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Abstract

THE EFFECTS OF VARYING LEVELS OF DIETARY LINOLEIC ACID ON SERUM CHOLESTEROL AND HDL-CHOLESTEROL IN HEALTHY MALE SUBJECTS

by Donna (Miller) Davidge

Sixteen healthy male subjects were randomly divided into four equal groups and fed a nutritionally balanced 30 calories % fat diet, with approximately 2500 calories and less than 15 mg cholesterol/day, for 28 days. Within each group a constant level of linoleic acid was given for the duration of the study--either 2, 6, 14 or 18 calories % linoleic acid. Rate of changes in total cholesterol, HDL-cholesterol and the ratio of total to HDL-cholesterol were subjected to analysis of variance and regression analysis. The results showed changes in total cholesterol and ratio of total to HDL-cholesterol to be significant. The more unsaturated fat intakes (14% and 18%) decreased total cholesterol over time while the more saturated fat intakes (2% and 6%) increased total cholesterol. No definite trends in HDL-cholesterol were observed, though all groups increased slightly. Ratio of total to HDL-cholesterol decreased in all groups except the group with the lowest linoleic acid intake, which had an increase in ratio. At 30 calories % fat intake the results of this study suggest that optimum

linoleic acid intake lies between 6 and 14%.

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THE EFFECT OF VARYING LEVELS OF DIETARY LINOLEIC ACID ON SERUM CHOLESTEROL AND HDL-CHOLESTEROL IN HEALTHY MALE SUBJECTS

by

Donna (Miller) Davidge

A Thesis in Partial Fulfillment
of the Requirements for the Degree Master of Science
in Nutrition

December 1979

Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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LITERATURE REVIEW

Introduction

Cardiovascular disease, particularly atherosclerosis, is the leading cause of mortality in the United States. Many experimental and epidemiological studies have shown an association between serum lipids and atherosclerosis. High cholesterol levels in the blood caused by high intakes of dietary fat have been related to atherosclerosis as well as to stroke and other cardiovascular diseases (Moss and Mayer, 1977; Anderson, Grande and Keys, 1973 and 1976).

A major stimulus to the use of epidemiological tools to study cardiovascular disease arose from the observance of a significant reduction in incidence of cardiovascular disease in a number of countries as a result of serious food restrictions during World War II (Moss and Mayer, 1977).

In the United States the Framingham Heart Study has devoted the past 28 years to detection of factors which increase the risk of coronary heart disease. The highly significant risk factors identified were elevated blood pressure, elevated blood cholesterol and cigarette smoking. Other factors such as lack of exercise, obesity and electrocardiogram abnormalities were also considered (Gordon et al, 1977; Castelli et al, 1977). To date, the underlying cause of coronary heart disease has not been identified though

much time and money has been spent on investigation (Moss and Mayer, 1977).

The inverse relationship between high-density lipoprotein (HDL) cholesterol levels and incidence of heart disease was observed as early as 1951 by Barr (Barr, 1951). Further study has confirmed that HDL cholesterol levels are of value as a negative risk factor in coronary heart disease (Albers, Cheung and Hazzard, 1978; Barboriak et al, 1979). The Framingham Study shows that individuals with HDL-cholesterol levels less than 35 mg/dl have eight times the incidence of heart disease over those with HDL levels of 65 mg/dl or above. At Framingham the average HDL level was 45 mg/dl, with 35 mg/dl as an established level of high risk (Gordon et al, 1977). Castelli recommends determining the ratio of total cholesterol to HDL-cholesterol for use as an indicator of heart disease risk (Marx, 1979).

Protection from heart disease associated with elevated HDL levels is an attractive theory, yet not totally confirmed. Walker and Walker (1978) cite that Norwegian skiers and American runners have HDL levels as high as African boys. Though the skiers and runners are at less risk of heart disease than most Westerners, they are still prone to heart disease compared to the Africans. The authors also point out that Norwegian, Finnish and Lapp men had similar HDL values, yet the Lapps have a lower incidence of heart disease

(Walker and Walker, 1978).

With the use of angiography, Kummerow observed that a comparison of HDL and total cholesterol levels are as variable in human subjects with proven coronary blockage as normal subjects of similar age, sex, blood pressure and total serum cholesterol levels. He suggests that coronary blockage is related to more than HDL and LDL serum cholesterol levels would indicate (Kummerow, 1979).

Technical error that may be associated with HDL determination in the laboratory is another consideration. Schonfeld states "studies of lipoprotein structure . . . suffer from the . . . methods of separation. Nevertheless, the techniques appear adequate for comparing the same person to himself (Schonfeld et al, 1976)." The error may result in a variance of 5 mg/dl or more which causes difficulty in assigning HDL as a risk factor (Wood et al, 1976).

Cholesterol Metabolism

Background on Cholesterol

Cholesterol is a lipid precursor for steroid hormones and a vital substance for cell integrity. Since it is insoluble in aqueous solutions, cholesterol requires special mechanisms of transport within the body. Lipoproteins transport plasma cholesterol, binding proteins are used for intracellular transfers and micellar solutions are used for biliary excretion and intestinal absorption. Precipitation

of cholesterol may occur in the arterial wall as an initiating factor for atherosclerosis (Grundy, 1978).

Absorption of Cholesterol

Cholesterol enters the intestinal tract from the diet and bile. A minor amount may come from secretion by intestinal mucosa. Dietary intake in America averages 500-750 mg cholesterol/day. Biliary output ranges from 750-1250 mg/day. Mucosal secretions have not been measured (Grundy, 1978; Vergroesen, 1975).

Before absorption occurs, cholesterol must be solubilized by the formation of micelles, which are particles consisting of bile acids and products of fat digestion. Reports suggest that maximum absorption of cholesterol is about 60% (varies from 30 to 60%) and occurs in the upper intestine. Cholesterol cannot be 100% absorbed because it is never completely dissolved (Grundy, 1978).

Micelles, in addition to solubilizing cholesterol, facilitate transport of cholesterol across the aqueous layer adjacent to the surface of the intestine's lumenal, or mucosal, cell. The micelle itself does not pass through the membrane but delivers the cholesterol to the membrane, where cholesterol passes through by monomolecular passive diffusion (Grundy, 1978).

Once in the membrane, the cholesterol moves into the cytoplasm of the mucosal cell and migrates to the endoplasmic

reticulum, where the chylomicron is synthesized. The chylomicron contains cholesterol esters and triglycerides in the core, and protein, phospholipid and free cholesterol in the outer coat (Grundy, 1978; Masoro, 1977; Vergroesen, 1975).

Once the chylomicron is formed it is secreted into the lymph and enters the body circulation via the thoracic duct (Masoro, 1968). As the chylomicron circulates with the blood it enters the capillary beds, where the triglyceride portion is hydrolyzed. The enzyme lipoprotein lipase, located on the surface of the endothelial cells, is responsible for this hydrolysis (Masoro, 1977; Grundy, 1978). Apoprotein CII, which is 75% of the apoprotein in the chylomicron, is required to activate lipoprotein lipase (Smith, Pownall and Gotto, 1978).

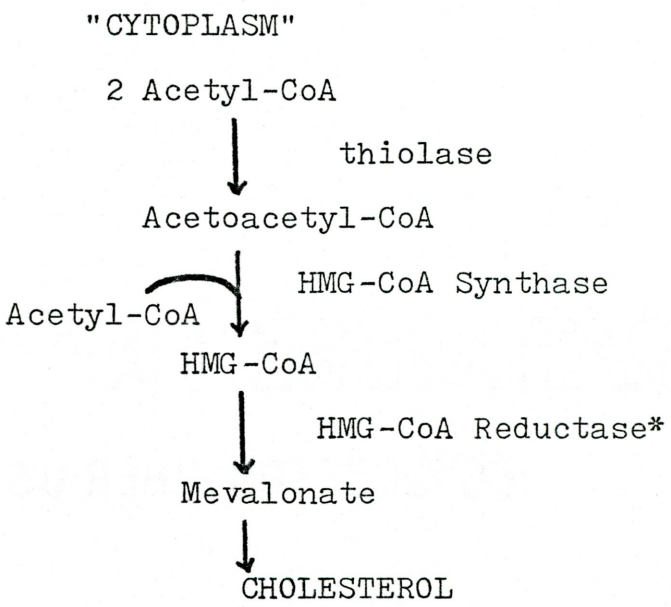
Hepatic Role in Cholesterol Metabolism

The clearance of the chylomicron caused by activation of lipoprotein lipase results in a remnant, which presumably contains all the cholesterol, and which is taken up by the liver. Cholesterol in the liver can come from (1) chylomicrons, (2) peripheral tissues via lipoproteins, or (3) synthesis within the liver cell. The liver is most likely the major source of newly-synthesized cholesterol. Total body synthesis of cholesterol is in the range of 9-13 mg/kg body weight/day. For a normal 70 kg man, this synthesis totals from 650-900 mg/day. It appears that there is a feed-

back inhibition of cholesterol synthesis in the liver which depends largely on cholesterol absorbed by the intestine and transported to the liver (Grundy, 1978).

Table 1

SYNTHESIS OF CHOLESTEROL



*Rate-limiting step

Hepatic overproduction of cholesterol is associated with high calorie intakes while restriction of calories reduces cholesterol synthesis. Whether saturation of dietary fat, independent of calories, influences cholesterol synthesis is unresolved (Moss and Mayer, 1977).

The fate of hepatic cholesterol can be (1) conversion to

bile acid, (2) directly into bile as cholesterol, (3) secreted into plasma with lipoproteins. Normally 200-300 mg/day (1/3 of daily production) is converted into bile acids (Grundy, 1978; Vergroesen, 1975).

Plasma Transport of Cholesterol via Lipoproteins

Although the primary lipoprotein of concern in this study is the high-density lipoprotein, the functions of all blood lipid carriers are important in understanding HDL.

Lipoprotein particles are classified usually by ultracentrifugal flotation or electrophoretic mobility. The lipoproteins include: (1) chylomicrons-carriers of exogenous dietary triglycerides, largest and lightest of the lipoproteins; (2) very-low-density (VLDL) or pre-beta-lipoproteins--major carrier of endogenously produced triglycerides; (3) low-density-lipoproteins (LDL) or beta-lipoproteins--products of VLDL catabolism, normally account for the bulk of plasma cholesterol; (4) high-density-lipoproteins (HDL) or alpha-lipoproteins--involved in cholesterol ester metabolism (Blum and Levy, 1975).

The lipoprotein moieties, apoproteins, are a heterogenous group of protein particles that differ from each other biologically, immunologically and physicochemically. Some have structural roles, others are functional in normal lipid metabolism as activators of enzyme activity (Blum and Levy, 1975). Table 2 describes the distribution of

Table 2

DISTRIBUTION OF APOPROTEINS IN LIPOPROTEINS

	Chylomicrons	VLDL	LDL	HDL
MAJOR	A-I	B	B	A-I
	B	C-I		A-II
	C	C-II		
		C-III		
		E		
MINOR	A-II	A-I	C	C-I
	E	A-II		C-II
	PRP	D		C-III
				D
				E

(Smith, Pownall, Gotto, 1978)

Table 3

CONSTITUENTS OF THE PLASMA LIPOPROTEINS IN PER CENT[1]

	HDL	LDL	VLDL	Chylomicron
Cholesterol	18	40-45	10-20	2-5
Protein	45-55	25	5-15	5-1
Triglyceride	2	5-10	50-70	85
Phospholipid	30	20-25	10-20	3-6

[1]Hernandez and Nicolosi, 1974.

apoproteins within the lipoproteins (Smith, Pownall and Gotto, 1978) and Table 3 describes the cholesterol, triglyceride, protein and phospholipid content of each type of lipoprotein (Hernandez and Nicolosi, 1974).

The major lipids of HDL are cholesterol, cholesterol esters and phospholipid, the principal phospholipid being lecithin (phosphatidyl-choline). Approximately 20% of HDL mass is cholesterol, 30% is phospholipid and 50% is protein (Schaefer, Eisenberg and Levy, 1978). Apoproteins AI and AII are the major peptides of HDL, constituting 90-98% of the total HDL protein (Blum and Levy, 1975; Schaefer, Eisenberg and Levy, 1978). The remaining few per cent are apoproteins C, D and E (Smith, Pownall and Gotto, 1978). Apoprotein AI is physiologically important as the activator for lecithin-cholesterol -acyltransferase (LCAT) the enzyme

responsible for most cholesterol esterification in man (Schaefer, Eisenberg and Levy, 1978).

HDL is divided into at least two density classes: HDL_2 which is 60% lipid and 40% protein, and HDL_3 which is 55% protein (Schaefer, Eisenberg and Levy, 1978). Some evidence shows that an increased level of HDL_2, which contains more free cholesterol than HDL_3, is associated with a decrease in heart disease (Sanders, 1979; Ononogbu, 1977). Since not enough is known about the different types of HDL, researchers like Robert W. Mahley, head of Comparative Atherosclerosis and Arterial Section of the National Heart, Lung and Blood Institute, feel it is premature to say that all HDL is beneficial (Sanders, 1979).

Evidence suggests that HDL or its precursors are produced by both intestine and liver (Smith, Pownall and Gotto, 1978). Recent studies confirm that the major apoprotein of HDL, apoprotein AI, is synthesized and secreted by human intestinal mucosa (Rachmilewitz and Fainaru, 1979). HDL precursors are both directly secreted into plasma and derived from surface components of chylomicrons and VLDL. Consequently, HDL circulates in both the plasma and the lymph. The LCAT reaction (Table 4) enables free cholesterol to be incorporated into the HDL particle as esters. Normal plasma contains spherical cholesterol-ester-containing HDL particles while LCAT deficient patients show discoidal HDL

Table 4

LCAT REACTION

Lecithin (phosphatidylcholine) + cholesterol $\xrightarrow{\text{LCAT}}$ Lysolecithin and cholesterol ester

particles, indicating that cholesterol-ester-containing HDL are not directly secreted. The spherical particles are probably derived from the discoidal apo-protein-lecithin-cholesterol precursors by the action of LCAT (Tall and Small, 1978).

Precursors for the discoidal HDL aggregates are assumed to come from (1) direct secretion of discoidal HDL from liver and intestine into plasma or lymph, (2) surface components of triglyceride-rich lipoproteins such as chylomicrons and VLDL. When chylomicron and VLDL are transformed to remnants, triglycerides, apoproteins A, apoprotein C, and phospholipids are all transferred to the plasma HDL (Tall and Small, 1978).

Although the exact metabolic link between HDL and atherosclerosis is not yet clearly understood, several mechanisms have been suggested:

(1) The LCAT reaction, gradually transforming free cholesterol (from remnant chylomicrons and VLDL) into cholesterol esters, forms the spherical HDL particle, which may serve as a route of transfer for cholesterol

to the liver for clearage (Tall and Small, 1978).

(2) HDL may mobilize cholesterol from the arterial wall. The extent of accumulation of cholesterol in atherosclerotic lesions is determined by balance between influx and removal of cholesterol. The regulation of neither process is well understood but this theory suggests HDL is a receptor site for cholesterol released from various cells, including atherosclerotic plaques (Grundy, 1978).

(3) Stein (Jerusalem) and Carew (San Diego, California) have shown that HDL can bind to cells and keep the LDL from binding to their receptor sites on the cell surface, a necessary step for accumulation of LDL cholesterol. Carew reports that cellular uptake of cholesterol is not nearly as much from HDL as it is from LDL (Marx, 1979).

It is important to remember that these theories have developed from <u>in vitro</u> studies and as yet cannot be assumed true for humans. The various HDL particles and their interactions with cholesterol and other lipoproteins require further investigation before definite conclusions can be made.

Non-Dietary Factors Affecting HDL-Cholesterol

Age, sex, genetics, obesity, diabetes, cigarette smoking, exercise and alcohol consumption have all been identified as factors which affect the level of serum HDL-cholesterol. An investigation of the metabolism of HDL and its transport of cholesterol as influenced by these factors is important to the final understanding of the relationship of HDL-cholesterol levels to the development of vascular disease.

Children have a higher percentage of cholesterol in the HDL fraction as compared to adults (Babar et al, 1976, and Srinvasan et al, 1978). Differences between the sexes are not as evident in childhood. For both sexes HDL-cholesterol rises from birth until puberty. Boys have higher HDL than girls up until age fourteen. At puberty, there is an especially large decrease in HDL for males, which is relatively stable for the rest of life. Females have correspondingly higher HDL levels from 14 to 18 years of age, with a progressive rise in HDL throughout life that peaks in the fifth decade (Tall and Small, 1978; Berensen et al, 1979; Ellefson et al, 1978).

Average values for the HDL-cholesterol in children from the Bogalusa Heart Study (on 5000 black and white children) are:

1 at birth, 36 ∓ 14 mg/dl.

2 at 6 months, 51 ∓ 22 mg/dl.

3 at 1 year, 53 ∓ 18 mg/dl.

4 at 2 1/2 to 5 1/2 years, 60 ∓ 19 mg/dl.

5 at 5 to 14 years, 68 ∓ 22 mg/dl.

(Berensen et al, 1978)

Race and genetics play a role in determining age-related trends in HDL. Black children have consistently higher total and HDL-cholesterol than white children at Bogalusa. There is also a relationship between HDL values in siblings (Berensen et al, 1979). The following results show that race and sex can affect HDL-cholesterol values within a given population.

I. AFRICANS FREE OF HEART DISEASE
(Walker and Walker, 1978)

Birth: 36 mg/dl.
10-12 years: 66 mg/dl.
16-18 years: 61 mg/dl.
60-69 years: 65 mg/dl.

II. TARAHUMARA INDIANS OF MEXICO
(Connor et al, 1978)

Boys 5-18 years: 23 ∓ 8 mg/dl.
Girls 5-18 years: 30 ∓ 11 mg/dl.
Men 19-70 years: 26 ∓ 7 mg/dl.
Women 19-70 years: 28 ∓ 14 mg/dl.

III. MEAN HDL-CHOLESTEROL BY AGE, RACE, AND SEX IN EVANS COUNTY (Castelli et al, 1977)

	Age			
	40-49	50-59	60-69	70+
Men				
White	52.3	48.3	48.1	50.9
Black	61.8	59	56.3	51
Women				
White	61.3	64.3	57.7	53.9
Black	66.4	53.3	66.4	67.5

The higher HDL-cholesterol levels in women appears to be hormonally related (Witztum and Schonfeld, 1979). Women taking estrogen treatment have increased levels of HDL-cholesterol (Marx, 1979; Tall and Small, 1978). The effect of oral contraceptive pills vary with the constituents of the pill (Roosner, 1978; Nutrition Abstracts and Reviews, January 1978). Pills containing progestin decrease serum HDL, which may be a factor in the increased risk of heart disease associated with oral contraceptive pill use (Marx, 1979).

Obesity decreases HDL (Berensen et al, 1979; Sanders, 1979). The Bogalusa Heart Study noted a significant inverse relationship between HDL-cholesterol and body weight (Berensen et al, 1979). Lees of the Massachusetts Institute of Technology has shown that HDL levels can be increased by

losing weight (Sanders, 1979). Some studies showing increased levels of HDL with weight loss also include interventions like exercise and cessation of smoking, which also increase HDL (Hulley et al, 1979). Witztum and Schonfeld (1979) point out the importance of comparing effects of chronic and acute weight loss on HDL.

Diabetes, a disease associated with increased risk of heart disease, has been shown to alter lipoprotein levels. Insulin-dependent diabetic children have higher total cholesterol and lower HDL-cholesterol (mean for HDL in mg/dl = 290, n = 40) than normal controls (mean for HDL in mg/dl = 368, n = 48) (Chase and Glasgow, 1976).

Calvert et al determined that the type of treatment for diabetes affects HDL levels. Insulin-controlled and diet-controlled diabetics had higher HDL-cholesterol levels than diabetics using sulphonylureas (oral medication) (Calvert et al, 1978). This is of concern because diabetics using sulphonylureas have a higher incidence of heart disease.

Whether a well-controlled diabetic will have higher levels of HDL-cholesterol has not been confirmed. High blood glucose is used to indicate uncontrolled diabetes. Elkeles, Wu & Hambley (1978) found no relationship between HDL and hemoglobin A, an index of blood glucose, in 40 insulin-dependent diabetics. Conversely, Calvert et al (1978) and Lopes, Stone & Colwell (1977) determined that HDL levels fell as blood glucose rose.

Cigarette smoking decreases HDL-cholesterol levels. However, upon cessation of smoking HDL levels return to normal (Garrison et al, 1978; Hulley et al, 1979).

Trained athletes, such as cross-country skiers (Enger et al, 1977) and long-distance runners (Wood et al, 1974 and 1976), have increased levels of serum HDL-cholesterol. Wood showed that runners had average HDL-cholesterol levels of 64 mg/dl versus 43 mg/dl in non-runners (Wood et al, 1974). A recent experiment used treadmill tests in a lab and found a small but significant increase in HDL-cholesterol levels with aerobic exercise (Schwane and Cundiff, 1979). Thresholds and explanations for the possible protective effect of exercise upon heart disease are not yet determined (Mann, 1977; Schwane and Cundiff, 1979).

Alcohol consumption apparently increases serum HDL-cholesterol levels (Garrison et al, 1978; Belfrage et al, 1977; Castelli et al, 1977; Barboriak et al, 1979; Sanders, 1979; Witztum and Schonfeld, 1979), increases lipoprotein lipase activity (Belfrage et al, 1977) and decreases triglyceride turnover (Lieber, 1974). In certain liver diseases associated with high intakes of alcohol, total lipids may increase or HDL may decrease (Feher, 1976). The relationship between this and other changes in lipid metabolism that result from alcohol consumption merit investigation before alcohol is advocated as a protective treatment in heart

disease (Castelli et al, 1977).

Diet and Its Relationship to HDL-Cholesterol

The relation of diet to serum cholesterol has been of interest for the last three decades (Vergroesen, 1975). It is generally agreed that a high saturated fat intake will increase serum cholesterol levels while a high polyunsaturated fat intake causes cholesterol levels to decrease in the serum (Anderson, Grande and Keys, 1973 and 1976; Kummerow, 1979). Keys and Hegstead have each shown that saturated fats, per weight basis, have twice the effect on change in serum cholesterol than polyunsaturated fats (Jackson et al, 1978; Anderson, Grande and Keys, 1973). Though the diet-cholesterol theory is not supported by all (Mann, 1977; Kummerow, 1979), the scientific community feels strongly enough about the relation of diet to serum cholesterol to have made certain recommendations. The American Medical Association, American Heart Association, Food and Nutrition Board of the National Research Council and Senate Select Committee on Nutrition and Human Needs (Dietary Goals for the U.S., 1977) have all recommended a lowering of cholesterol, and saturated fats especially, in the American diet (Shepherd et al, 1978).

The need for investigation of the effect of diet on specific cholesterol carriers in the blood has been realized. Smith, Pownall and Gotto state that "the dynamics and

contribution of each process of catabolism /of lipoproteins/ are poorly defined under various nutritional and physiological states" (Smith, Pownall and Gotto, 1978). Tall and Small suggest that specific details of how diet affects apoprotein synthesis and intestinal HDL secretion are needed. They feel that modifications of diet or enterohepatic circulation may affect HDL production since the small intestine is a source of HDL components (Tall and Small, 1978). The need to evaluate the effect of low fat diets on lipid/protein ratios in lipoproteins is pointed out by Spritz and Mishkel (1969).

Researchers have found it difficult to determine definite dietary effects on HDL. Hulley et al (1977) found major deviations, up and down, in the Multiple Risk Factor Intervention Trial (MRFIT) study, a heart disease risk intervention program. Though he suggested that diet may be an important factor in affecting HDL, other facets of the program, such as weight loss, cessation of smoking and increased physical activity, may have contributed to the increased HDL as much as the high polyunsaturated fat intake did. In the Bogalusa Heart Study diet did not account for the majority of the variability in HDL levels; genetics and race were more significant effectors (Berensen et al, 1979).

The effects of polyunsaturated fats versus saturated fats on HDL-cholesterol has had conflicting results (Spritz

and Mishkel, 1969; Shepherd et al, 1978). Spritz and Mishkel (1969), using a 40% fat formula diet, found no effect on serum HDL levels with either polyunsaturated or saturated fat. Shepherd et al, who also studied the effect of a 40% fat diet on serum HDL (cholesterol intake of 400 mg/day), found the HDL-cholesterol to be significantly reduced by the high polyunsaturated diet, P/S* of 4.0. Since the per cent of cholesterol found in the HDL was unaffected by the dietary fat, he assumed the fall in HDL-cholesterol concentration was a result of an overall decrease in plasma HDL concentration (Shepherd et al, 1978).

Brunner et al (1979) studied 26 Yemenite Jews who traditionally ate a low-caloric, low-fat (26% fat, 512 mg cholesterol/day) diet. While living in their usual environment, the subjects were fed a high-calorie, high-fat diet (50% fat, 911 mg cholesterol/day) for seven months, resumed their normal diet for three months, then were again placed on the high-fat, high-calorie diet. Though body weight changed little, serum HDL-cholesterol was significantly affected, following proportionately the increase in total serum cholesterol that occurred on the high-fat diet (HDL 29, total cholesterol 150 to 58 and 203, respectively). The per cent cholesterol in HDL was unchanged (29%). The stability in per cent cholesterol relative to protein found in HDL was supported by Shepherd's findings (see previous paragraph).

---

*P/S = ratio of polyunsaturated/saturated fatty acids

Brunner suggests that HDL-cholesterol % must be quite stable in view of the stress of overeating present in his study (Brunner et al, 1979).

Like the Yemenite Jews, the Tarahumara Indian of Mexico consume a low-fat diet, 12% fat, 75% carbohydrate, and 13% protein. The average intake of saturated fat is 2% and of cholesterol is 71 gm/day, which is extremely low compared to the average North American. Connor et al studied 523 children and adult Tarahumara Indians, carrying out lipoprotein fractionation on 61 subjects. Total cholesterol was low at 134 mg/dl, as was the HDL-cholesterol. Despite a high level of physical activity and absence of heart disease in this population, HDL concentrations were not elevated in relation to total cholesterol, as might be expected (Connor et al, 1978).

A high carbohydrate diet decreases HDL-cholesterol levels (Schonfeld, 1976). Schaefer, Eisenberg and Levy (1978) reported a 32% decrease in HDL-cholesterol on an 80% carbohydrate diet, which was attributed to an increased rate of HDL catabolism. Schonfeld used an 80% carbohydrate formula diet (less than 1% fat) which resulted in a decrease in HDL-cholesterol and apoprotein AI levels. He pointed out that high carbohydrate diets also increase the rate of hepatic secretion of VLDL, but that information on apoproteins under these dietary conditions is scarce (Schonfeld et al,

1976).

Burslem (1978) did a study of vegetarian males whose normal diet was 32 calories % fat, P/S of 1.9 and a cholesterol intake of less than 10 mg/day. The control males ate a 42 calories % fat diet, P/S of .44 and had a cholesterol intake of 424 mg/day. Total and HDL-cholesterol were lower in the vegetarian group (Burslem et al, 1978). Despite the low HDL in vegetarians, this group had a lower ratio of total to HDL-cholesterol than the controls, which would indicate less risk of heart disease in vegetarians.

The effect of dietary polyunsaturated fat on the serum HDL-cholesterol levels remains confusing. The present investigation was undertaken to clarify the role of dietary polyunsaturated fat in serum HDL-cholesterol homeostasis. In an attempt to do this, subjects were fed diets containing 30 calories % fat with linoleic acid content varying; either 2, 6, 14 or 18 % of calories was derived from linoleic acid. The total serum cholesterol and serum HDL-cholesterol were measured at weekly intervals in the subjects that were fed the different levels of linoleic acid.

MATERIALS AND METHODS

Experimental Method

Male subjects were recruited from a group that responded to a posted announcement. A group meeting was held to discuss the purpose, basic methods and requirements of the study. In addition, each subject was subsequently interviewed individually to determine food habits and relevant information such as drug and vitamin intakes.

Sixteen male subjects who were in good health were selected. Ages ranged from 21 to 29, with one 36 year old. Subjects consented to eat a vegetarian diet for the 28 day period of the study. Though being on a vegetarian diet was not a requirement of the study, subjects were all Seventh-day Adventists, who as a group consume less meat than the average American, if meat is consumed at all.

Subjects were randomly divided into four groups, each group containing four subjects. All subjects received a nutritionally adequate diet and were requested not to take any vitamin supplements.

The computed diet contained approximately 30 calories % fat, as recommended by the United States Dietary Goals, with 58% carbohydrate and 12% protein. Total calories were approximately 2500, based on average age and size of the subjects. Sour dough bread and jelly were provided ad lib

for provision of extra calories for those who had higher caloric needs. This ad lib regimen provided little linoleic acid and avoided unwanted weight changes.

Each group received the same level of linoleic acid in their fat source for the entire four weeks. The levels of linoleic acid were 2, 6, 14 and 18 calories %. The table below shows how this was determined.

Table 5

AVERAGE COMPOSITION OF BASAL DIET AS IT OCCURRED IN 5 DAY MENU

Nutrient	% Total Calories
Protein	12
Carbohydrate	58
Fat	2.7

Table 6

ADDITION OF FAT TO THE BASAL DIET TO MAKE DIET CONTAIN 30 CALORIES % TOTAL FAT

Group	Safflower Oil	Corn Oil	Coconut	(Including Diet Fat Linoleic Acid* % Total Cals.
I	83.3 gm	-	-	18
II	-	83.3 gm	-	14
III	-	42 gm	41 gm	6
IV	-	-	83.3 gm	2

*Linoleic acid content includes contribution of basal diet.

Table 7
GRAMS AND PER CENT (% 2500 CALORIES) OF SATURATED, MONOUNSATURATED AND POLYUNSATURATED FATTY ACIDS IN FAT SOURCE*

Group	Saturated	Monounsaturated	Polyunsaturated
I	7 gm	12.5 gm	60 gm
	2%	4%	21%
II	8.3 gm	23 gm	44 gm
	3%	8%	16%
III	47 gm	14.5 gm	26.5 gm
	14%	4%	8%
IV	70 gm	.83 gm	0 gm
	25%	.3%	0%

*Calculated from USDA Home Economics Research Report No. 7, "Fatty Acids in Foods"; Information on Hydrogenated Coconut Oil from PVO International, Inc., Veg-Oil, Inc., Emoryville, CA 94608.

Meals were prepared in the Nutrition Research Kitchen at Loma Linda University. Weekend sack lunches were prepared for the subjects so they could eat them at home. No foods except those on the diet were to be eaten for the duration of the study. Food portions were weighed to the nearest five grams. Subjects were weighed weekly and food portions adjusted slightly if necessary. However, amount of fat administered was constant. Menu items[1] were very low in

[1]Menu included in Appendix, Exhibit 3.

linoleic acid and provided an average of 2.7 calories % total fat per day. Cholesterol content of the diet ranged from 8 to 15 mg/day. The specific amount and type of fat were added to each subject's meal prior to serving and consumption of each meal.

Method of Collection

Blood samples were drawn the morning of the first day of the study and once per week thereafter. The final drawing was taken the morning after the last day of the diet. Five total samples were taken throughout the study.

Blood Sample

Fasting blood samples were drawn into 10 ml vacutainer tubes and immediately centrifuged. The serum obtained was frozen for later analysis. When analyzed, the frozen samples were thawed overnight to allow for proper equilibration.

Reagents

A single vial reagent was used for both HDL and total cholesterol, as provided by Calbiochem, La Jolla, Ca.

A 4% phosphotungstic acid solution and a magnesium chloride solution were prepared, as instructed by Calbiochem methods, and used to precipitate the HDL-cholesterol.

Method of Extraction

Using the procedure described by Allain (1974) and tested by Calbiochem and Lopes-Virella (1977), the following

steps were taken to separate HDL-cholesterol from the other serum lipoproteins:

1. pipet 1 ml of serum into clean centrifuge tube.
2. pipet 70 microliters $MgCl_2$ into same tube.
3. pipet 100 microliters phosphotungstic acid into the tube.
4. mix with vortex mixer 10-15 seconds.
5. centrifuge the solution at room temperature for 40 minutes (bench centrifuge).
6. decant supernatant into clean, labeled test tube.

Method of Assay

Both HDL and total cholesterol used the same assay procedure. The only difference was the amount of sample used--20 microliters of serum for total and 50 microliters of supernatant for HDL-cholesterol. HDL-cholesterol was done at least in duplicate and total cholesterol at least in triplicate, as follows:

1. pipet 2 ml of the prepared reagent into a clean, dry cuvet with a 1 cm light path.
2. place the cuvet into Beckman spectrophotometer, temperature controlled cell compartment at 37°C.
3. equilibrate cuvet with reagent at least three minutes, until stable.
4. zero spectrophotometer before adding the sample to the cuvet.

5. add sample, mix quickly by gentle inversion with a square of parafilm over the mouth of the cuvet.
6. place cuvet back in the spectrophotometer, read absorbance (at 500 nm) ten minutes later.

Standard

To assure standardization of results a standard curve was run on a cholesterol solution (See Appendix for diagram of standard curve). A 100 mg standard was made by dissolving .1 gram cholesterol in 100 ml propranol and tested each day before starting the assay.

Changes in Suggested Procedure

HDL Precipitation

1. $MgCl_2$ increased from 25 to 70 microliters, facilitating more accurate measure with available equipment and did not alter results when tested.
2. Centrifugation time increased from 30 to 40 minutes since bench centrifuge operates at 1000 x g instead of the recommended 1500 x g in the directions.
3. Each sample served as its own blank for more accuracy. Though water was not used as the blank, reagents were periodically tested against water to be certain of no contamination.

Assay

1. Cuvets were not pre-incubated in water bath, as suggested. When tested, reagent equilibrated as well in temperature controlled cell of spectrophotometer as when pre-incubated in water bath.
2. As mentioned with HDL (above), each sample served as its own blank for both total and HDL-cholesterol.

RESULTS AND DISCUSSION

Sixteen normolipemic subjects were randomly divided into four equal groups and fed a thirty calories % fat diet for twenty-eight days. Each group received one of the following: Group 1, 18 calories % linoleic acid; Group 2, 14 calories % linoleic acid; Group 3, 6 calories % linoleic acid; Group 4, 2 calories % linoleic acid. Blood was drawn from each subject five times (weekly) throughout the study for determination of total cholesterol and HDL-cholesterol.

A plot of the mean for each group versus time was constructed, week 1 being the first day of the study and week 5 the final blood drawing on the day after the last evening meal. Overall the plot in Figure 1 shows a decrease in total cholesterol from weeks 1 to 5 in the groups receiving the higher levels of linoleic acid, Groups 1 (-16.5% change) and 2 (-18.4% change). An overall increase was seen in the groups eating the more saturated fat diet, Groups 3 (3.6%) and 4 (32.4%).

Slopes of cholesterol over time for each individual were used to calculate the mean slopes for each group. Table 8 shows that the change in mean slope from negative to positive in Groups 1 to 4 indicates that cholesterol increases with increases in dietary saturated fat. These were compared by one-way analysis of variance (ANOVA), which

Table 8

MEAN SLOPES OVER TIME AND STANDARD DEVIATION PER GROUP FOR ANALYSIS OF VARIANCE

Group	Mean Slopes Over Time	Standard Deviation
Total Cholesterol		
1	-2.9	3.18
2	-7.6	4.72
3	1.7	2.45
4	9.8	3.15
HDL Chol		
1	2.6	1.21
2	.35	1.67
3	2.0	1.07
4	1.4	1.77
Ratio Total/HDL Chol		
1	-.23	.15
2	-.28	.20
3	-.15	.15
4	.13	.18

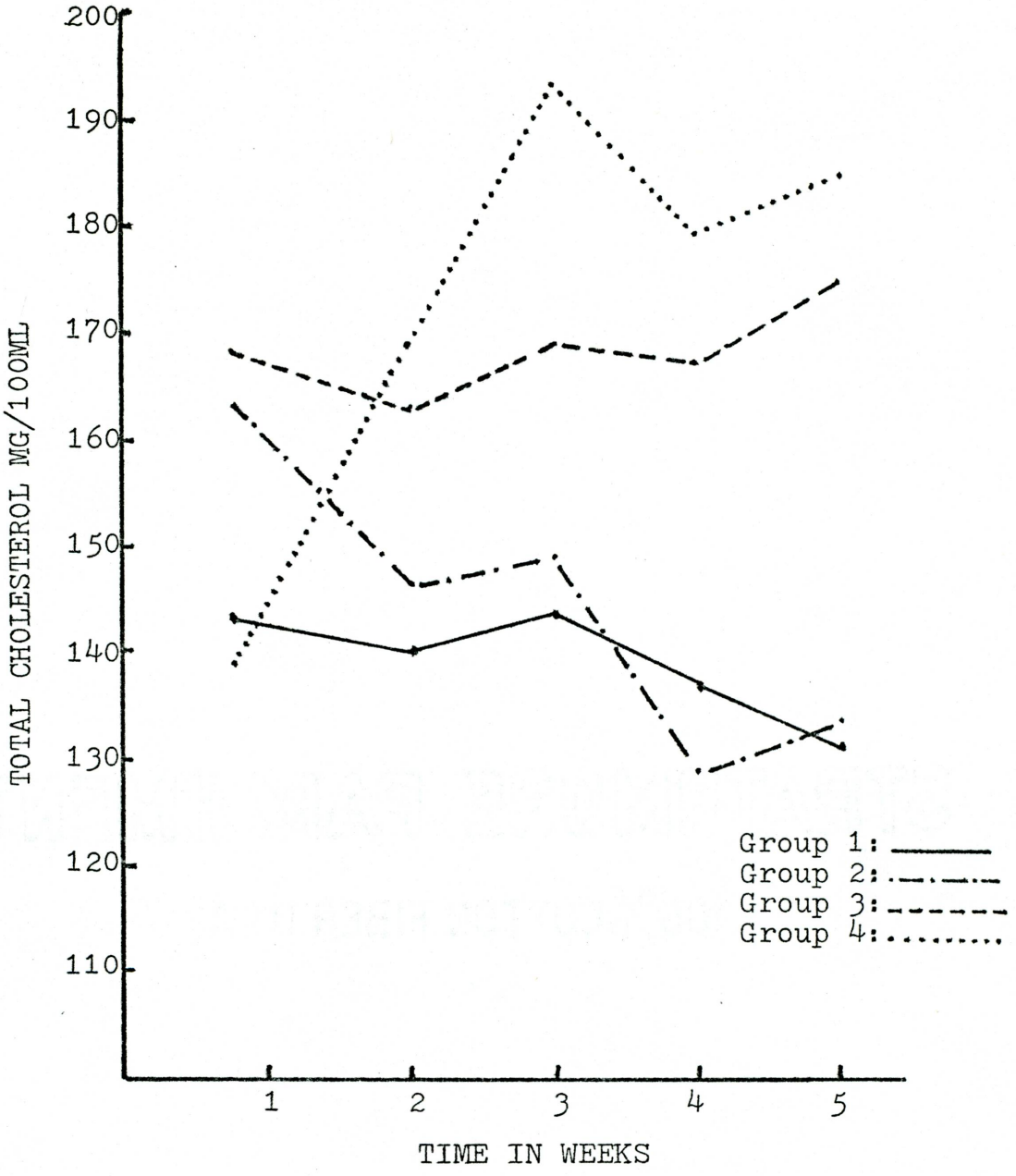


Figure 1. Total cholesterol per group over 28 days Calories % linoleic acid: Group 1 = 18%, Group 2 = 14%, Group 3 = 6%, Group 4 = 2%.

showed the difference in rate of change of total cholesterol to be significantly different among groups ($p = .0001$). Regression analysis showed that the observed trend in slopes for total cholesterol was significant ($p < .0005$).

The plot in Figure 2 shows mean HDL-cholesterol per group versus time, and though clear trends were not observed each group did increase slightly by the fifth week.

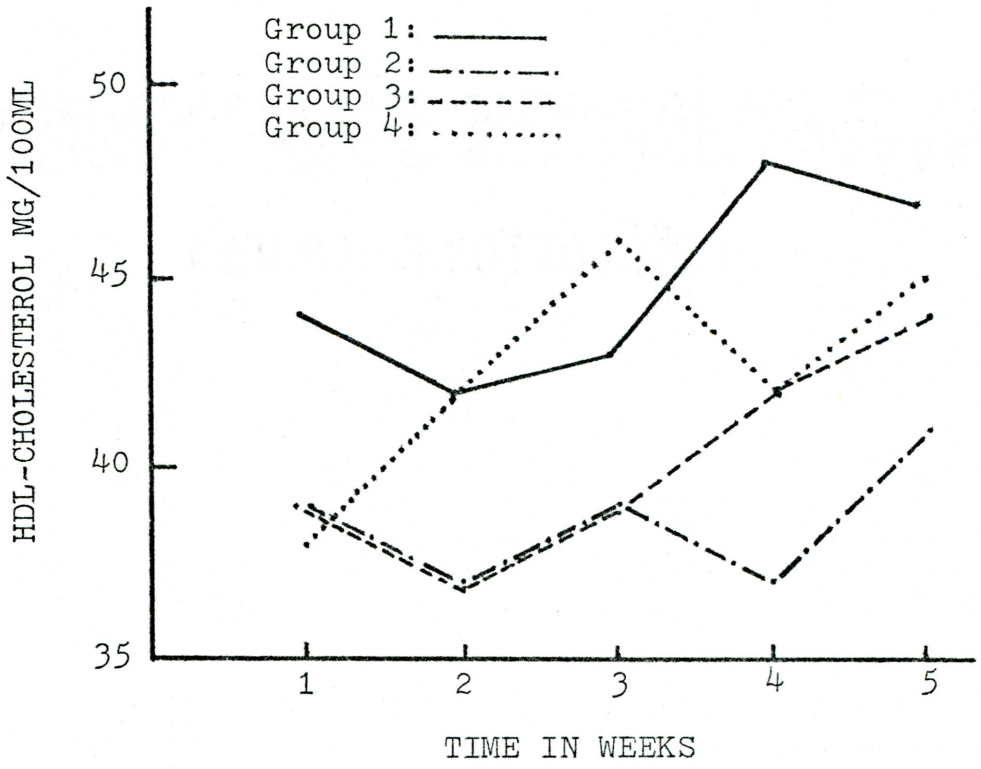


Figure 2. HDL-cholesterol per group over 28 days. Calories % linoleic acid: Group 1 = 18%, Group 2 = 14%, Group 3 = 6%, Group 4 = 2%.

ANOVA and regression analysis were carried out for HDL-cholesterol using the same procedure as with total cholesterol. Neither analysis showed HDL-cholesterol to be significantly affected by the time period of the diet.

A ratio of total cholesterol to HDL-cholesterol per subject was calculated. A downward trend is seen for the mean ratio in all groups except Group 4 (2% linoleic acid). As Figure 3 shows, Groups 1 and 2 changed the most, -17.8% and -26.5% respectively. Group 3 decreased 12%, indicating that a 6 calories % linoleic acid intake improves the ratio of total to HDL-cholesterol despite a slight increase in total cholesterol value (3.6%). Group 4, the most saturated fat diet, had an increased ratio of 15.9%.

The rate of change of the ratio of total cholesterol to HDL-cholesterol was analyzed by ANOVA, which showed the change in this dependent variable to be significant ($p = .03$). Regression analysis showed the observed trend in slopes to be significant at $p < .05$.

The data presented suggests that a level of 2% linoleic acid in a 30 calories % fat diet is not beneficial with respect to effect on total cholesterol and the ratio of total to HDL-cholesterol. An intake of 6 calories % linoleic acid increases total cholesterol slightly yet improves the ratio for total to HDL-cholesterol.

Total cholesterol was decreased in the groups receiving

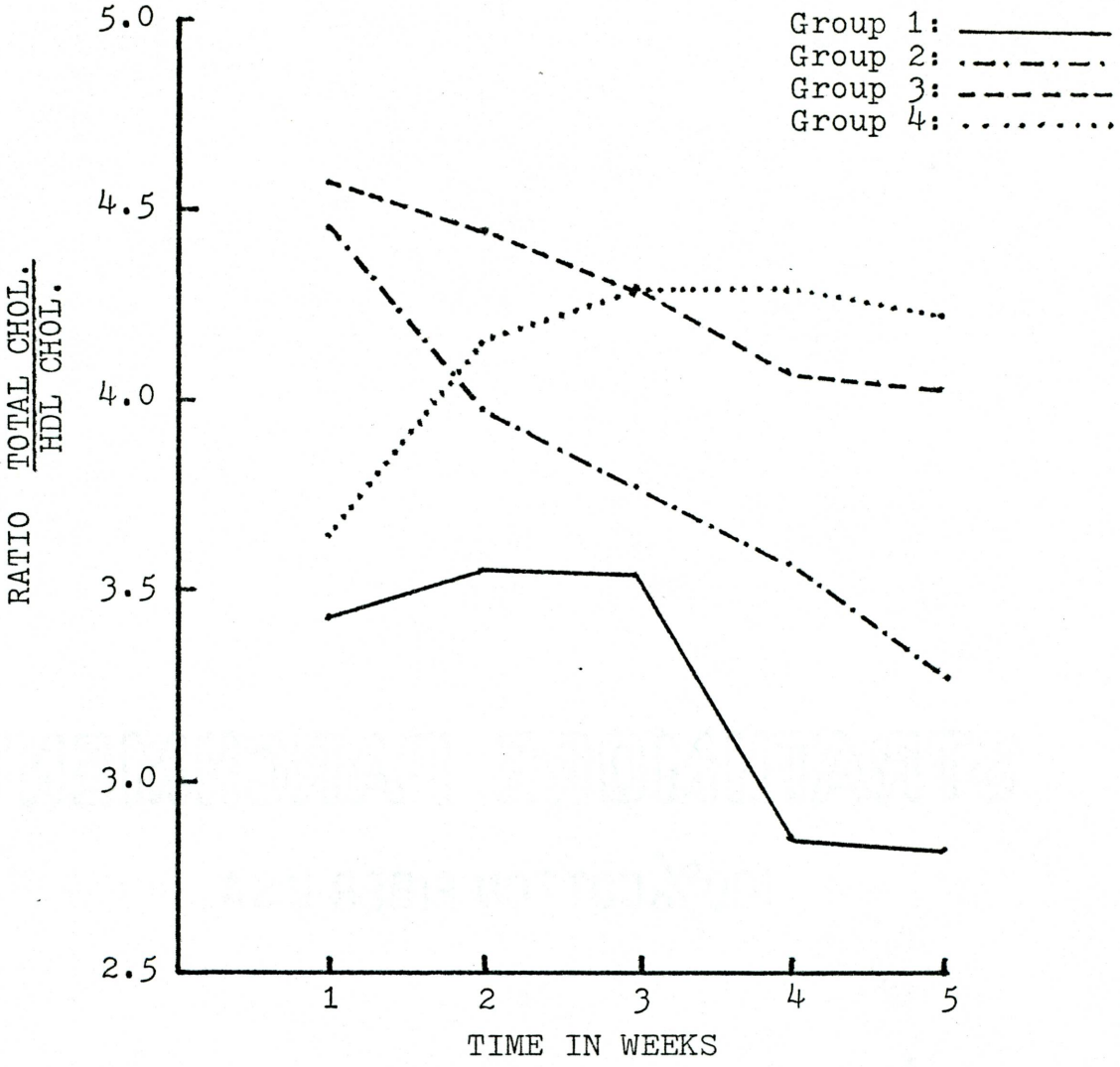


Figure 3. Ratio of total cholesterol to HDL-cholesterol per group over 28 days. Calories % linoleic acid: Group 1 = 18%, Group 2 = 14%, Group 3 = 6%, Group 4 = 2%.

14 and 18 calories % linoleic acid. The 18% group showed no enhanced lowering of cholesterol over the 14% group. However, the mean initial value for total cholesterol per group was different. Had they been equal, noticeable differences in trends among groups caused by the diet may have been easier to identify. It should be noted that the subjects in all groups had relatively low total and HDL-cholesterol initially. This is expected and agrees with Burslem's findings (1978) since the subjects' normal diets were vegetarian or at least lower in fat than the average American diet.

Our results suggest that alterations in saturation of fat may cause an increase in HDL levels. However, this does not agree with Shepherd, who found HDL-cholesterol to be lowered by polyunsaturated fat at a level of 40% fat. Spritz and Mishkel, who also studied a 40% fat diet found no alteration in HDL with either high saturated or polyunsaturated fat. The relationship of HDL to fat intake is further confused by Brunner's study, where HDL-cholesterol rose correspondingly with total cholesterol when fat was increased from 26 to 50% of the diet. Thus, HDL cholesterol levels may relate to fat transport requirements rather than specific dietary changes.

The results of the study suggest that an optimum intake of linoleic acid lies between 6 and 14% for the population from which the subjects were selected and based upon the

level and nature of fat in the diet. Studying the effects of 8, 10 and 12 calories % linoleic acid in the diet, using a larger sample size, would help determine the optimal intake of linoleic acid.

SUMMARY

Sixteen normolipemic males received a 30 calories % fat diet for 28 days. Subjects were randomly divided into four groups, each group receiving a constant level of linoleic acid for the duration of the study. Changes in total cholesterol and HDL-cholesterol were assessed in each subject each week and group mean values were used to analyze the data. Analysis of variance and regression analysis showed changes in total cholesterol and ratio of total to HDL-cholesterol to be significantly different among groups and over time. No significant trends were observed for HDL-cholesterol alone. The results of the study suggest that an optimal intake of linoleic acid in the diet lies between 6 and 14%. These conclusions are assumed to apply to the population from which the subjects came, a largely vegetarian group, and with the fats used in the study, all of which were of vegetable origin (including the saturated fat).

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APPENDIX

EXHIBIT 1

Subject's name:

Nutritional History

Bkf:

Lunch:

Supper:

Snacks:

Do weekends differ significantly from this pattern?

Vitamin supplements and/or drugs (eg: Vitamin E, Aspirin, alcohol, smoking, etc.)

Major food allergies or intolerances:

Height: _____ Weight: _____

Activity pattern--Any regular exercise program?

Subject signed consent form?

EXHIBIT 2

CONSENT FORM

I have been informed of the nature of my participation in this special diet* study at Loma Linda University Nutrition Department for 28 days, from _____ to _____. During the length of the study, all my food and meals will be provided by and eaten at the Nutrition Department. I will have approximately _____ mls of blood drawn once per week by a licensed venipuncturist, starting at the beginning of the study and for the duration of the study, for a total of five times. Blood will be analyzed for: 1) vitamin E, 2) linoleic acid (an essential fatty acid) content in plasma and red blood cell membranes, 3) LCAT (an enzyme), and 4) total cholesterol and HDL cholesterol, and 5) Malondialdehyde (prostaglandin production). The minimal risks in drawing blood have been explained to me.

The purpose and methods of the experiment have been explained for my satisfaction. It has been explained to me that this information will be informative to me and may be beneficial to mankind in evaluating the effect that types of fat have on metabolism. I have been informed that there is no financial reimbursement for my participation.

The information and data obtained in the study will be confidential and, if published, the names of the subjects will not be disclosed in the report. I understand that I am under no obligation to participate and I maintain the right to withdraw from the study at any time without prejudice to

my educational program.

Signed _____

Witness _____Date_____

*Diet will be nutritionally adequate (30% fat, 12% protein, 58% carbohydrate) consisting of natural food sources.

For further information, contact: Dr. James Blankenship, Principal Investigator, Phone 796-7311, ext. 3761.

EXHIBIT 3

5-DAY CYCLE MENU

Day 1

Breakfast:

Hot farina, 360 gm., topped with raisins, 36 gm.

Canned sliced peaches, 256 gm.

Sourdough English muffins (2), 119 gm.

Skim milk, 245 gm.

Lunch:

Rice casserole, 480 gm.

Mixed lettuce salad, 75 gm. iceberg and 50 gm. romaine (fat-free dressing)*

Sourdough French rolls

Fresh cantaloupe balls, 240 gm.

Apple juice, 248 gm.

Dinner:

Minestrone soup, 450 gm.

Sliced sourdough bread**

Frozen strawberries, 149 gm.

Skim milk, 245 gm.

Day 2

Breakfast:

Buckwheat pancakes, 198 gm. with syrup ad lib.

Applesauce, 255 gm., with cinnamon

Hot cocoa, 283 gm.

Lunch:

Spaghetti with sauce, 200 gm and 285 gm. respectively

Sliced tomato and lettuce salad, 35 gm. and 75 gm. respectively

Fresh peach, 175 gm.

Fruit punch, 249 gm.

Dinner:

Scrambled egg sandwich

Pita bread, 2 (126 gm.)

Egg white with chicken seasoning, 165 gm.

Lettuce, 75 gm.

Tomato, 135 gm.

Cucumber chunks, 28 gm.

Pineapple, carrot and raisin salad, 160 gm.

Skim milk, 245 gm.

Day 3

Breakfast:

Fruit toast:

2 sourdough English muffins, 119 gm.

Fruit sauce, 270 gm.

Banana slices, 75 gm.

Apricot nectar, 251 gm.

Skim milk, 245 gm.

Lunch:

Lentil soup, 335 gm.

Lettuce and tomato salad, 75 gm. and 35 gm. respectively

Sourdough French rolls

Grape juice, 253 gm.

Dinner:

Tostadas:

Corn tortillas, 2 (52 gm.)

Bean filling, 190 gm.

Lettuce, 75 gm.

Tomatoes, 135 gm.

Onions, 43 gm.

Canned sliced peaches, 256 gm. topped with chopped dates, 32 gm.

Skim milk, 245 gm.

Day 4

Breakfast:

Hot cornmeal mush, 480 gm. topped with chopped dates, 32 gm.

Toast with honey or jelly

Skim milk, 245 gm.

Watermelon, 160 gm.

Lunch:

Lasagna, 290 gm.

Vegetable platter:

Lettuce chunks, 78 gm.

Celery sticks, 50 gm.

Cucumber circles, 28 gm.

Fresh apple, 135 gm.

Lemonade, 245 gm.

Dinner:

Chinese stir-fry, 250 gm.

Rice, 205 gm.

Pineapple chunks, 255 gm.

Peach nectar, 245 gm.

Day 5

Breakfast:

French toast, 180 gm., with syrup ad lib

Applesauce, 255 gm., topped with raisins, 36 gm.

Hot cocoa, 283 gm.

Lunch:

Curry casserole, 360 gm.

Broccoli spears, 90 gm.

Lettuce and tomato salad, 75 gm. and 35 gm. respectively

Canned apricots, pitted, 160 gm.

Grape juice, 253 gm.

Dinner:

Potato soup, 345 gm.

Three-bean salad, 190 gm.

Bagels (no fat added) (2), 132 gm.

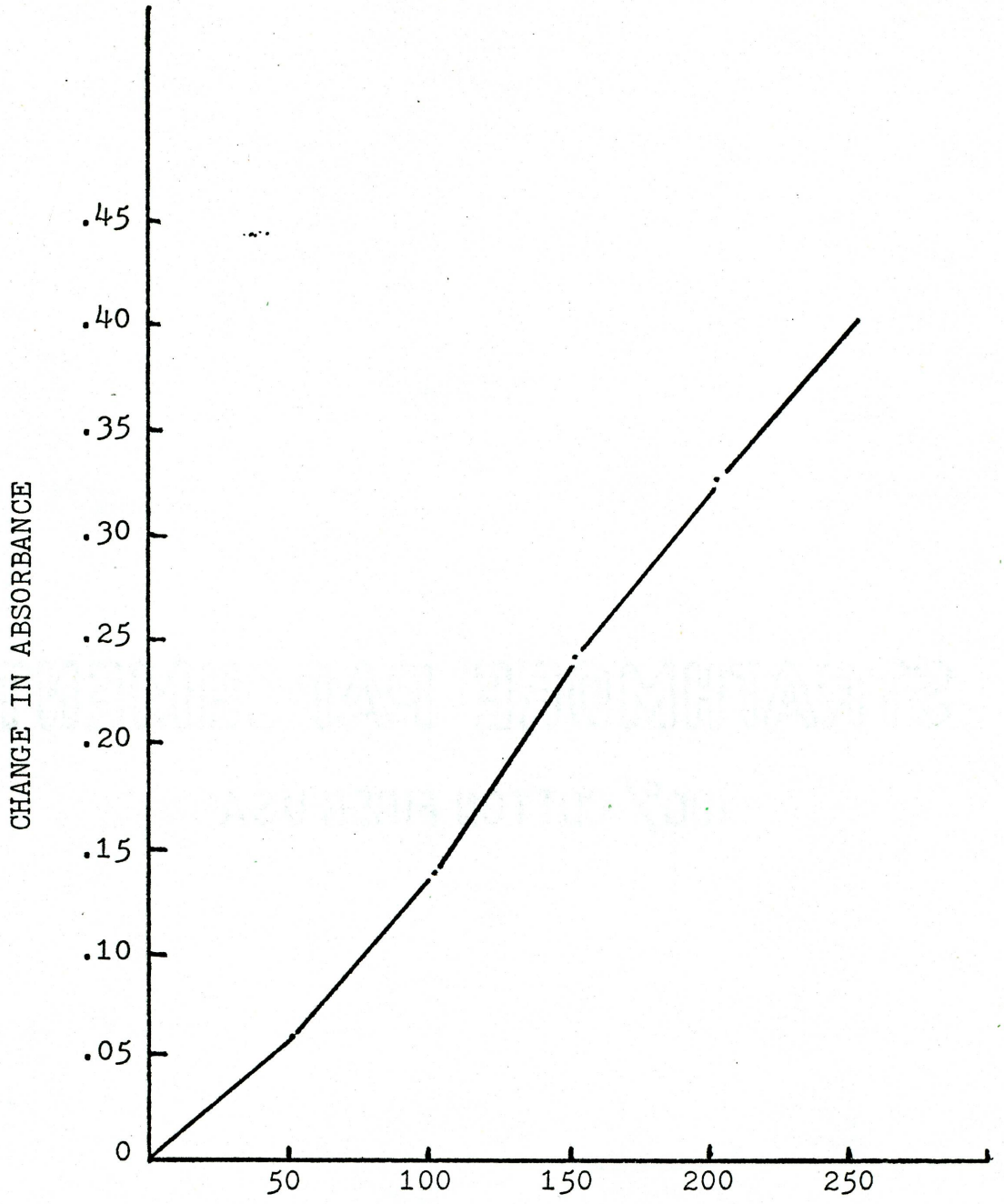
Orange juice, 287 gm.

*Fat-free dressing available with all salads.

**Sourdough bread available at all meals.

***As the study progressed, portion sizes were adjusted to maintain body weight; however, each subject received a constant intake level of fat.

STANDARD CURVE FOR TOTAL CHOLESTEROL[1]



[1]correlation = 0.998

NOTE:

Values are included in the following table for Group 1 both with and without subject #1's values. This subject was unique because his ordinary diet was 15 calories % total fat. The 30 calories % fat diet resulted in an increased total cholesterol for week 1-3, with a subsequent decrease weeks 4 and 5. The final value was still 9 mg. higher than the initial value, even considering the equilibration that appeared to occur after week 3. It is suggested that his value may have continued to decrease, but for the duration of the study his results were understandably atypical of Group 1.

EXHIBIT 4

MEAN CHOLESTEROL PER GROUP PER WEEK

Linoleic Acid Content	Group	Week 1	Week 2	Week 3	Week 4	Week 5	% Change
18%	I	143	140	143	137	129	-9.79
	I (minus subject #1)	133	120	122	117	111	-16.54
14%	II	163	146	149	129	133	-18.40
6%	III	168	163	169	167	174	+3.57
2%	IV	139	169	193	179	184	+32.37

EXHIBIT 5

MEAN HDL-CHOLESTEROL PER GROUP PER WEEK

Linoleic Acid Content	Group	Week 1	Week 2	Week 3	Week 4	Week 5	% Change
18%	I	44	42	43	48	47	6.81
	(minus subject #1)	36	37	37	42	42	13.88
14%	II	39	37	39	37	41	5.12
6%	III	39	37	39	42	44	12.82
2%	IV	38	42	46	42	45	18.42

EXHIBIT 6

MEAN RATIO TOTAL/HDL-CHOLESTEROL PER GROUP PER WEEK

Linoleic Acid Content	Group	Week 1	Week 2	Week 3	Week 4	Week 5	% Change
18%	I	3.43	2.54	3.53	2.86	2.82	-17.78
	I (minus subject #1)	3.69	3.43	3.44	2.78	2.81	-23.84
14%	II	4.46	3.99	3.79	3.58	3.28	-26.45
6%	III	4.58	4.44	4.30	4.09	4.03	-12.00
2%	IV	3.64	4.15	4.28	4.28	4.22	+15.93

EXHIBIT 7

"MEAN MG CHOLESTEROL/100ML, HDL-CHOLESTEROL MG/100ML AND RATIO $\frac{\text{Total}}{\text{HDL}}$ CHOLESTEROL PER SUBJECT PER GROUP"

Group	Subject	Week	MG Chol	MG HDL	Ratio
1	1	1	175	60	2.92
1	1	2	200	53	3.78
1	1	3	205	55	3.70
1	1	4	198	64	3.08
1	1	5	184	64	2.87
1	2	1	167	39	4.29
1	2	2	138	41	3.36
1	2	3	154	43	3.61
1	2	4	149	46	3.25
1	2	5	145	46	3.14
1	3	1	103	33	3.09
1	3	2	114	33	3.50
1	3	3	105	32	3.28
1	3	4	103	45	2.27
1	3	5	85	47	1.78
1	4	1	128	bl*	bl
1	4	2	107	bl	bl
1	4	3	107	bl	bl
1	4	4	100	35	2.84
1	4	5	104	30	3.51

*Due to inadequate equilibration time, the HDL-supernatant gave an abnormally low reading for these and the data is considered BLANK.

EXHIBIT 8

"MEAN MG CHOLESTEROL, HDL-CHOLESTEROL AND RATIO $\frac{\text{TOTAL}}{\text{HDL}}$ PER SUBJECT PER GROUP"

Group	Subject	Week	MG Chol	MG HDL	Ratio
2	1	1	172	32	5.37
2	1	2	143	36	3.94
2	1	3	139	37	3.79
2	1	4	127	33	3.82
2	1	5	122	43	2.85
2	2	1	200	32	6.23
2	2	2	185	33	5.69
2	2	3	199	43	4.66
2	2	4	188	36	5.21
2	2	5	187	38	4.88
2	3	1	126	48	2.63
2	3	2	107	42	2.56
2	3	3	117	40	2.94
2	3	4	91	39	2.31
2	3	5	109	41	2.66
2	4	1	155	43	3.60
2	4	2	148	39	3.79
2	4	3	140	37	3.78
2	4	4	112	38	2.97
2	4	5	115	42	2.75

EXHIBIT 9

"MEAN MG CHOLESTEROL, HDL-CHOLESTEROL AND RATIO $\frac{\text{TOTAL}}{\text{HDL}}$ PER SUBJECT PER GROUP"

Group	Subject	Week	MG Chol	MG HDL	Ratio
3	1	1	139	bl	bl
3	1	2	133	30	4.46
3	1	3	134	30	4.54
3	1	4	137	30	4.50
3	1	5	146	36	4.08
3	2	1	190	36	5.22
3	2	2	172	36	4.71
3	2	3	199	48	4.15
3	2	4	178	44	4.00
3	2	5	180	49	3.69
3	3	1	185	49	3.75
3	3	2	182	46	3.94
3	3	3	190	42	4.51
3	3	4	190	56	3.37
3	3	5	204	54	3.76
3	4	1	157	33	4.78
3	4	2	164	35	4.65
3	4	3	151	38	4.00
3	4	4	166	37	4.51
3	4	5	166	36	4.58

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EXHIBIT 10

"MEAN MG CHOLESTEROL, HDL-CHOLESTEROL AND RATIO $\frac{\text{TOTAL}}{\text{HDL}}$ PER SUBJECT PER GROUP"

Group	Subject	Week	MG Chol.	MG HDL	Ratio
4	1	1	134	36	3.67
4	1	2	170	53	3.21
4	1	3	184	56	3.30
4	1	4	158	48	3.30
4	1	5	171	40	4.25
4	2	1	133	39	3.42
4	2	2	146	40	3.68
4	2	3	181	42	4.35
4	2	4	161	44	3.64
4	2	5	170	57	2.98
4	3	1	142	41	3.42
4	3	2	197	40	4.92
4	3	3	212	42	5.10
4	3	4	206	40	5.18
4	3	5	191	47	4.03
4	4	1	149	37	4.05
4	4	2	163	34	4.80
4	4	3	194	44	4.39
4	4	4	190	38	4.99
4	4	5	204	36	5.64

*Due to inadequate equilibration time, the HDL-supernatant gave an abnormally low reading for these and the data is considered BLANK.