohydrate, fat, cholesterol, and fatty acid composition of the diet were minimal between dietary treatment. The study design was different from previous reports examining the effect of source of protein on serum triglycerides and VLDL.

No studies have investigated the effect of feeding non-fat dairy protein in a controlled setting on serum lipid levels. Rather previous investigations have examined the effect of feeding supplemental amounts of milk, yogurt and cream. In these studies other dietary components including fat, fatty acid composition and carbohydrate content were not controlled. Furthermore, no studies have dealt with the effect of feeding egg white protein under controlled conditions on serum lipid levels.

The findings from the present study indicated that no significant differences between serum lipid levels in individuals fed either soy, non-fat dairy products or egg white existed during the experimental period. Rather serum triglyceride and VLDL-triglyceride levels of individuals on all treatment groups responded in the same fashion with respect to dietary treatment. All groups experienced decreases in serum triglyceride from week one to week two and from week

three to week four. However, from week two to week three and week four to follow-up, all groups experienced increases in serum triglyceride concentrations. All groups displayed similar trends with regard to VLDL-triglycerides. VLDL-triglycerides decreased from the initial baseline value to week one and from week three to week four. However, from week one to week two and week four to week five, serum VLDL-triglycerides increased. The observation that no significant differences occurred between treatment groups is in agreement with similar studies. Serum triglycerides do no appear to be predominantly influenced by protein source but may respond to other dietary components most notably total caloric fat and carbohydrate content.

The observation that all treatment groups experienced the same rises and falls in serum triglyceride and VLDL-triglycerides may be due to other experimental factors including spontaneous variation within individuals.

Based on present results and retrospective thought, the experimental design could have been improved. Subjects could have been on a controlled house diet prior to the beginning of treatment intervention. This would alleviate the problem of previous heterogeneity in dietary patterns which can influence serum triglyceride concentrations. In a future study the source of carbohydrate could be varied from

one treatment to another. This change would verify that changes (or lack thereof) in serum lipids are primarily influenced by carbohydrate content of the diet. In addition, longer feeding periods may be beneficial. Serum triglycerides might have reached a stabilization point, with longer dietary treatment, rather than continuing the increases and decreases from week to week that were observed. Further, a larger population sample is suggested. Each treatment group included eight subjects; the problem of individual variability and spontaneous fluctuation is compounded when only small samples are used.

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APPENDICES

Appendix A

FLYER PROVIDING BASIC INFORMATION ABOUT THE STUDY

EAT FREE FOR A MONTH

That's right! Wanted: Dedicated non-smoking male volunteers in good health, of normal weight and between the ages 18-28 to participate in a month long diet study. Every day each subject will receive 3 delicious vegetarian meals FREE. The investigation will involve assessment of plasma lipoprotein fractions as affected by dietary source of protein. Each subject will be randomly assigned to 1 of 3 treatment groups in which primary source of protein will come from soy, dairy and egg white respectively. All subjects will eat at the metabolic kitchen - Solitude. Physical examinations will be provided FREE before the study's onset and measurements of plasma cholesterol and triglycerides will be made at regular intervals. In addition, all subjects will receive $90 compensation upon successful completion of the study. The project will run from March to May. For further information on this exciting project contact:

Department of Human Nutrition and Foods 961-5987

Appendix B

METROPOLITAN LIFE INSURANCE TABLES FOR DETERMINING NORMAL BODY WEIGHT FOR HEIGHT

WEIGHT IN POUNDS ACCORDING TO FRAME (IN INDOOR CLOTHING)

Source: Metropolitan Life Insurance Company

	HEIGHT		SMALL FRAME	MEDIUM FRAME	LARGE FRAME
	5	1	112-120	118-129	126-141
	5	2	115-123	121-133	129-144
	5	3	118-126	124-136	132-148
	5	4	121-129	127-139	135-152
	5	5	124-133	130-143	138-156
MEN	5	6	128-137	134-147	142-161
of	5	7	132-141	138-152	147-166
AGES 25	5	8	136-145	142-156	151-170
and	5	9	140-150	146-160	155-174
OVER	5	10	144-154	150-165	159-179
	5	11	148-158	154-170	164-184
	6	0	152-162	158-175	168-189
	6	1	156-167	162-180	173-194
	6	2	160-171	167-185	178-199
	6	3	164-175	172-190	182-204

	HEIGHT		SMALL FRAME	MEDIUM FRAME	LARGE FRAME
	4	9	94-101	98-110	106-122
	4	10	96-104	101-113	109-125
	4	11	99-107	104-116	112-128
	5	0	102-110	107-119	115-131
WOMEN	5	1	105-113	110-122	118-134
of	5	2	108-116	113-126	121-138
AGES 25	5	3	111-119	116-130	125-142
and	5	4	114-123	120-135	129-146
OVER	5	5	118-127	124-139	133-150
	5	6	122-131	128-143	137-154
	5	7	126-135	132-147	141-158
	5	8	130-140	136-151	145-163
	5	9	134-144	140-155	149-168
	5	10	138-148	144-159	153-173

Appendix C

WRITTEN EXPLANATION OF STUDY PROVIDED TO ALL PERSONS INTERESTED IN PARTICIPATING AS SUBJECTS

Did you know that certain lipoprotein fractions found in the blood are correlated to incidence of coronary artery disease and reports have shown that vegetarians who regularly consumed low-fat, low-cholesterol diets showed reduced levels of the lipoprotein lipid fractions? The objective of our research stems from that premise. We will attempt to evaluate the source of dietary protein on plasma lipids and lipid proteins. Subjects will be divided into 1 of 3 dietary treatment groups in which primary source of protein will come from: soy, dairy and egg white respectively. The subjects will be assigned to each treatment by randomized block design using plasma cholesterol levels to rank the experimental subjects. In addition, screening based on physical examination and questionnaires will also be considered. The diets will be consumed at the metabolic kitchen - Solitude. Three meals a day will be provided at Solitude. No food or drink (that includes alcohol, guys) except water can be consumed outside the kitchen. All three dietary groups will consume caloric values of 2800. Weight ideally will be kept constant; if you begin to lose or gain weight, your calories will be adjusted.

APPENDIX C

WRITTEN EXPLANATION OF STUDY PROVIDED TO ALL PERSONS INTERESTED IN PARTICIPATING AS SUBJECTS (CONTINUED)

A certified Medical Technologist will draw fasting blood samples from all subjects for plasma lipid determinations: 30 ml at screening, 60 ml at the initiation of the study, 30 ml week 2, week 3, and week 4. 60 ml at the termination of the study. A follow-up blood sample of 60 ml two weeks after the termination of the study will also be taken. (No, we are not vampires, we need the blood to access the lipoprotein fractions.) If you have any questions or concerns, please let us know.

Appendix D

QUESTIONNAIRE REQUIRED OF ALL INTERESTED PERSONS USED TO DETERMINE SUBJECT ELIGIBILITY

Name_____ Age_____

Address_____

Phone_____

1. History of Cardiovascular disease (If yes, specify what type of condition).

   Personal_____
   Grandfather_____
   Maternal_____
   Paternal_____
   Father_____
   Brother(s)_____
   Uncle(s)_____
   No known history_____

2. Have you ever been diagnosed as having hyperlipoproteinemia?
   No_____ Yes_____

3. If yes, specify which type_____

4. Have you ever been diagnosed as having hyperglycemia or diabetes mellitus?
   No_____ Yes_____

5. Are you taking any medications on a regular basis?
   No_____ Yes_____

6. If yes, specify the name and daily dosage of the medication. _____

7. Are you on a special diet?
   No_____ Yes_____
   If yes, specify what type_____

8. Do you supplement your diet with either vitamin(s), mineral(s) or protein?
   No_____ Yes_____

APPENDIX D
QUESTIONNAIRE REQUIRED OF INTERESTED PERSONS USED TO DETERMINE SUBJECT ELIGIBILITY (CONTINUED)

9. If yes, specify the brand names and the daily dosage of each supplement.
   vitamin(s)_____ vitamin-mineral_____
   mineral(s)_____ protein_____

10. Do you smoke? No_____ Yes_____
    If yes, which of the following do you use?
    cigarette_____ pipe_____
    cigar_____ chewing tobacco_____

11. Do you use any recreational drugs? (ie. pot, LSD, cocaine, etc.)
    No_____ Yes_____

12. Are you a vegetarian?
    No_____ Yes_____

13. Are you allergic to any specific foods?
    No_____ Yes_____
    If yes, list foods you are allergic to_____
    _____

14. Would you like the results of your analysis?
    No_____ Yes_____

15. Do you plan or anticipate being out of town from April 20th to May 20th?
    No_____ Yes_____

16. What will your address be in June of 1981?
    _____
    _____
    _____
    Social Security#_____

Blood Pressure_____mm Hg
Height_____cm
Weight_____kg
Total plasma cholesterol_____mg%
Total plasma triglycerides_____mg%
Urinalysis_____
Hemoglobin_____
Hematocrit_____

Appendix E

FOOD FREQUENCY QUESTIONNAIRE REQUIRED OF ALL INTERESTED PERSONS USED TO DETERMINE SUBJECT ELIGIBILITY & EATING HABITS

I. Instructions:

Indicate whether or not you eat the following foods by checking the columns "DON'T EAT" or "DO EAT" for each item. For each food you have checked "DO EAT" write the approximate number of times you eat it in a week. If you eat any particular food less than once a week, do not write anything in the column "TIMES EATEN PER WEEK".

FOOD	Don't Eat	Do Eat	Times Eaten/Week
Chicken			
beef, hamburger, veal			
liver,kidney,tongue,etc.			
lamb			
cold cuts			
hot dogs			
pork,ham sausage			
bacon			
fish			
shellfish			
kidney beans,pinto,etc.			
soybeans			
nuts or seeds			
peanut butter			
tofu			
cottage cheese			
cheese			
ice cream			
yogurt			
pudding and custard			
milkshake			
sherbert			
ice milk			
whole grain bread			
white bread			
rolls,biscuits,muffins			
bagel			
crackers,pretzels			
pancakes,waffles			
cereals			

APPENDIX E

FOOD FREQUENCY QUESTIONNAIRE REQUIRED OF ALL INTERESTED PERSONS USED TO DETERMINE SUBJECT ELIGIBILITY & EATING HABITS (CONTINUED)

FOOD	Don't Eat	Do Eat	Times Eaten Per Week
white rice			
brown rice			
noodles,macaroni,grits			
pizza			
potato chips,fried snacks			
tomato,tomato sauce/juice			
tangerine			
grapefruit			
lemonade			
white potato			
turnip			
peppers			
orange juice			
oranges			
lettuce			
asparagus			
cabbage			
broccoli			
brussels sprouts			
spinach			
greens(collard,kale)			
carrots			
artichoke			
corn			
sweet potato/yam			
zucchini			
summer squash			
winter squash			
green peas			
green/yellow beans			
hominy			
beets			
cucumbers or celery			
peach			
apricot			
apple			
banana			
pineapple			
cherries			
cakes,pies,cookies			
sweet roll,doughnuts			
hard candy			
chocolate candy			

APPENDIX E

FOOD FREQUENCY QUESTIONNAIRE REQUIRED OF ALL INTERESTED PERSONS USED TO DETERMINE SUBJECT ELIGIBILITY & EATING HABITS (CONTINUED)

FOOD	Don't Eat	Do Eat	Times Eaten Per Week
jelly			
cocoa			
wine			
beer			
cocktails			
fruit drink			

II. Below are a list of food items. Please indicate the foods you consume DAILY.

FOOD	Don't Eat	Do Eat	Times Eaten/Day
whole milk			
2% milk			
skim milk			
sweetened carbonated beverage			
unsweetened carbonated beverage			
coffee or tea			
sugar			
cream or half & half			
non-dairy creamer			
butter			
margarine			
mayonnaise			
mayonnaise or similar type dressing			
liquid vegetable oils			
salad dressings			

List types of salad dressings.	Times Eaten/Day
_____	_____
_____	_____
_____	_____
_____	_____

APPENDIX E

FOOD FREQUENCY QUESTIONNAIRE REQUIRED OF ALL INTERESTED PERSONS USED TO DETERMINE SUBJECT ELIGIBILITY & EATING HABITS (CONTINUED)

III. 1. Approximately how many eggs per week do you eat?
_____

2. Approximately how many times per week do you eat fried foods?_____

3. Are you on a high fiber diet? No_____ Yes_____

4. Do you eat bran as a dietary supplement? No__Yes__

5. On the average, how many meals per day do you consume? _____

IV. Likes and Dislikes

The foods listed below constitute a large part of the study menu. In otherwords, there is a strong likelihood you will be required to eat many of these foods. Are you willing to eat this food? (ie. if you are allergic to it or hate it, check no.)

Food	Yes	No
tomatoes	_____	_____
coconut	_____	_____
skim milk	_____	_____
hard boiled eggs	_____	_____
fried eggs	_____	_____
scrambled eggs	_____	_____
omlets	_____	_____
mushrooms	_____	_____
spicy foods	_____	_____
green pepper	_____	_____
onions	_____	_____
cottage cheese	_____	_____
diet drinks	_____	_____
saccharin	_____	_____

Appendix F

TWENTY-FOUR HOUR FOOD RECORD REQUIRED OF ALL INTERESTED PERSONS USED TO DETERMINE SUBJECT ELIGIBILITY & EATING HABITS

A food record is a valuable educational tool which can be used by you and the dietitian to examine your present eating habit and your caloric and nutritional intake.

Please write down your food intake for yesterday. If this was not what you consider a "typical" day, (i.e. weekend, eating in restaurants) choose another recent day that you can remember fairly well.

List each food that you ate, including all beverages, snacks and condiments. Estimate serving sizes in measurements such as teaspoons, tablespoons, cups or ounces. Describe any foods you are unsure about by brand name, size, etc.

Thank you for your cooperation.

When & Food	Amount	Food	Amount
Orange juice,unsweet.	8 oz.		
Coffee	16 oz.		
Cream-half & half	4 Tbsp.		
Sugar	4 tsp.		
Egg, fried	1 large		
Toast, Hollywood diet	2 slices		
Margarine(for tst,egg)	4 tsp.		
Ritz crackers	4		
Cheese-cheddar	1"square		
Coke	12 oz. can		

EXAMPLE:

Appendix G

EXERCISE AND ACTIVITY LEVEL QUESTIONNAIRE

Name_____

Below are a list of exercises and activities. Each question has 3 parts to it. If you participate in the activity or exercise, please complete all 3 parts. If you do not participate in the activity, please leave blank. (Consider what you will do or are doing from January to June 1981.)

1. Baseball
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light___ mild___
      moderate___ vigorous___
   C. How long? _____

2. Basketball
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous____
   C. How long? _____

3. Bowling
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

4. Calisthenics
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

5. Canoeing
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

APPENDIX G
EXERCISE AND ACTIVITY LEVEL QUESTIONNAIRE (CONTINUED)

6. Dancing (square, clogging, ballroom, modern)
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

7. Chopping wood
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

8. Gardening
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

9. Golfing
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

10. Racquetball
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

11. Cross Country Skiing
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      moderate___ vigorous___
   C. How long? _____

12. Downhill Skiing
   A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
   B. What level of intensity: light____ mild____
      nocerate___ vigorous___
   C. How long? _____

APPENDIX G
EXERCISE AND ACTIVITY LEVEL QUESTIONNAIRE (CONTINUED)

13. Soccer
    A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
    B. What level of intensity: light____ mild____
       moderate___ vigorous___
    C. How long? _____

14. Sprinting
    A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
    B. What level of intensity: light____ mild____
       moderate___ vigorous___
    C. How long? _____

15. Rugby
    A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
    B. What level of intensity: light____ mild____
       moderate___ vigorous___
    C. How long? _____

16. Tennis
    A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
    B. What level of intensity: light____ mild____
       moderate___ vigorous___
    C. How long? _____

17. Volleyball
    A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
    B. What level of intensity: light____ mild____
       moderate___ vigorous___
    C. How long? _____

18. Weight lifting
    A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
    B. What level of intensity: light____ mild____
       moderate___ vigorous___
    C. How long? _____

19. Recreational Biking
    A. Days/week 1___ 2___ 3___ 4___ 5___ 6___ daily___
    B. What level of intensity: light____ mild____
       moderate___ vigorous___
    C. How long? _____

APPENDIX G
EXERCISE AND ACTIVITY LEVEL QUESTIONNAIRE (CONTINUED)

Part II: Please answer the following questions.

1. What is your main form of transportation?
   Car_____
   Bike_____
   Walk_____
   Other_____ Explain_____

   What is the distance you walk/bike per day?
   less than 1 mile_____
   1-2 miles_____
   2-3 miles_____
   greater than 3 miles_____

2. Do you hike? No_____ Yes_____
   If yes, how many times per month_____
   On the average, what is the distance covered?_____

3. Do you run? No_____ Yes_____
   Types: Cross Country_____
   days/week_____
   miles/run_____
   how long_____
   Field House or Track_____
   days/week_____
   miles/run_____
   how long_____
   Graded Surface_____
   days/week_____
   miles/run_____
   how long_____

4. Do you swim? No_____ Yes_____
   Types: Recreational_____
   days/week_____
   #laps/swim____
   how long_____
   Team Swim_____
   days/week_____
   #laps/swim____
   how long_____

Appendix H

PHYSICAL EXAMINATION CHECKLIST

Name_____

Skin:	Color		Texture	Moisture
	Eruptions		Other lesions	
Head:	Appearance			
	Hair		Distribution	Character
	Scalp			
Face:	Appearance		Color	Tenderness
Eyes:	Crows		Lids	
	Eyeball motion		Prominence	Tension
	Conjunctivae	Sclerae	Cornea	
Irises				
	Pupils	Size	Shape	Regularity
Reactions				
	Fundi			
Ears:	Appearance		Hearing	
	Tenderness	Discharge		
	Drums	Canals		
Nose:	Appearance		Discharge	
	Nasal cavity		Septum	
Mouth:	Breath			
	Lips	Color	Fissures	Lesions
	Tongue	Tremor		Deviation
		Color	Moisture	Texture
	Gingivae	Color	Bleeding	Pus
	Teeth	Number	Condition	Dentures
Throat:	Palate & Uvula		Tonsils	
	Posterior Pharynx			
Neck:	Lymphnodes			
	Thyroid			
	Veins			

Shoulder Girdle: Swelling Tenderness Joints

APPENDIX B
PHYSICAL EXAMINATION CHECKLIST (CONTINUED)

Arms and Hands: Deformities Tenderness
Fingers:

Back: Deformities Motion Skin Swelling Tenderness

Chest: Shape Respiratory Movements

Heart: Apex Impulse Location Character
Thrill
Sounds Rate Rhythm Intensity
Quality
Murmurs
Pulses Equality Character
Blood Pressure

Lungs:

Abdomen: Size Shape Masses Tenderness
Spasm
Liver Spleen Kidneys Herniae
Inguinal
lymphnodes

Legs and Feet: Position Tenderness
Abnormal Motions Joints Edema
Local Swellings

Reflexes: Biceps
Triceps
Knee
Ankle
Romberg

Genitalia:

Any abnormal findings otherwise.

Appendix I

METHOD FOR BLOOD HEMOGLOBIN DETERMINATIONS

Hemoglobin Std. Curve

Mark 2 tubes for each of the following concentrations:

Blank, 5 gms/100 ml, 10 gms/100 ml, 15 gms/100 ml, 100 ml/100 ml, 20 gms/100 ml

Pipette the following volumes of Cyanmethemoglobin Standard and Cyanmethemoglobin Reagent into the correspondingly marked tubes and mix well.

Hemoglobin Concentration (gms/100 ml)	Blank	5	10	15
Cyanmethemoglobin Standard (ml)	0.0	1.5	3.0	4.5
Cyanmethemoglobin Reagent (ml)	5.0	4.5	3.0	1.5

Transfer the solutions to well-matched cuvettes and measure the absorbance of each dilution against the blank at 540 nm.

Plot the absorbance of each standard (ordinates) against its concentration (abscissae).

Hemoglobin Samples (unknowns)

Mark 2 tubes with your initials.

Pipette 5.0 ml of Cyanmethemoglobin Reagent into each tube.

Fill 2 hemocap tubes (20 microliters) with blood and carefully drop one into each of the 2 tubes of Cyanmethemoglobin Reagent. Mix well and allow to stand at least 10 minutes at room temperature.

Transfer the contents of the tubes to cuvettes (unless mixing was done in cuvettes) and measure the absorbance against the Cyanmethemoglobin Reagent at 540 nm.

Appendix J

METHOD FOR BLOOD HEMATOCRIT DETERMINATIONS

Duplicate or triplicate micro-hematocrits of each individual's blood will be determined simultaneously with blood hemoglobins.

Procedures:

1. From the lanced finger tip blood is drawn into 2 or 3 heparinized micro-hematocrit (capillary) tubes.
2. Fill tubes to within 1/4 -1/2 inch of end.
3. Seal the filled end of tube with "seal-ease."
4. Place the tubes in the micro-centrifuge being careful to make sure the sealed ends of all tubes are touching the outside edge of the centrifuge.
5. Secure the lid of the centrifuge and set the timer for 10 minutes. Centrifuge at 3000 rpm.
6. Allow the centrifuge to stop without use of the brake.
7. Read the bottom of the meniscus of the plasma and the top of the packed red cells.
8. Express the volume of red blood cells as percent of whole blood.

Appendix K

WRITTEN AUTHORIZATION FOR PARTICIPATION IN THE STUDY REQUIRED FOR ALL SUBJECTS

I have received an oral and written explanation of the study and have had an opportunity to ask questions. I understand the following:

Purpose: The purpose of the study is to provide information on the effect of dietary protein source on blood lipids in adult males.

Procedure: Twenty-four subjects (aged 18-28 years) will be recruited during March 1981. Screening will be based on results of pre-experimental questionnaire, pre-experimental total and HDL cholesterol measurements and a physical examination by a physician.

The subjects will be matched in 3 dietary groups (8 subjects per group) using a randomized block design. The primary source of protein for each dietary group will come from soy, egg white or dairy. The subjects will consume three meals a day (approximately 2800 kilocalories per meal) that will be served at Solitude, the metabolic kitchen. The diets are well-balanced and meet the daily dietary requirements for the major nutrients. No food or drink except water may be consumed outside of Solitude.

A certified Medical Technologist will draw fasting blood samples from all subjects for lipid determinations 6 times: 5 ml at screening and Weeks 2, 3, 4, and 5; and 60 ml at Weeks 1, and follow-up. Cholesterol and triglyceride concentrations will be determined in total plasma and various plasma lipoprotein fractions.

All information obtained will be held strictly confidential and will be used for statistical purposes only.

No compensation will be offered if injury is incurred as a result of participation in this project. The subjects will be expected to advise the researchers of any medical problems that arise during the study and are free to withdraw consent and discontinue participation at any time.

APPENDIX K
WRITTEN AUTHORIZATION FOR PARTICIPATION IN THE STUDY REQUIRED FOR ALL SUBJECTS (CONTINUED)

Any inquiries about procedures by the subjects will be answered at any time.

Upon successful completion of the study, subjects will receive three dollars per day (approximately ninety dollars) for participation.

I understand the above and agree to participate in this study to be conducted at Virginia Polytechnic Institute and State University during Spring quarter of 1981.

_____ _____

(DATE) (NAME)

Principle Investigators: Dr. F. W. Thye (961-6220),
Dr. J. Taper (961-5549),
Dr. S. J. Ritchey (961-6779),
Dr. L. F. Ferreri (961-6331).

Other Investigators: Mary Lou Johnston, Mary Lou Price, Mary Smith

Chairman, Institutional Review Board for Research Involving Human Subjects: Dr. Milton Stombler (961-5283).

APPENDIX L

Description of Study Participants by Age, Weight, Hemoglobin, Hematocrit and Urinanalysis

Subject Initial	Age	Weight (kg)	Hemoglobin (mg/100ml)	Hematocrit (%)	Urinanalysis
C.G.	24	69.2	16.3	47%	NN
M.W.	28	88.2	18.8	50%	NN
C.K.	27	59.8	17.3	48.5%	NN
B.H.	24	81.2	19.6	57%	NN
B.F.	24	77	20.4	55.5%	NN
B.P.	22	81.5	19.3	56%	NN
K.M.	22	70.5	19.3	53.5%	NN
J.T.	21	74.0	19.7	56%	NN
C.W.	28	70.5	18.5	61%	NN
L.C.	24	63.7	19.9	56%	NN
A.W.	21	63.7	21.7	59%	NN
B.C.	19	72	17.6	52.5%	NN
P.D.	21	67.5	17	48%	NN
D.D.	22	62.9	18	50%	NN
L.S.	23	64.7	17.6	50%	NN
M.S.	26	56	18.5	51.5%	NN
C.S.	21	88.7	18	51%	NN
G.M.	22	75.9	16.9	47%	NN
J.S.	21	83.8	16	46%	NN
B.M.	25	77.8	18.8	50%	NN
K.L.	22	76.5	17.2	48%	NN
T.M.	24	80	19.8	52%	NN
T.C.	26	72.5	18	49%	NN
J.L.	28	67.8	18.3	56.5%	NN
$\bar{X} \pm SD$	23.5 ± 4	72.7 ± 8	18.4 ± 1	52.2 ± 4%	

NN Normal-No glucose present; Hemoglobin based on mg/100 ml; Hematocrit based on % Red Blood Cells.

APPENDIX M

Assignment of Study Participants to Treatment Groups Using Screening Values of Cholesterol and Body Weight

Subject#	Cholesterol (mg/100ml)	wt. (kg)	Subject#	Cholesterol (mg/100ml)	wt. (kg)	Subject#	Cholesterol (mg/100ml)	wt. (kg)
1	201.2	72.0	9	151.2	61.0	17	179.3	56.0
2	164.5	74.0	10	146.4	88.7	18	188.0	67.5
3	157.1	72.5	11	206.7	69.2	19	161.9	76.0
4	185.2	64.7	12	193.4	70.5	20	156.6	64.8
5	154.1	83.3	13	167.5	77.8	21	193.4	88.2
6	156.7	81.2	14	162.1	72.9	22	148.6	81.5
7	180.0	80.0	15	162.9	67.8	23	169.8	76.5
8	164.7	59.8	16	159.4	77.0	24	178.7	70.5
$\bar{X} \pm SD$	171.1 ± 9	73.4 ± 7		168.7 ± 20	73.1 ± 3		172.0 ± 15/72.5±9	

APPENDIX N

Food Items and Serving Sizes (in grams) of the Four Daily Menus Used Throughout the Experimental Period

MENU I

SOY		NON-FAT DAIRY		EGG WHITE	
Food Item	Weight	Food Item	Weight	Food Item	Weight
scrambled eggs		Egg omelet		scrambled eggs	
egg yolks	17	egg yolks	34	egg whites	198
coffeemate	11.4	coconut oil	9.2	egg yolk	17
soy flour	5	cottage cheese	113	coconut oil	21
bacos	23.1	pimento	14.3	thin toast	28.4
margarine	7.4	bagel	28.4	margarine	7.4
Grapefruit	268.0	margarine	4.7	sugar	7
Bagel	56.8	honey	15	grapefruit	268
Margarine	7.4	grapefruit	268	honey	
		sugar	7	lettuce	10
Celery soup	56	skim milk	122.4		
bacos	23.1			potato salad	
soy flour	5	carrot salad		sliced potatoes	155
pumpkin bread		carrots	110	egg yolk	17
flour	37.5	pineapple	61.5	egg white	99
sugar	72	raisins	54	chopped celery	30
soy flour	10	cottage cheese	113	egg free mayo	32.7
coconut oil	29	Wheatsworth cracker	25	tomato soup	180
egg yolk	17	lemon pudding (dry)	21.3	escort cracker	21.3
pumpkin	56.8	sugar	24	mandarin orange	113.6
soy granules	25	Ginger snaps		coconut macaroons	
lettuce	90	margarine	18.7	coconut	14.2
mandarin oranges	113.6	sugar	44.4	coffeemate	5.7
Green bean casserole		molasses	9.1	dried egg white	13.5
soy granules	50.7	flour	46.8	fresh egg whites	33
green beans	65	cornstarch	7.1	sugar	24
mushrooms	52.4				
macaroni	70	spinach souffle		green bean casserole	
peas and carrots	80	cottage cheese	142..	green beans	65
roll	25.2	golden image cheese	85	cm. mushroom soup	122.5
margarine	4.7	mushrooms	35	dried egg white	22.5
Chocolate pudding	29	spinach (cooked)	110	chp. mushroom	52.4
soy flour	5	onions	10	chp. onions	40
soy nuts	28.4	coconut oil	18	cooked macaroni	70
raisins	13.5	roll	25.2	peas and carrots	80
dried apples	141.5	margaine	4.7	roll	25.2
		corn	85	parkay margarine	9.4
		mandarin orange	113.6	lemon pudding	33.4
		ice milk	95.3	dried egg white	12.5
		strawberries (frozen)	61.2	sugar	24
		lemonade mix	21.3	margarine	9.4
				banana	175
				jelly	14
				cake	354

APPENDIX N

Food Items and Serving Sizes (in grams) of the Four Daily Menus Used Throughout the Experimental Period (continued)

MENU II

SOY		NON-FAT DAIRY		EGG WHITE	
Food Item	Weight	Food Item	Weight	Food Item	Weight
soy pancakes		banana pancakes		orange julious	
soy granules	38	egg yolks	25.5	orange juice conc.	124
flour	46.9	cottage cheese	56.5	honey	21
sugar	4	cornstarch	36	sugar	24
molasses	13	sugar	12	dried egg whites	36
coffeemate	13	dry milk	17	coffeemate	13.3
coconut oil	14.2	banana puree	25	coconut oil	16.4
banana puree	25	coconut oil	15.1	english muffin	56.8
egg yolk	17	syrup	14.9	margarine	18.7
margarine	14	margarine	14.0	jelly	14
maple syrup	29.6	orange juice	124.5	sliced hot peaches	113.6
orange juice	124.5	uneeda biscuit	8.0		
		Mock Waldorf Salad		fried eggs	100
soy burger		raisins	9	bread	33.6
soy granules	28	cottage	113	egg free mayo	14
onions	43	diced apple	55	vegetarian soup	120
soy sauce	18	vegetarian soup	120	shredded cabbage	45
coconut oil	12.1	cole slaw		corn oil	13.6
tomato sauce	15	shredded cabbage	45	sugar	8.3
egg yolk	17	crisco oil	13.6	apple	180
whole wheat flour	7.3	vanilla	85.3		
sauce				lasagna	
egg free mayo	9.4	Lasagna		coconut oil	11.8
catsup	15	golden image cheese	95	egg whites	132
pickle relish	15	cottage cheese	169.5	dried egg white	27
vegetable soup	120	lasagna noodles (ck)	254.7	tomato puree	346.8
shredded cabbage	45	tomato sauce	221	mushrooms	13.1
corn oil	4.5	mushrooms	8.7	onions	15
safflower oil	4.5	green pepper	10	green pepper	15
		yellow squash	65.3	cooked lasagna noodles	142
Lasagna		lettuce wedge	90	lettuce	90
TVP	46.5	fruit cocktail	113.6	soybean oil	6.8
Tofu	113.6	coconut (shredded)	38	fruit cocktail	113.6
coconut oil	4.5	skim milk	244.8	Forgotten Angel Cookies	
lasagna noodles	170.4	chocolate cookies		egg white	17
tomato puree	231.2	coconut oil	5.8	sugar	48
chp. mushrooms	8.7	unsw. chocolate	6.3	chocolate chips	28.4
chp. onions	10	sugar	19.4		
green pepper	10	egg yolks	8.3		
squash yellow	65.3	flour	6.9		
lettuce wedge	90	cornstarch	6.3		
fruit cocktail	113.6	10 x sugar	2.2		
soy crescents					
crisco	8				
margarine	11.7				
honey	10.5				
soy nuts	11.7				
soy flour	6.3				
white flour	10.4				
10 x sugar	1.6				

APPENDIX N

Food Items and Serving Sizes (in grams) of the Four Daily Menus Used Throughout the Experimental Period (continued)

MENU III

SOY		NON-FAT DAIRY		EGG WHITE	
Food Item	Weight	Food Item	Weight	Food Item	Weight
coffee cake		coffee cake		coffee cake	
sugar	48	sugar	48	sugar	48
coconut oil	21.4	coconut oil	30.5	coconut oil	14.4
egg yolk	17	egg yolk	17	crisco oil	6.8
flour	62.6	flour	62.6	dried egg whites	18
soy flour	8.8	yogurt	56.8	coffeemate	34.2
soy granules	17	brown sugar	20.6	flour	93.9
coffeemate	5.4	skim milk	244.8	cornstarch	36
brown sugar	20.6	orange	252	brown sugar	20.6
orange juice	249	white bread	56.8	orange juice	248
		diet margarine	7	margarine	18.7
ham salad		golden image cheese	56.7	egg salad	
vegetarian hamchunks		tomato	50	chp. egg whites	231
TVP	11	lettuce	10	egg yolk	17
coconut oil	14.2	celery sticks	50	egg free mayo	42
egg free mayo	25.2	carrot sticks	28	earth grain bread	28.4
pickle relish	15	pineapple ring	37.3	tomato	50
bacos	23.1	cottage cheese	28.3	lettuce	10
white bread	56.8	skim milk	244.8	celery sticks	50
celery sticks	50	stir fry vegetables		carrot sticks	28
carrot sticks	28	yellow squash	35.4	banana	175
tomato	10	pea pods	21.3	pickle relish	15
lettuce leaf	10	brocolli	70.8	stir fry vegetables	
banana	175	water chesnuts	18.8	yellow squash	35.4
stir fry vegetables		fresh carrots	13.8	pea pods	9.4
fresh carrot	13.8	sliced celery	15	brocolli	20.6
fresh celery	15	pimento	14.3	water chesnuts	18.8
pimento	14.3	soy sauce	6	carrots	13.8
yellow squash	35.4	coconut oil	3.4	celery	15
pea pods	21.3	minute rice (dry)	19	canned pimento	14.3
chopped broccoli	70.8	golden image cheese	85	cornstarch	2.7
water chesnuts	8.8	wheatsworth cracker	12.5	soy sauce	6
cornstarch	2.7	pumpkin custard		coconut oil	11.6
soy sauce	6	egg yolk	17	minute rice (dry)	28.5
coconut oil	7.1	pumpkin	56.3	hard boiled egg whites	264
minute rice (dry)	19	sugar	18	wheatsworth cracker	12.5
tofu	71	dry milk powder	22.7	pumpkin custard	
pumpkin custard		cool whip	14	egg yolk	17
egg yolk	17	nabisco cookies	9.5	canned pumpkin	56.8
canned pumpkin	56.8	milk	244.8	sugar	18
sugar	18	jelly beans	28.4	dried egg whites	9
soy granules	9.5			coffeemate	2.3
soy flour	2.5			cool whip	14
coffeemate	1.9				
cool whip	14.8				
nabisco cookies	9.5				
dried apricots	28.4				
soy nuts	56.8				

APPENDIX N

Food Items and Serving Sizes (in grams) of the Four Daily Menus Used Throughout the Experimental Period (continued)

MENU IV

SOY		NON-FAT DAIRY		EGG WHITE	
Food Item	Weight	Food Item	Weight	Food Item	Weight
soy oatmeal		corn flakes	21	oatmeal	
dry oatmeal	20	sugar	7	dried egg white	27
soy granules	46.6	fruit yogurt	113.2	raisins	13.5
raisins	13.5	skim milk	244.8	rolled oats	20
applesauce	63.8	white bread	28.4	brown sugar	14.2
brown sugar	14.3	jelly	14.0	coffeemate	22.8
coffeemate	7.6			margarine	18.8
pineapple chunks	6.15	chopped egg yolks	34	applesauce	127.5
orange juice	124.5	lettuce chunks	75	pineapple chunks	123
		lo cal dressing	30	orange juice	124.5
lettuce	75	cottage cheese	56.5	egg white omelet	
white bread	56.8	canned pears	135	crisco oil	13.6
jelly	28	mexican chile		egg white (fresh)	19.8
soy nut butter		coconut oil	15	egg yolks	34
soy nuts	28.4	onions	30	spinach	71
honey	7.5	tomatoes	120	cm. celery soup	56
margarine	35	grated golden image		bagel	28.4
coconut oil	11.1	cheese	56.1	margarine	24.6
soy carrot soup		hamburger bun		grapejelly	14
soy flakes	52.5	carrot sticks	30	brownies	
carrots	55	dinner roll	50	fried egg white	9
coffeemate	11.4	margarine	9.4	cocoa	7
brown sugar	13.7	green bean casserole		white sugar	48
egg yolks	25.5	coconut oil	21	flour	23.4
pear	180	green beans	65	crisco	24
green pepper with		cm. mushroom soup	122.5	pear	180
soy burger		canned mushrooms	52.4	spicy rice burger	
BBQ sauce	93.8	chp. onions	40	hamburger bun	52.8
tomato sauce	84.9	golden image cheese	56.7	dried egg white	18
green pepper	5	gum drops	28	coconut oil	8.8
onion	5	macaroni (cooked)	70	green pepper	10
TVP	33	lemon cheese cake (mock)		onion	10
hamburger bun	52.8	vanilla pudding	27.5	BBQ sauce	62.5
jello salad		sugar	33	tomato sauce	56.6
dry jello	37.9	evaporated skim milk	163	rice (cooked)	65
fruit cocktail	56.8	lemon juice	25.3	squash	65.3
raisin rock cookies				margarine	9.3
egg yolk	8.5			jello salad	
brown sugar	17.1			dried egg white	13.5
margarine	7			fruit cocktail	27.5
coconut oil	6.8			dry jello	37.9
soy flour	7.3			candy corn	22
white flour	18.8				
chocolate chips	10.5				
soy nuts	14.2				

APPENDIX O

Partial Nutritive Analysis of Experimental Diets (Handbook 456)

NUTRIENT	MENU I			MENU II		
	Soy	Dairy	Eggwhite	Soy	Dairy	Eggwhite
Energy (kcal)	2726	2851	2782.3	2704.4	2756.5	2908
Protein (total)	101.3	98.9	99	106.4	99	106.2
Protein (treat.)	74.9	75.8	75.6	77.5	75.4	75.6
Protein (non-treatment	26.4	23.1	23.4	29	24.6	30.6
Carbohydrate (grams)	351.7	369.9	339.2	347.4	349.8	346.2
Total Fat (grams)	108.1	100.2	110.8	109.2	109.7	107.1
Saturated fatty acids (grams)	47.4	41.7	49.3	50	56	52.9
Polyunsaturated fatty acids (grams)	18.9	15.7	18.8	19.6	22.4	21.7
*P/S ratio	.399	.376	.381	.392	.400	.41
Cholesterol (mg)	508	580	508	508	580	508

*Calculated as linoleic acid/total saturated fatty acid

NUTRIENT	MENU III			MENU IV		
	Soy	Dairy	Eggwhite	Soy	Dairy	Eggwhite
Energy (kcal)	2717.9	2894.3	2796.9	2757	2768	2783
Protein (total)	100.5	105.8	101.6	103.7	105.9	102.4
Protein (treat.)	75.0	75.8	75.6	79.0	71.1	75.6
Protein (non-treatment)	25.5	30	26	24.7	34.8	26.8
Carbohydrate (grams)	349.1	350.1	353.8	366.9	325.7	353.7
Total Fat (grams)	109.7	113.1	113.2	108.9	109.6	108.9
Saturated Fatty acids (grams)	56.4	50.5	57.8	43.4	52.3	38.2
Polyunsaturated fatty acids (grams)	23.3	20.2	21.3	17.3	22.4	15.3
*P/S ratio	.413	.400	.37	.399	.43	.401
Cholesterol (mg)	508	580	508	508	580	508

*Calculated as linoleic acid/total saturated fatty acid

Appendix P

SERVING SIZE OF FOODS (IN GRAMS) ADDED TO BOTH DIETS TO ADJUST FOR BODY WEIGHT

FOOD ITEM	SERVING SIZE	
	276 KCAL	552 KCAL
Roman meal bread	85.2	170.4
Parkay margarine	9.2	18.4

APPENDIX Q

Average Daily Nutrients Consumed per Subject With Foods Added to Adjust for Body Weight Loss

276 kcal adjustment

NUTRIENT	SOY	NON-FAT DAIRY	EGG WHITE
Energy (kcal)	3002.3	3093	3093
Protein (gm total)	111.9	111.4	111.3
Protein (gm treatment)	76.6	74.5	75.6
Protein (gm non-treatment)	35.4	37.1	35.7
Protein (% of Calories)	14.9	14.4	14.4
Carbohydrate (gm)	394.2	383.8	383.7
Carbohydrate (% of Calories)	52.5	49.6	49.6
Fat (gm)	119.3	118.9	120.8
Fat (% of Calories)	35.7	34.5	35.1
P/S ratio	.42	.42	.41
Cholesterol (mg)	504	580	504

552 kcal adjustment

NUTRIENT	SOY	NON-FAT DAIRY	EGG WHITE
Energy (kcal)	3278.3	3369.4	3369.9
Protein (gm total)	129.9	120.4	120.3
Protein (gm treatment)	76.6	74.5	75.6
Protein (gm non-treatment)	44.4	46.1	44.7
Protein (% of Calories)	15.8	14.3	14.2
Carbohydrate (gm)	432.7	418.8	418.8
Carbohydrate (% of Calories)	51.7	49.7	49.7
Fat (gm)	129.7	128.9	130.8
Fat (% of Calories)	35.6	34.5	34.9
P/S ratio	.44	.44	.43
Cholesterol (mg)	504	580	504

APPENDIX R

Average Daily Nutrients Consumed Per Subject

NUTRIENT	SOY GROUP	NON-FAT DAIRY GROUP	EGG WHITE GROUP
Energy (kcal)	2726.3	2817.4	2817
Protein (gm total)	102.9	102.4	102.3
Protein (gm treatment)	76.6	74.5	75.6
Protein (gm non-treatment)	26.4	28.1	26.7
Protein (% of Calories)	15.1	14.5	14.5
Carbohydrate (grams)	353.7	348.8	348.7
Carbohydrate (% of Calories)	51.8	49.5	49.5
Fat (grams)	108.9	108.1	110
Fat (% of Calories)	35.9	34.5	35.1
P/S ratio	.401	.40	.39
Cholesterol (mg)	504	580	504

APPENDIX S

Description of Reagents Used in Triglyceride Determinations[1]

(1) isopropyl alcohol - high purity isopropyl alcohol

(2) stock saponification reagent - an aqueous solution of potassium hydroxide (20% w/v).

(3) oxidizing Reagent - a solution of sodium meta-peridate (0.065% w/v) and ammonium acetate (7.7% w/v) in aqueous glacial acetic acid (6% v/v).

(4) color reagent - a solution of 2.4-pentanedione (0.75% v/v) in isopropyl alcohol.

(5) stock triglyceride standard - a solution of triolein (1% w/v) in isopropyl alcohol.

(6) extraction reagent - high purity n-heptane

(7) acid alcohol reagent - a solution of 0.08 N aqueous H_2SO_4 (24% v/v) in isopropyl alcohol.

[1]Stanbio Laboratory ESC Triglyceride Test Kit, Texas.

APPENDIX T

Proximate Analysis of Experimental Diets

	Soy	NFDM	Egg White
Day 1 (Menu A)			
% Ash[a]	2.9	2.5	2.4
% Moisture	63.7	63.8	70.0
% Fat[a]	7.5	7.2	7.5
Protein gm	121.4	117.8	115.8
Day 2 (Menu B)			
% Ash	2.4	2.2	2.3
% Moisture	68.8	68.8	68.0
% Fat	7.4	7.8	7.7
Protein gm	110.6	113.7	116.4
Day 3 (Menu C)			
% Ash	*	*	*
% Moisture	72.4	75.4	74.4
% Fat	6.5	6.2	5.5
Protein gm	91.9	99.6	117.6

*Data unavailable

Day 4 (Menu D)			
% Ash	*	*	*
% Moisture	73.8	75.0	73.6
% Fat	5.5	5.0	5.2
Protein gm	108.7	109.6	107.6

*Data unavailable

[a]Percent ash and fat on a wet sample basis.

APPENDIX U

Body Weights (kg): Individual Data

Group	Subject #	Screening	Week				
			Initial	Experimental			
			0	1	2	3	4
A	1		71.5	70.9	69.9	69.8	69.2
	2	74.0	74.7	74.2	73.4	73.2	72.7
	3	72.5	71.6	71.3	71.4	71.6	71.8
	4	64.7	63.0	62.9	62.4	62.4	62.1
	5	83.3	82.5	82.4	81.6	80.8	80.4
	6	81.2	80.6	80.7	80.3	79.5	78.8
	7	80.0	80.0	79.4	78.8	78.7	78.4
	8	59.8	59.8	59.8	59.4	59.2	58.9
	$\bar{X}$ ± SEM	73.6 ± 3	73.0 ± 3	72.7 ± 3	72.3 ± 3	71.9 ± 3	71.5 ± 3
B	9	61.0	60.6	61.0	60.7	60.7	60.6
	10	88.7	87.6	87.2	86.2	85.7	85.2
	11	69.2	68.8	68.7	68.1	67.7	67.4
	12	70.5	71.0	70.4	69.5	68.9	69.6
	13	77.8	78.6	78.4	77.9	77.6	77.2
	14	72.9	74.7	73.4	72.7	72.0	71.3
	15	67.8	66.7	66.6	66.8	66.9	66.8
	16	77.0	76.4	76.7	76.5	76.0	75.6
	$\bar{X}$ ± SEM	73.1 ± 3	73.1 ± 3	72.8 ± 3	72.3 ± 3	71.9 ± 3	71.7 ± 3
C	17	67.5	66.3	66.5	66.6	66.5	66.4
	18	56.0	55.8	56.2	56.5	56.5	56.5
	19	75.9	74.8	73.9	72.7	72.4	72.0
	20	63.7	64.1	63.7	63.7	63.7	63.6
	21	81.5	90.3	88.2	87.0	86.4	86.0
	22	88.2	80.2	79.9	79.2	78.7	78.1
	23	76.5	76.4	75.6	75.2	74.8	75.1
	24	70.5	70.1	69.9	69.9	69.7	69.8
	$\bar{X}$ ± SEM	72.4 ± 3	72.2 ± 3	71.7 ± 3	71.3 ± 3	71.1 ± 3	70.9 ± 3

APPENDIX V

Serum Triglyceride Concentrations: Individual Data

Group	Subject #	Week Initial 0	Experimental 1	2	3	4	Follow-up 6
A	1	78.4	72.4	44.3	50.4	45.3	69.6
	2	126.4	102.7	82.7	119.5	73.6	143.9
	3	109.3	107.8	75.6	123.2	65.1	85.7
	4	89.1	76.4	57.2	71.6	86.2	65.7
	5	99.7	66.2	58.5	85.4	59.7	123.1
	6	60.0	43.4	30.1	76.4	62.7	71.0
	7	121.6	163.1	147.9	155.6	173.2	159.7
	8	70.1	68.5	49.1	62.9	95.2	64.9
	$\bar{X}$ ± SEM	90.4 ± 10	86.2 ± 10	66.8 ± 10	91.9 ± 10	81.3 ± 10	96.6 ± 10
B	9	50.5	73.2	45.3	68.8	44.4	40.8
	10	95.8	109.3	80.8	87.4	76.3	91.8
	11	83.8	90.1	82.3	84.3	71.6	65.3
	12	58.0	018.6	45.6	98.4	103.9	169.9
	13	80.4	97.9	76.5	86.7	72.7	72.8
	14	128.4	101.9	85.9	85.0	70.9	101.9
	15	47.8	84.2	43.2	87.6	31.3	50.9
	16	63.6	64.2	63.2	69.6	46.7	70.3
	$\bar{X}$ ± SEM	76.1 ± 10	91.2 ± 10	65.4 ± 10	82.3 ± 10	64.8 ± 10	83.2 ± 10
C	17	62.2	99.9	44.8	54.2	76.3	67.8
	18	61.8	68.1	24.2	a	36.7	62.8
	19	96.6	67.3	20.8	64.5	25.8	92.6
	20	56.0	48.9	38.1	85.2	50.7	53.8
	21	87.4	79.1	93.2	71.6	52.9	54.5
	22	32.9	41.1	76.0	93.2	47.5	51.3
	23	78.5	88.2	111.6	112.0	70.9	109.8
	24	69.6	68.7	88.4	93.6	46.1	70.3
	$\bar{X}$ ± SEM	70.9 ± 10	70.4 ± 10	62.4 ± 10	80.6 ± 10	51.1 ± 10	70.6 ± 10

[a] Data unavailable

APPENDIX W

Plasma VLDL-Triglyceride Levels: Individual Data

Group	Subject #	Week Experimental					
		Initial 0	1	2	3	4	Follow-up 6
A	1	43.8	28.1	35.0	28.1	18.3	44.4
	2	83.5	36.7	56.4	55.2	40.1	121.2
	3	70.0	59.7	57.2	61.1	36.7	45.3
	4	39.7	26.5	37.7	28.1	24.1	25.6
	5	68.9	27.3	33.5	54.7	22.2	a
	6	27.9	a	24.0	21.7	17.3	33.5
	7	64.8	71.9	64.4	89.5	81.3	92.6
	8	38.4	14.0	31.6	18.3	16.5	28.0
	$\bar{X}$ ± SEM	55.0 ± 6	35.5 ± 6	42.9 ± 6	45.0 ± 6	32.8 ± 6	41.8 ± 6
B	9	9.9	14.0	43.8	31.9	11.0	6.5
	10	49.6	13.2	41.2	45.0	a	53.4
	11	57.7	19.9	40.4	29.7	23.6	32.9
	12	46.0	27.7	25.1	44.1	30.9	95.3
	13	61.5	28.8	45.7	37.4	20.2	30.9
	14	110.2	33.9	40.8	26.4	34.7	57.3
	15	38.4	23.0	40.4	36.1	13.9	14.3
	16	54.2	11.3	33.5	25.1	a	30.9
	$\bar{X}$ ± SEM	40.9 ± 6	21.4 ± 6	38.8 ± 6	34.4 ± 6	22.5 ± 7	40.1 ± 6
C	17	43.8	51.9	53.0	28.1	37.2	25.1
	18	44.2	14.0	33.1	44.2	79.0	25.1
	19	67.3	a	17.9	25.5	a	40.7
	20	35.3	18.2	59.8	54.3	28.9	15.3
	21	53.0	26.4	30.1	36.9	20.2	26.0
	22	37.2	a	24.0	46.7	23.6	27.0
	23	31.4	44.6	45.0	30.1	27.0	67.0
	24	43.8	27.3	43.1	46.3	17.8	43.6
	$\bar{X}$ ± SEM	49.9 ± 6	29.2 ± 7	38.5 ± 6	41.8 ± 6	23.8 ± 6	34.0 ± 6

[a]Data unavailable

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THE EFFECT OF FEEDING EITHER EGG WHITE, SOY AND NONFAT DAIRY PROTEIN IN MALE SUBJECTS ON PLASMA LEVELS OF TRIGLYCERIDES AND VERY LOW DENSITY LIPOPROTEINS UNDER CONTROLLED CONDITIONS

by

Mary Lou Price

(ABSTRACT)

Twenty-four male university students were fed vegetarian diets containing 100 grams of protein. Seventy-five grams of protein came either from soy, non-fat dairy products or egg white. Diets were adjusted so that differences in total caloric intake, protein, carbohydrate, fat and fatty acid composition were minimal between the dietary treatments. Plasma total triglyceride and very low density lipoprotein-triglycerides were measured at the beginning, weekly throughout the experimental period, and two weeks after completion of the study. No significant differences existed in serum lipid values between treatment diets nor was any interaction between diet and week observed. A significant week effect was observed indicating that subjects fed soy, non-fat dairy products or egg whites responded in the same fashion to the diet from week to week. This relationship was true for both variables: serum triglycerides and VLDL-triglycerides. Serum triglyceride concentrations for all treatment groups combined at baseline were 79 mg/100 ml, increasing to 82 mg/100 ml at week 1 and decreasing to 64 mg/100 ml at week two. An increase of 84 mg/100 ml was noted

at week three. Decreases were observed at week four, with serum concentrations of 65 mg/100 ml. From week four to follow-up serum triglyceride concentration rose to 83 mg/100 ml.

Similar trends were noted in serum VLDL-triglyceride levels when mean concentration were combined for all treatment groups. Serum VLDL-triglyceride concentrations at baseline were 48 mg/100 ml. At week one serum serum VLDL-triglyceride concentrations remained unchanged with values of 40 mg/100 ml in both instances. Decreases were observed at week 4 woth serum VLDL-triglyceride concentrations increased to 38 mg/100 ml. The results indicate that plasma triglycerides and VLDL-triglycerides are influenced by other dietary factors rather than by the protein source.