EFFECTS OF NATURAL AND PARTIALLY HYDROGENATED SAFFLOWER OIL ON LIPID AND PROTEIN CONSTITUENTS OF HUMAN SERUM

by

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STATEMENT BY AUTHOR

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INTRODUCTION

Lipid metabolism is one of the most active of research fields today and the subject of transport of lipids from one organ to another is a major phase being investigated. Interest in this area has been heightened by reports that elevated serum lipids seem to be related in some manner to the incidence of coronary heart disease and atherosclerosis, one of the primary degenerative diseases and a major cause of death in our population. Epidemiological studies of various population groups throughout the world have shown a close association between low incidence of atherosclerotic disease, low-fat diet and low serum lipid level (1). This has led to intensive research concerning important factors which effect changes in fat transport, thus altering the serum lipid pattern and its relation to nutritional and endocrine status. To date, studies have emphasized changes in the serum lipids of subjects with abnormal patterns and levels; more information is needed, however, on these factors in clinically healthy people.

It is now well accepted that all lipids present in serum combine with protein to produce a variety of lipid-protein complexes which exhibit a more or less constant composition. These complexes while containing a characteristic protein, carry varying proportions of one or more types of lipids. Three general methods are employed for isolating or quantitatively determining serum lipoproteins: Gofman's ultracentrifugation, Tiselius' electrophoresis, and Cohn's solubility

method. There are many variations of each general method, but they are principally variations in density of the medium as used in ultracentrifugation, in supporting media, pH, and dye in zone electrophoresis, and chemicals in the solubility method. It is difficult to compare the results of studies on lipoprotein levels involving these methods, since each separates the lipid-protein complexes differently and expresses results in units characteristic of the method. Table 1, prepared by Straus and Wurm correlates these methods on the basis of five groups related to density range.

The effect of diet on serum lipids levels may be evaluated by determining quantitatively the concentration of the individual serum lipids such as phospholipids, cholesterol, and triglycerides, and the concentration of the different lipoprotein fractions. To date, because of the relative ease of analysis, most investigations have utilized only total serum cholesterol as an index of the serum lipid concentrations. However, it is necessary to know the effect not only on serum lipid but lipoprotein fractions as well, since unusual elevations of specific lipids, especially of the triglycerides, as reflected in changes in lipoprotein fractions, may occur as a result of dietary changes despite normal serum cholesterol levels (2, 3).

The effects of a number of dietary nutrients on human lipid levels has been investigated. Elevated serum cholesterol concentration is often associated with the degenerative diseases and cholesterol is a major constituent in arterial deposits. Therefore, the influence of dietary cholesterol, the importance of which is still controversial, was among the first to be studied. Since the endogenous production of

Table 1 Interrelation of Various Lipoprotein Designations[1]

Density	a S_f	b S_f	Electrophoresis a	b	c	d	e	f Ethanol Fractions
1.006	20	70	Chyl.	Chyl.	a_2	a_2	N.F.	I + III
1.006-1.019	12-20	40-70	Chyl.		a_2	B_1	a_2	I + III
1.019-1.063	0-12	25-40	B_1	B_1	B	B_1	B	I + III
1.063-1.125	HDL_2	20-25	a_2	a_2	a_1	a_1	a_1	IV + V
1.125-1.210	HDL_3	1-10	a_1	a_1	a_1	a_1	L.A.	IV + V

a = Density 1.063 after De Lalla and Gofman *et al.* (1954), Lindgren, Elliott and Gofman (1951).

b = Density 1.21 after Lewis and Page (1953).

c = Starch electrophoresis (Kunkel and Trautman, 1956).

d = Eder (1957)

e = Straus and Wurm (1958).

f = Oncley (1956).

[1]Straus, M., and Wurm, M.: Lipoproteins in serum. A. Characterization and methods of analysis; in Lipids and the Steroid Hormones in Clinical Medicine (ed. F.W. Sunderman and F.W. Sunderman, Jr.). Lipinncott, Philadelphia - Montreal, 1960, pp. 47-57.

cholesterol by the liver in the homeostatic regulation of biosynthesis of cholesterol is greater than the amount normally consumed, it has been concluded by most investigators that cholesterol *per se*, as present in the "average" American diet, is of little importance. However, recent experiments by Beveridge *et al.* (4) have shown that, over the lower ranges of cholesterol ingestion, beginning at zero level, there is a positive correlation between intake and serum cholesterol concentration. This, he states, is modified by the type of triglyceride present in the diet. Recently, Connor *et al.* (5) and Steiner *et al.* (6) reported that cholesterol, added to the diet in the form of egg yolk powder or as a mixture with olive oil, elevated serum cholesterol significantly. They attributed the increase to better absorption from the gastrointestinal tract than when cholesterol was fed in crystalline form as was used in earlier experiments.

Serum lipid concentrations are affected by the quality of the fat in the diet. A decade ago Groen *et al.* (7) observed that vegetarians had considerably lower serum cholesterol values than non-vegetarians, and that this effect was independent of the level of total fat ingested. In the same year, Kinsell *et al.* (8) reported that the isocaloric substitution of vegetable fat for animal fat dramatically reduced the serum cholesterol and phospholipid concentrations. But the terms 'animal" and 'vegetable' do not distinguish between fats which raise and lower serum lipids; coconut oil and butter both raise serum cholesterol. The hypercholesterolemic effect of butter has been attributed to the chain length of the saturated fatty acids, C_6 to C_{14}.

The development of new analytical methods for easily separating and identifying the fatty acids in lipids, namely, gas-liquid chromatography, makes it possible to determine easily the kind and amounts of each fatty acid present in the experimental diet. Ahrens (9) and others have shown that unsaturated fats lower serum cholesterol and Bronte-Stewart et al. (10) and Gofman et al. (11) reported that such fats lower the concentration of the beta lipoproteins, corresponding to S_f 0-20. Non-essential and essential polyunsaturated fatty acids are both effective in this respect (12).

Keys (13) proposed the "formula" given below for describing quantitative effects of variation in kind and amount of dietary fat upon serum cholesterol in men who are normocholesterolemic (ca. 230 mg. per cent) and who consume a diet containing about 40 per cent fat calories, an amount considered to be characteristic of the diet of middle-aged adults in this country. His formula specified that change in serum cholesterol elicited by a change in dietary fat equals the change in saturated fatty acids, plus the change in monoethenoid fatty acids plus the change in polyethenoid fatty acids; all fatty acid intakes being expressed in terms of per cent of total calories. According to this concept, saturated fatty acids are twice as effective in raising serum cholesterol as equal amounts of polyunsaturated fatty acids in lowering them, oleic acid being neutral. However, Ahrens was of the opinion that the formula represented little more than an expression of the iodine number of the fat which was fed (14).

There are only a few reports on the effect of monoethenoid fatty

acids on serum lipids. In 1958 Keys et al. (15) reported that oleic acid had a neutral effect on serum cholesterol; Armstrong et al. (16) found that olive oil, rich in triolein, was about one-half as effective as corn oil, rich in linoleic acid.

In the average American diet a large portion of the fat calories derive from partially hydrogenated fat. Hydrogenation saturates the double bonds of the natural oils, thus introducing unnatural isomers; the type and amount vary with the process employed. Little is known about the mechanism for metabolizing these unnatural isomers or their effect on serum lipids. It appears (17) that humans are capable of metabolizing ingested ethenoid fatty acids with the trans configuration. Bronte-Stewart (18) reported that hydrogenation of the polyunsaturated vegetable oils greatly reduced their effectiveness in lowering serum cholesterol, while Ahrens et al. (2) reported no difference in the effect of corn oil, natural and partially hydrogenated, on the serum lipids of one normocholesterolemic subject. Recently Horlick (19) reported that feeding partially hydrogenated oil at the level of 40 per cent of the calories, following a period on a low-fat diet, resulted in a gradual rise in serum cholesterol.

The present investigation has been made because it was considered valuable to know how fat, which has been partially hydrogenated with no change in chain length, affects the serum lipids of clinically healthy young women. Since 12 to 15 per cent of the serum proteins are associated with lipoproteins, it would also be important to know if any changes in the lipoprotein fractions are accompanied by corresponding changes in the plasma protein fractions.

PROCEDURE

The experiment was divided into three periods, eight weeks each in Periods I and III and nine in Period II. The effect of a controlled adequate diet, the fat content of which had a high proportion of poly-unsaturated oil, was measured in Period I, and that of the same diet with the same oil partially hydrogenated was measured in Period III. The subjects consumed a self selected diet during Period II which served as an adjustment period, allowing blood values to return, presumably, to approximately the same level as that which preceeded Period I.

A. Subjects

Three female graduate students in nutrition, 34 to 38 years of age, volunteered to serve as subjects. All were judged clinically healthy, after a comprehensive medical examination at a local medical center which included an electrocardiogram, a complete blood count, and urine analysis (Appendix Tables 1a and 1b).

Subjects were weighed twice weekly for the purpose of maintaining constant weight. Blood pressure was measured monthly. Hematologic examinations, including hemoglobin, hematocrit, differential white cell count and cell maturation, were repeated at the end of Periods I and III. The basal metabolism of each subject was measured in the present laboratory at the beginning and end of the experiment. Each subject kept a daily record of activities and of any events which might affect in any way her state of health.

B. Methods of Analysis

Fasting finger-tip blood was taken on the first, third, and fifth days of each week throughout the three periods. Aliquots of serum were frozen for cholesterol determination at termination of the experiment. Sera for protein and lipoprotein measurements were refrigerated at 34-36°F. and analyses begun within twelve hours.

Serum Cholesterol

Serum total cholesterol concentrations of each subject were measured in triplicate three times weekly by Adamson's micromodification of the Pearson, Stern, and McGavack method.[1] Pooled human serum was used as the control for cholesterol analyses.

Serum Proteins and Lipoproteins

Serum proteins and lipoproteins were measured in duplicate twice weekly, using the Spinco Model R Paper Electrophoresis System. Sera samples, 0.006 ml. for proteins and 0.020 ml. for lipoproteins, were fractionated in Durrum type electrophoretic cells on paper strips in Veronal buffer, with a pH of 8.6 and 0.075 ionic strength. A constant current of 2.5 milliamperes for proteins and 4.0 milliamperes for lipoproteins was maintained by a Duostat power regulator and applied across paper strips for seventeen hours. Serum proteins were separated at room temperature and lipoproteins at 4-10°C.

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[1] Adamson, L.F., Leeper, G.K., and Ross, E.: Influence of dietary fats and cholesterol on tissue lipids in chickens. J. Nutrition 73:247, 1961.

The paper strips containing the separated protein fractions were then dyed with alcoholic bromphenol blue, using the Spinco "Procedure B" method.[1] The dye solution was newly prepared weekly. Lipoprotein fractions were stained with freshly prepared Fat Red 7 B (Ciba) dye solution according to Straus and Wurm's method (Am. J. Clin. Path. 29: 581, 1958). Strips were scanned immediately in a Spinco model RB analytrol equipped with a B-5 balancing cam and two 500 millimicron interference filters.

The relative concentration of each of the five protein fractions per strip was calculated as a percentage of the total area under the curve as recorded by the integrator. When Fat Red 7 B is used for staining lipoproteins five fractions, which correspond to the protein fractions, are demonstrated. These, like the serum protein fractions, were evaluated in terms of relative concentration. Beta/alpha ratios represent the ratio of the beta fraction to the combined $alpha_2$, $alpha_1$, and lipalbumin fractions. Beta/lipalbumin ratios were also derived.

The total protein concentration of each subject's serum was measured by refractometry[2] twice weekly throughout Period III.

C. Experimental Dietaries

The diet for Periods I and III consisted of three simply prepared and palatable menus (Appendix Tables 2a, 2b, and 2c) rotated

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[1] A Method for Serum Proteins using Bromphenol Blue Dye in Alcoholic Solution, Technical Bull. No. TB 6050A. Beckman Spinco Division, PaloAlto, Calif., April, 1958.

[2] TS Meter for Protein and Index, American Optical Co., Buffalo, N.Y.

daily for variety. Each menu met the recommended dietary allowances of the Food and Nutrition Board of the National Research Council.[1] The nutritive values of the diets were calculated from standard food composition tables (20). The amount of cholesterol in the diets was also approximated (21). Dietary computations were based on the needs of Subject 2, since she required fewer calories. As observed during the first adjustment period, all nutrients for the other two subjects required 122 per cent of the basic diet. In each case, fat constituted 40 per cent of the total dietary calories; protein, 16 to 17 per cent, computed on the basis of 1.5 gm/kg of body weight; the balance, 43 to 44 per cent, was derived from carbohydrate (Table 2).

Fat Content

During Period I, 77 per cent of the total fat calories, or 30 per cent of the total dietary calories, was derived from safflower oil. For Period III the natural oil was replaced by the same amount of the same oil partially hydrogenated. All other constituents were quantitatively the same in both periods, thus minimizing possible variables in the diet. In the process of partial hydrogenation of the oil, most of the linoleic acid was converted to elaidic acid and the stearic acid was increased from 2.5 per cent to 13.4 per cent (Table 3). Thus the polyunsaturated/saturated acid ratio (P/S) for the natural oil was changed from 9.26 to 0.18 for the partially hydrogenated.

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[1] Food and Nutrition Board: Recommended Dietary Allowances, Pub. 589, revised 1958. National Academy of Sciences--National Research Council, Washington, D.C., 1958.

Table 2 Dietary Nutrients, Amounts and/or Distribution

Subject No.	Calories	Protein		Carbohydrate		Fat		Fatty acids			Cholesterol
								Saturated	Unsaturated		
									Mono	Poly	
		gm.	%[1]	gm.	%[1]	gm.	%[1]	gm.	gm.	gm.	mg.
					Period I						
1	2,200	86	16	244	44	98	40	14	20	60	401
2	1,800	78	17	192	43	80	40	12	16	49	327
3	2,200	92	17	238	43	98	40	14	20	60	401
					Period II						
1	1,435	74	20	162	45	56	35	21	22	5	331
2	1,436	67	18	170	46	58	36	20	23	7	433
3	1,857	76	16	214	44	85	40	34	31	8	469
					Period III						
1	2,200	86	16	244	44	98	40	24	66	4	401
2	1,800	78	17	192	43	80	40	20	53	3	327
3	2,200	92	17	238	43	98	40	24	66	4	401

[1]Per cent of total calories

Table 3 Composition and Constants of Safflower Oil[1]

Description	Safflower Oil	
	Natural	Partially Hydrogenated
Fatty acids in percentage		
Caprylic		trace
Capric		trace
Lauric	trace	0.5
Myristic	0.1	0.3
Palmitic	5.8	7.2
Stearic	2.5	13.4
Palmitoleic	trace	
Oleic	13.7	12.7
Elaidic		62.0
Linoleic-Linolenic	77.8	3.9
Experimental iodine value	142.2	74.3
Calculated iodine value	146.3	71.0
Color	0.7r-6y	0.5r-4y
Refractive index at 48°C.	59.20	46.7
Mod. W. melting point	----	102.5°F.
Fatty acid ratios		
P/S[a]	9.26	0.18
M/S[b]	1.63	3.49
P + M/S[c]	10.89	3.67

[a] P/S = Polyunsaturated/Saturated

[b] M/S = Monounsaturated/Saturated

[c] P + M/S = Poly and Monounsaturated/Saturated

[1] Information furnished by Pacific Vegetable Oil Corporation, Research Laboratory, Richmond, California

Sixty-one per cent of the total fat calories was derived from polyunsaturated fatty acids in Period I, four per cent in Period III (Table 4); a negligible percentage from monounsaturated fatty acids in Period I, 67 per cent in Period III. Forty-eight per cent of the monounsaturated fatty acids in Period III had the trans configuration; elaidic acid contributing approximately 19 per cent of the total calories in the diet. The P/S ratio of the dietary fat for Period I was 4.15, and for Period III, 0.16 (Table 5).

Precautionary measures were taken that the same percentage and type of fat be consumed by each subject during Periods I and III. One lot of extra lean ground round beef was purchased, portioned into individual servings, and frozen. Similarly, all eggs were purchased from the same source in one lot, mixed thoroughly, portioned into individual servings, and frozen. Turkey breasts were also prepared in advance. Since packaged foods, such as nonfat dry milk, canned fruit and frozen vegetables, may differ in nutrient value, the same brand and lot number were purchased for the entire experiment.

Edible portions of each food were weighed to the nearest gram after cooking. Each subject added the safflower oil, natural or partially hydrogenated, to the food as served; to eggs and cereal at breakfast, soup and salad at lunch, and potatoes and vegetables at dinner.

D. Self Selected Dietaries

Each subject kept a record of the food consumed during the third and sixth weeks of Period II. The total nutritive value of each week's

Table 4 Percentage Distributions of Fat and Fatty Acid Calories

Subject No.	Percent total calories of the diet					Percent fatty acid calories in total fat calories			
		Fatty acids				Fatty acids			
			Unsaturated				Unsaturated		
	Total	Saturated	Mono[1]	Elaidic[2]	Poly	Saturated	Mono[1]	Elaidic[2]	Poly
				Period I					
1, 2, 3	40	6	8		24	15	16		61
				Period II					
1	35	13	14		3	38	39		9
2	36	13	14		4	35	40		12
3	40	17	15		4	40	37		9
				Period III					
1, 2, 3	40	10	27	19	2	25	67	48	4

[1]Includes both trans and cis configuration.

[2]Known elaidic in the partially hydrogenated oil.

Table 5 Ratios of the Fatty Acids

Subject No.	P/S	M/S	P + M/S
		Period I	
1,2,3	4.15	1.36	5.50
		Period II	
1	0.24	1.05	1.29
2	0.35	1.15	1.50
3	0.24	0.91	1.15
		Period III	
1,2,3	0.16	2.70	2.87

diet was obtained by computer, using the program of Thompson and Tucker (22). Average nutritive values of the subjects' individual diets for Period II are given in Table 3 of the Appendix.

All subjects consumed fewer calories on the self selected than on the experimental diet. Since there was little variation in body weight it appears that there may have been less expenditure of energy or perhaps less accurate estimation of total calories during Period II. In general, the diets were well balanced and, with few exceptions, met the recommended daily dietary allowances of the National Research Council. The diet of Subject 2 was somewhat low in calcium, namely, 549 mg., and diets of Subjects 1 and 3 were below recommended allowances in iron, namely, 9 mg. and 11 mg., respectively. The percentages of dietary calories derived from protein, carbohydrate, and fat were not significantly different from the experimental diets of Periods I and III (Table 2). The P/S ratios of dietary fat in the three diets for Period II, namely, 0.24, 0.35, and 0.24, respectively, were slightly larger than those of Period III.

RESULTS

A. Serum Total Cholesterol Concentration

Period I

The serum total cholesterol of all subjects diminished in concentration during Period I. Weekly means (Table 6) represent the average of fasting serum samples obtained on three alternating days each week. After three weeks, the serum cholesterol weekly means of Subject 1 stabilized to within ± 3 mg. per cent of the mean (202 mg. per cent) of the last five weeks. Those of Subjects 2 and 3 stabilized after four weeks to within ± 5 and ± 1 mg. per cent, respectively, of the means (204 and 149 mg. percent, respectively) of the last four weeks. The variability (Fig. 1) was similar in all cases. Weekly changes during Period I, expressed as per cent of the mean value of the first week, are shown in Table 8. The average weekly decreases over the first four weeks of the period for Subjects 1, 2, and 3, respectively, were 14.0 (statistically significant, $P < 0.05$), 6.5, and 2.8 mg. per cent. The standard deviations (S.D.) were ± 19, ± 18, and ± 14 mg. per cent, respectively; the standard errors (S.E.), ± 3.89, ± 3.61, and ± 2.92 mg. per cent, respectively; the coefficients of variation (C.V.), 9, 8, and 9 per cent, respectively (Table 7).

Period II

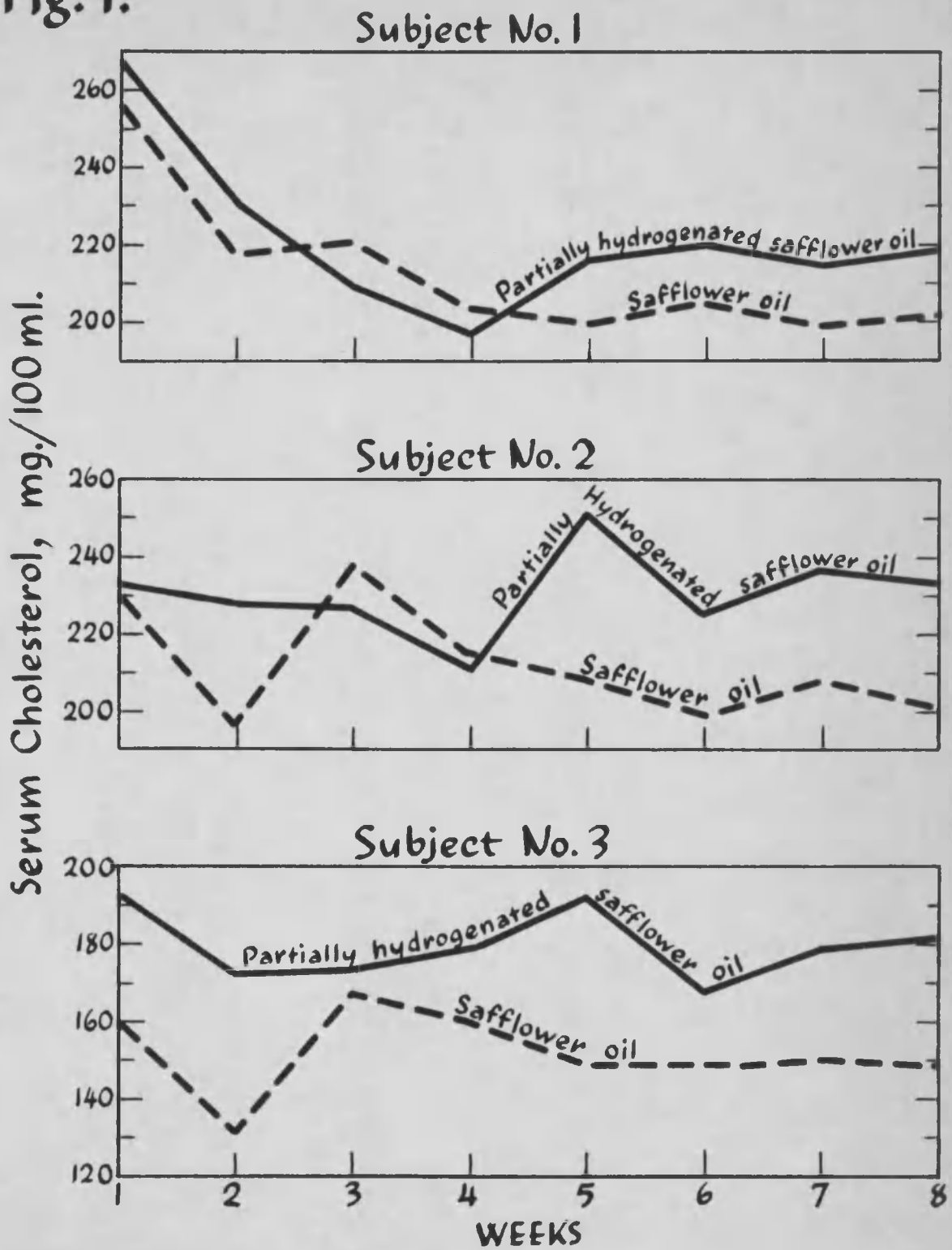
The weekly serum cholesterol concentrations of all subjects varied at random during Period II with no definable pattern for any of

Table 6 Weekly Means of Serum Total Cholesterol Concentrations, mg. %

Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
				Subject No. 1					
I	257	217	220	203	199	205	199	202	
II	218	246	217	221	237	250	255	223	219
III	268	230	209	197	216	220	215	219	
				Subject No. 2					
I	230	197	238	215	208	199	208	201	
II	215	228	242	225	246	243	239	208	232
III	233	228	227	211	251	225	237	233	
				Subject No. 3					
I	160	131	167	160	149	149	150	149	
II	158	176	165	175	191	195	190	171	179
III	193	172	173	179	192	168	179	182	

Weekly Means of Serum Cholesterol Concentrations, Periods I & III

Fig. 1.



the subjects. In each case, however, there was little difference in the S.D., S.E., and C.V. for Periods I and II (Table 7). The means for Period II were 10, 9, and 18 per cent, respectively, higher than those for Period I.

Period III

The serum total cholesterol of Subjects 1 and 3 diminished in concentration during Period III. The pattern of change for Subject 1 was similar to that for Period I (Fig. 1), showing a continuous decrease in concentration through the fourth week, increasing at the fifth week, at which level it stabilized to within ± 3 mg. per cent of the mean (218 mg. per cent) of the last four weeks. The average weekly serum cholesterol values of Subject 3 suggest a pattern in variability which seemed to repeat itself (Fig. 1). It dropped during the second week and then increased gradually for the next three weeks, reaching the initial value for Period III in the fifth week. There was another decrease during the sixth week and, as before, a gradual increase followed, stabilizing in the seventh week to within ± 2 mg. per cent of the mean (180 mg. per cent) of the last two weeks. No significant change in serum cholesterol concentration was observed for Subject 2 during this period (Table 8). The mean of the last week (233 mg. per cent) was the same as the mean of the first week and the average of the means of the last four weeks (236 ± 15 mg. per cent) was not significantly different.

The average weekly decreases over the first four weeks of the period for Subjects 1 and 3, respectively, were 12.2 and 3.2 mg. per cent. Standard deviations are correlated with the mean and for the

Table 7 Variability in Serum Cholesterol Concentration, mg. %

Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 1			
I	8	24	210	±19.04	±3.89	9
II	9	27	232	±20.23	±3.97	9
III	8	24	222	±28.60	±5.84	13
			Subject No. 2			
I	8	24	211	±17.71	±3.61	8
II	9	27	231	±16.68	±3.21	7
III	8	24	231	±16.25	±3.32	7
			Subject No. 3			
I	8	23	151	±14.00	±2.92	9
II	9	27	178	±15.38	±2.96	9
III	8	24	180	±13.35	±2.72	7

subject with the highest cholesterol concentration, the week to week variation was greater than for the subjects with the lower cholesterol concentrations. Standard deviations are for Subjects 1, 2, and 3, respectively, ± 29, ± 16, and ± 13 mg. per cent; standard errors, ± 5.84, ± 3.32, and ± 2.72 gm. per cent; coefficients of variation, 13, 7, and 7 per cent (Table 7).

Table 8 Variability in Serum Total Cholesterol, Per Cent of Mean Concentration of First Week

Subject No.	Weeks 2nd	3rd	4th	5th	6th	7th	8th
			Period I				
1	-16	-16	-21	-23	-20	-23	-21
2	-14	+ 3	- 7	-10	-13	-10	-13
3	-18	+ 4	0	- 7	- 7	- 8	- 7
			Period III				
1	-14	-22	-26	-19	-18	-20	-18
2	- 2	- 3	- 9	+ 8	- 3	+ 2	0
3	-11	-10	- 7	0	-13	- 7	- 6

B. Serum Lipoprotein Percentages

Changes in the lipoprotein fractions in the individual experimental periods did not correlate with the changes in cholesterol concentration. However, the mean percentage concentrations of the beta fractions of the three subjects for the three periods were directly proportional to their initial total cholesterol concentrations. Conversely, the alpha (alpha$_1$ plus alpha$_2$) fractions were inversely proportional to total cholesterol concentrations and the other fractions, namely, gamma plus neutral fat, and lipalbumin, showed similar trends, but less significant (Table 9).

Table 9 Mean Percentage of Lipoprotein Fractions for the Three Experimental Periods Referred to the Initial Total Cholesterol Concentration

Subject No.	Total Cholesterol	Lipoprotein Fraction			
		Lipalbumin	Alpha$_1$ + Alpha$_2$	Beta	Gamma + Neutral fat
	mg. %	%	%	%	%
1	257	22.0	17.5	40.8	19.6
2	230	26.7	18.2	35.3	20.1
3	194	26.8	20.2	31.5	21.5

Period I

The average weekly decline of the beta fraction of Subject 2 was statistically significant ($P<0.05$), namely, 0.39 per cent, during Period I and the alpha$_2$ fraction increased significantly ($P<0.05$),

namely, 0.28 per cent (Appendix Table 4). Two ratios, beta/alpha$_1$ plus alpha$_2$ and beta/alpha$_1$ plus alpha$_2$ plus lipalbumin, decreased significantly ($P < 0.05$) (Appendix Table 5). There were no significant changes in the fractions or ratios of Subjects 1 and 3 during Period I (Appendix Tables 4 and 5). Variability in S.D., S.E., and C.V. was negligible in all cases (Appendix Tables 6 and 7).

Period II

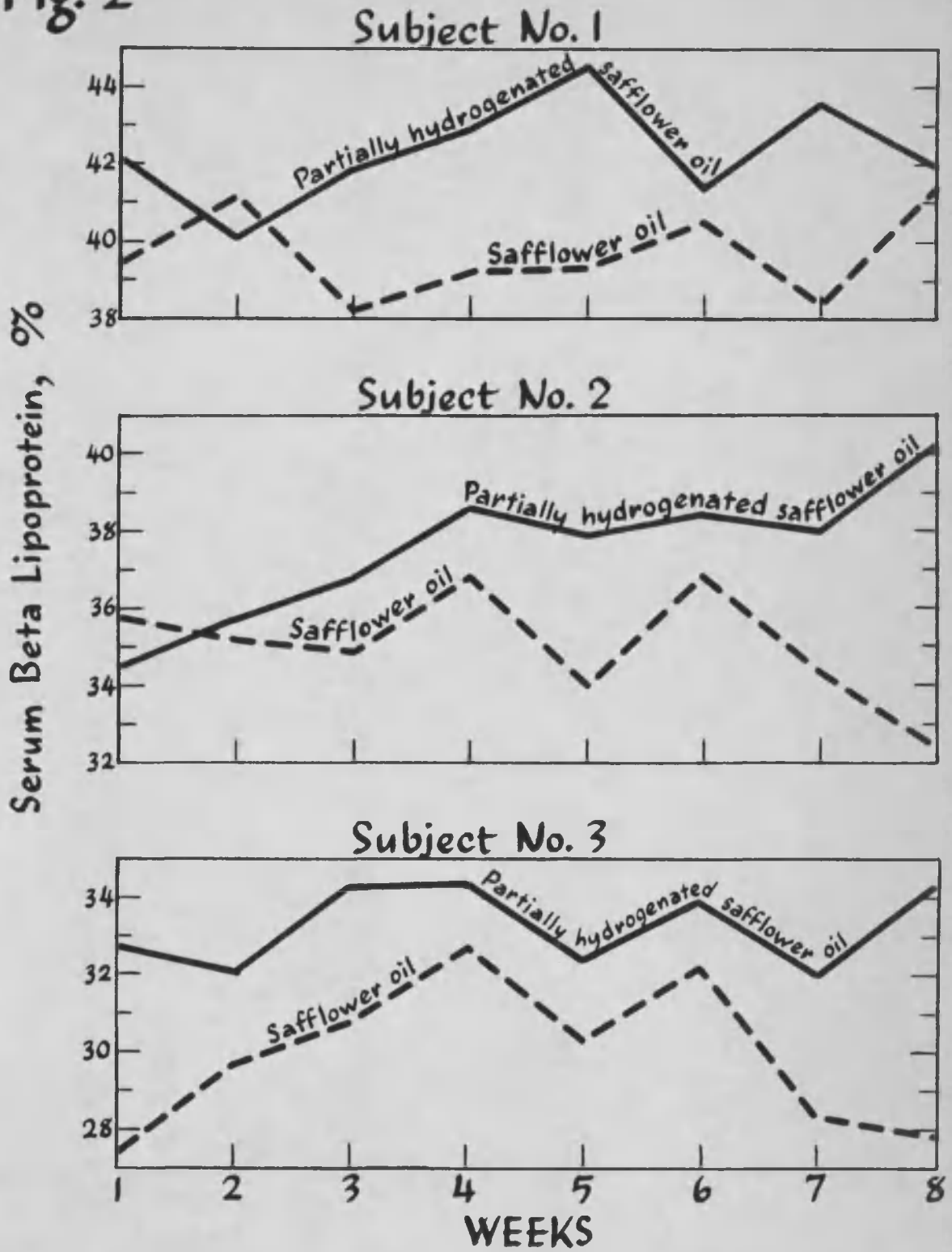
Percentage concentrations of the various lipoprotein fractions for all subjects were approximately the same for Periods I and II (Appendix Tables 6 and 7). However, variability in most fractions for all subjects was greater in Period II.

Period III

The lipoprotein patterns of Subjects 1 and 2 changed significantly ($P < 0.05$) during Period III; the average weekly decline of the lipalbumin fraction was 0.29 per cent and 0.60 per cent, respectively. This decrease in Subject 1 was compensated by an increase in the gamma plus neutral fat fraction and in Subject 2 in the beta fraction (Fig. 2); the average weekly increases were 0.27 per cent and 0.67 per cent, respectively. For Subject 2, three of four of the ratios increased equally significantly: beta/lipalbumin, beta/alpha$_1$ plus alpha$_2$ plus lipalbumin, and beta/gamma plus neutral fat. There was little change in the lipoprotein pattern of Subject 3 during Period III. With the exception of alpha$_1$, variability in S.D., S.E., and C.V. was negligible in all cases (Appendix Table 6).

Weekly Means of Serum Beta Lipoprotein Fraction, Periods I & III

Fig. 2



C. Serum Protein Percentages

Change in diet did not appear to modify, to any extent, the electrophoretic serum protein patterns of any of the three subjects (Appendix Tables 8 and 9); all fractions, with one exception, were relatively constant throughout the experiment. The gamma globulin fraction of Subject 2 increased significantly ($P<0.05$) during Periods II and III. It appears that this increase is the result of a response to the virus infection which she had during the fourth week of Period II, prior to the increase. In all cases, the variability was small (Appendix Tables 10 and 11); as would be expected, albumin, the largest fraction, had the least variability (3 per cent), and alpha$_1$ globulin, the smallest fraction, showed the greatest variability (15 per cent). There was slightly more variability in the fractions during Period III, but in all cases, the coefficients of variation of the subjects' sera and of the standard were similar.

Total protein concentration, measured during Period III, was also constant (Table 10); the mean values for Subjects 1, 2, and 3, were, namely, 6.9 (6.7 to 7.2), 6.8 (6.6 to 7.1) and 6.5 (6.1 to 6.9) gm. per cent, respectively. The first two were within ± 0.3 gm. per cent of the individual means for the period, and the third was within ± 0.4 gm. per cent.

Table 10 Serum Total Protein Concentration, Period III, gm. %

Week	Subject No. 1	2	3
1	7.1	6.9	6.5
	7.0	6.6	6.6
2	7.2	6.8	6.6
	6.7	6.7	6.6
3	6.9	7.0	6.6
	6.8	6.9	6.4
4	6.9	7.1	6.6
	7.0	6.8	6.5
5	6.8	6.7	6.1
	7.0	6.6	6.1
6	6.9	6.6	6.5
	6.7	6.8	6.5
7	6.8	6.8	6.9
	7.1	6.9	6.4
8	---	6.8	6.5
	7.0	6.8	6.6
Av.	6.9	6.8	6.5

DISCUSSION

In 1957, Lawry et al. (23) reported that the average serum total cholesterol value for fifty-six healthy American women between the ages of 30 and 39 years was 209 ± 32 mg. per cent. By this standard, Subject 1 would be considered hypercholesterolemic; Subjects 2 and 3, average.

Few studies have been reported on the electrophoretic pattern of serum lipids stained with Fat Red 7 B dye. Straus and Wurm (24) reported the following lipoprotein values for 40 normal individuals (25 males ranging in age from 10 to 80 years, and 15 females from 11 to 60 years): lipalbumin, 19.8 ± 5.86; alpha$_1$, 4.67 ± 2.07; alpha$_2$, 7.06 ± 2.35; beta, 52.5 ± 9.20; and gamma plus neutral fat, 15.5 ± 4.56 per cent. The ratios were: beta/alpha$_1$ plus alpha$_2$, 1.81 ± 0.68; and beta/alpha$_1$ plus alpha$_2$ plus lipalbumin, 3.02 ± 1.36. In comparison, the lipoprotein patterns of the three subjects in this experiment contain less percentages of beta and more of all other fractions (Appendix Table 6).

Minor variations are reported by investigators in the electrophoretic pattern of serum protein in apparently healthy persons. Wall (25), using paper electrophoresis to separate the serum protein fractions and bromphenol blue dye for staining, reported the following for normal adults: albumin, 66 (60-70); alpha$_1$, 3 (2-5); alpha$_2$, 7 (5-10); beta, 9 (8-12); and gamma, 12 (10-15) per cent. The serum protein fraction percentages for the three subjects are in close agreement with the above percentages throughout the experiment (Appendix Table 8). The

absolute values of serum total protein of the three subjects (Table 10) also fall within the normal range, 6.0 to 8.0 mg./100 ml. (26).

In agreement with other workers (6, 7, and 8), in the present study, safflower oil lowered the serum total cholesterol concentration of all subjects. Swell et al. (27) fed sixteen clinically healthy subjects a diet which contained 30 gm. of linoleic acid daily (25 per cent of the fat calories). At the end of 10 weeks, those subjects with normal initial cholesterol values (150-250 mg. per cent) had an average decline in serum cholesterol of 12.3 per cent (26 mg. per cent). Individual response to the high linoleic acid diet varied; one subject showed a decrease of 28.9 per cent whereas another showed no decrease at all. In the group with initially elevated blood cholesterol levels (above 250 mg. per cent) there was a significant decline in the serum cholesterol of every subject at the end of 10 weeks. The average decline for this group was 62 mg. per cent (21.2 per cent). Joliffe et al. (28) also reported that decreases in cholesterol were greatest in those subjects who initially had the highest values and least in those with lower initial values. The results of this experiment are in agreement with this finding.

Brown and Page (29) reported that a diet having 30 per cent of the total calories as cottonseed oil and 8 per cent as saturated fatty acids significantly lowered the serum cholesterol level of forty-six normal men and women, aged 25 to 55 years.

While consuming a diet rich in safflower oil, the lipoprotein pattern of one subject (Subject 2) changed; the beta fraction decreased

and the alpha$_2$ fraction increased. Horlick (19) reported that a diet having 40 per cent of the calories as corn oil produced an increase in alpha lipoprotein. Entenman (30) found that there was no change in beta lipoprotein when corn oil furnished 25 per cent of the dietary calories but with 40 per cent of the calories from corn oil, there was a marked decrease in beta. Ahrens (9) and Bronte-Stewart et al. (10) found that feeding polyunsaturated fat lowered beta lipoprotein cholesterol. In this experiment, two subjects had decreases in serum cholesterol with no change in lipoprotein patterns.

As Lewis (31) pointed out, heredity, diet, hormonal balance and hepatic function all affect the serum lipoprotein pattern. She reported that when a 40 per cent fat diet (30 per cent cottonseed oil) was fed to patients with elevated blood lipids those of the group having hypercholesterolemia (as in the case of Subject 1) showed variable changes. However, she reported that decreases in serum cholesterol (7 to 40 per cent) were 'usually' associated with a proportionate decrease in beta lipoprotein concentrations.

Boyle et al. (32) studied the lipoprotein patterns of 2,306 persons and found six distinctive types, only one type of which he considered normal. Brown and Page (33) reported, after two years of experimentation with the effect of low-fat and vegetable oil food patterns on the serum lipids of thirty-four patients, that abnormal serum lipid levels responded to dietary treatment according to the type of abnormality present.

The varied response to the controlled diet of the serum lipids

of the three subjects in this experiment bear out the above-mentioned reports; lipoprotein patterns and serum lipid levels vary among subjects. Thus, response to experimental diet is diverse.

The different responses to partially hydrogenated safflower oil continue to support the hypothesis that the fat metabolism pattern is individualistic. The serum cholesterol of Subjects 1 and 3 was lowered; that of Subject 2 showed no change. On the other hand, the lipoprotein patterns of Subjects 1 and 2 changed; that of Subject 3 remained constant.

The reports in the literature are also controversial. Horlick (19) fed two types of hydrogenated vegetable oil to healthy subjects (linoleic/saturated ratios, 1.3 and 0.6) as 40 per cent of the dietary calories following a basic diet containing 4 per cent fat. In one of three trials serum cholesterol levels returned to control levels; in the other two trials they stabilized at 10 per cent below the control levels. Anderson et al. (34) replaced 30 gm. of safflower oil in the basic diet of twenty-seven healthy male subjects by the hydrogenated oil. The mean increase in serum cholesterol was 10 mg. per cent. When 100 mg. of safflower oil were replaced with hydrogenated oil, the mean increase in serum cholesterol of twelve of the group was 25 mg. per cent. Replacement of 100 gm. of corn oil by an equal amount of hydrogenated corn oil caused a mean increase of 21 mg. per cent of cholesterol in thirteen of the group.

Anderson compared the effect of two dietary fats, matched in total saturated, total monoene and total polyene fatty acids. One of the mixtures, hydrogenated corn oil (HCO), contained unnatural isomers

produced by hydrogenation (36 per cent trans-monoene and 12 per cent trans-diene). The other, a mixture of vegetable oils, was made up of naturally occurring isomers. When HCO replaced the natural mixture in the diet of twenty-three men, there were significant mean increases in cholesterol, phospholipids and triglycerides. It is interesting to note that the trans isomers caused a rise in triglycerides. Subject 1 of this experiment had a rise in the gamma plus neutral fat fraction, which is rich in triglycerides. Little is known about the mechanism for metabolizing trans isomers; some believe that the pathway is different from that of natural isomers.

McOsker et al. (35) fed four different partially hydrogenated vegetable fats to healthy male subjects as 40 per cent of the dietary calories. Three of the fats having an iodine value (I.V.) of 95 or greater, and P/S ratio, 1.2 or greater, produced significantly lower serum cholesterol levels than those maintained on a diet representative of the "average" American diet. The results were interpreted to suggest no difference between effects of the partially hydrogenated and the natural oil. One margarine, the fourth fat fed, having an I.V. of 61 and P/S, 0.3, produced no change in serum cholesterol. The latter margarine compares more closely with the partially hydrogenated fat used during Period III of this experiment. The above margarine contained 52 per cent oleic acid, 35.3 per cent saturated acids (16 and 18 C) and 11.6 per cent linoleic acid; 7.8 per cent of the fatty acids had the trans configuration.

Beveridge et al. (36) concluded from an eight day experiment

with eighty healthy university students that hydrogenated corn oil (I.V., 69.6) had significant hypocholesterolemic effects when added to a diet containing a hypercholesterolemic fat (butter) and no effect when added to a fat free diet. Ahrens et al. (2) fed hydrogenated corn oil (I.V., 80) to one normocholesterolemic patient and reported that it produced no increase in cholesterol level over that of the unhydrogenated oil. However, the feeding of hydrogenated oils to hypercholesterolemic patients resulted in increases in serum cholesterol, varying with the I.V. of the fat. He concluded that fats with an I.V. of 100 or more produced equally low levels of cholesterol, suggesting a limit to the effect produced by ingestion of unsaturated fats. He reported that by feeding fats with iodine values below 90, the cholesterol content of the serum was increased as the saturation of the dietary fat increased.

Bronte-Stewart et al. (10) fed hydrogenated ground nut fat (I.V., 55) to Bantus in two experiments and in both there occurred a slight rise in serum lipid levels, including that of serum cholesterol.

SUMMARY

In this investigation, when safflower oil contributed 30 per cent of the calories in the experimental diet, the serum total cholesterol concentration was lowered in the three subjects. The change was statistically significant ($P < 0.05$) in the case with the highest initial weekly mean of 257 mg. per cent; the other two subjects having initial weekly means of 230 mg. per cent and 160 mg. per cent, respectively. The means of the last four weeks for Subjects 1, 2, and 3, respectively, were 22, 12, and 7 per cent below those of the first week. There were no significant changes in the lipoprotein patterns of Subjects 1 and 3; the beta fraction of Subject 2 decreased significantly ($P < 0.05$) while the $alpha_2$ fraction increased significantly.

When the safflower oil was replaced by the partially hydrogenated product, the serum cholesterol concentration was lowered in two of the three subjects. As before, the greatest change occurred in the case with the highest initial weekly mean value. The means of the last four weeks for Subjects 1 and 3 were below those of the first week, 19 per cent and 7 per cent respectively; that of Subject 2 showed no change. The serum cholesterol of Subject 1 was more variable during Period III (C.V., 13 per cent) than during Period I (C.V., 9 per cent; that of Subjects 2 and 3 showed slightly less variability during Period III (C.V., 7 per cent) than during Period I (C.V., 8 per cent and 9 per cent, respectively).

The lipoprotein patterns of Subjects 1 and 2 changed significantly during Period III; that of Subject 3 showed little change. Increases in the gamma plus neutral fat fraction of Subject 1 and the beta fraction of Subject 2 compensated for decreases in the lipalbumin fraction of both subjects. Throughout the experiment, the $alpha_1$ fraction was more variable than the others.

Changes in the lipoprotein fractions in the individual experimental periods did not correlate with the changes in cholesterol concentration. The initial total cholesterol concentrations of the three subjects, however, were directly proportional to the mean percentage concentrations of the respective beta fractions for the three periods; but they were inversely proportional to the alpha concentrations ($alpha_1$ plus $alpha_2$).

Change in dietary fat did not appear to modify the electrophoretic serum protein patterns of the three subjects. Total protein concentrations, measured during Period III, were approximately constant.

CONCLUSION

The results in this investigation point up the fact that fat metabolism varies among subjects. It appears that the initial serum lipid level and the characteristic pattern of the individual are important factors in determining the effect of dietary fat on serum lipids. It would seem therefore that no single measurement of dietary fat, such as P/S ratio, iodine value, or percentage of *trans* isomers will predict the effect on serum lipids of all individuals.

Since the subjects maintained constant weight when 19 per cent of the dietary calories was supplied by *trans*-monoene fatty acids, the conclusion is made that, under the conditions in this experiment, *trans*-monoene fatty acids are utilized by the human body but the change in the lipoprotein pattern of two of three subjects might indicate that the metabolic pathway is different for this isomer. More detailed measurements of the different lipid components in the individual lipoprotein fractions would be most interesting and informative.

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A P P E N D I X

Appendix Table 1a

Physical and Physiological Measurements

Subject No.	Period	Physical					Physiological				
				Weight						Basal Metabolism	
		Age	Height	Beg.	End	Surface Area	Pulse Rate	Blood Pressure	Electro-cardio-gram	Cal/m^2/hr	% Deviation Mayo Foundation
		yr.	cm.	kg.		m^2	/min.	mm. Hg.			
1	I	34.2	164	57	56	1.62	88	124/74	normal	34.23	- 4.09
	III			55	54					35.23	- 1.32
2	I	38.6	158	52	51	1.51	80	132/78	normal	30.88	-13.50
	III			50	50					29.84	-16.42
3	I	38.6	180	62	61	1.78	88	128/74	normal	32.64	- 8.57
	III			62	62					34.19	- 4.23

Appendix Table 1b Biochemical Measurements--Blood and Urine

Subject No.	Period	Blood										Urine
		Hemoglobin		Hematocrit		White Cell Count		Cholesterol		Serum Protein		Specific Gravity
		Beg.	End	Beg.	End	Beg.	End	Beg.	End	Beg.	End	
		gm./100 ml.		%		cu.mm.		mg./100 ml.		gm./100 ml.		
1	I	13.6	13.6	42.5	42.0	7,750	6,500	237	199			1.015
	III		13.6		44.5		6,400	235	231	6.7	7.0	
2	I	13.2	12.8	37.5	38.5	5,450	5,400	243	199			1.025
	III	12.8	12.8		39.0		3,850	237	232	7.2	6.8	
3	I	13.6	14.0	42.0	43.5	8,600	8,050	173	137			1.009
	III		13.2		43.0		6,800	173	179	6.5	6.6	

Appendix Table 2a — Menu No. 1 of the Experimental Diet[1]

Food	Weight	Calories	Protein	Fat	Fatty acids: Saturated	Fatty acids: Monounsaturated	Fatty acids: Polyunsaturated	Carbohydrate	Calcium	Iron	Vitamin A Value	Thiamine	Riboflavin	Niacin[2]	Vitamin C	Cholesterol
	gm.		gm.	gm.	gm.	gm.	gm.	gm.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.	mg.
Breakfast:																
Orange and grapefruit juice	124	55	0.5	Tr.	----	----	----	13.0	10	0.1	135	0.08	0.01	0.4	51	0
Oatmeal, cooked	118	75	2.5	1.5	0.5	0.5	0.5	13.0	11	0.9	0	0.11	0.02	0.2	0	0
Egg, raw	50	80	6.0	6.0	2.0	3.0	Tr.	Tr.	27	1.1	590	0.05	0.15	Tr.	0	248
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Milk, dry, nonfat	13	45	4.5	Tr.	----	----	----	6.5	149	0.1	5	0.05	0.22	0.1	1	1
Safflower oil	12															
Lunch:																
Soup																
Rice, raw	6	21	0.4	Tr.	----	----	----	4.5	1	Tr.	0	0.11	0.02	0.2	0	0
Tomato juice	145	30	1.2	Tr.	----	----	----	6.0	10	0.6	1524	0.07	0.04	1.1	23	0
Salad																
Cottage cheese, dry	56	50	10.0	Tr.	----	----	----	2.0	52	0.2	Tr.	0.02	0.16	Tr.	0	0
Carrot	28	11	0.3	Tr.	----	----	----	2.5	11	0.2	3300	0.02	0.02	0.2	2	0
Lettuce	25	3	0.5	Tr.	----	----	----	.5	6	0.1	135	0.01	0.02	0.1	2	0
Pineapple, canned, crushed	87	68	0.3	Tr.	----	----	----	18.3	25	0.5	70	0.07	0.01	0.1	8	0
Gelatin, dry, dessert pwd.	14	54	1.3	Tr.	----	----	----	12.7	0	0	0	0	0	0	0	0
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Milk, dry, nonfat	25	90	9.0	Tr.	----	----	----	13.0	298	0.1	10	0.10	0.44	0.2	0	1
Safflower oil	18															
Dinner:																
Ground lean beef, cooked	85	185	23.0	10.0	5.0	4.0	Tr.	0	10	3.0	20	0.08	0.20	5.1	0	77
Potato, baked, peeled	78	71	2.4	Tr.	----	----	----	16.6	7	0.6	Tr.	0.08	0.03	1.3	16	0
Peas, green, cooked	100	68	3.7	0.6	----	----	----	11.8	22	1.9	713	0.25	0.14	2.3	15	0
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Pudding, dry, instant	21	80	Tr.	0.1	----	----	----	20.3	0	0	0	0	0	0	0	0
Strawberries, frozen	36	38	0.3	0.1	----	----	----	9.4	8	0.2	15	0.01	0.02	0.1	15	0
Milk, dry, nonfat	10	36	3.6	Tr.	----	----	----	5.2	119	Tr.	4	0.04	0.18	0.1	1	Tr.
Safflower oil	28.5															
Total, safflower oil, omitted		1240	75.5	21.3	8.7	8.7	0.8	191.3	823	11.4	6521	1.33	1.83	13.3	134	330
" " " , included		1767	75.5	79.8	13.6	16.7	46.3	191.3	823	11.4	6521	1.33	1.83	13.3	134	330
" " " , partially hydrogenated, included		1767	75.5	79.8	21.2	52.4	3.1	191.3	823	11.4	6521	1.33	1.83	13.3	134	330

[1] Quantities are for Subject No. 1

[2] Niacin equivalent, 25 mg.

Appendix Table 2b — Menu No. 2 of the Experimental Diet[1]

Food	Weight	Calories	Protein	Fat	Fatty acids: Saturated	Fatty acids: Monounsaturated	Fatty acids: Polyunsaturated	Carbohydrate	Calcium	Iron	Vitamin A Value	Thiamine	Riboflavin	Niacin[2]	Vitamin C	Cholesterol
	gm.		gm.	gm.	gm.	gm.	gm.	gm.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.	mg.
Breakfast:																
Orange and grapefruit juice	124	55	0.5	Tr.	----	----	----	13.0	10	0.1	135	0.08	0.01	0.4	51	0
Farina, cooked	119	52	1.5	Tr.	----	----	----	11.0	16	0.4	0	0.05	0.04	0.5	0	0
Egg, raw	50	80	6.0	6.0	2.0	3.0	Tr.	Tr.	27	1.1	590	0.05	0.15	Tr.	0	248
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Milk, dry, nonfat	13	45	4.5	Tr.	----	----	----	6.5	149	0.1	5	0.05	0.22	0.1	1	1
Safflower oil	12															
Lunch:																
Beans, kidney, canned	128	115	7.5	0.5	----	----	----	21.0	37	2.3	0	0.07	0.07	0.8	Tr.	0
Catsup	17	15	Tr.	Tr.	----	----	----	4.0	2	0.1	320	0.02	0.01	0.4	2	0
Salad																
Green pepper	42	10	0.6	Tr.	----	----	----	2.0	4	0.3	174	0.04	0.04	0.2	53	0
Lettuce	50	5	1.0	Tr.	----	----	----	1.0	11	0.2	270	0.02	0.04	0.1	4	0
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Apricots, canned in heavy syrup	61	53	0.5	Tr.	----	----	----	13.5	7	0.2	1065	0.01	0.02	0.2	3	0
Milk, dry, nonfat	25	90	9.0	Tr.	----	----	----	13.0	298	0.1	10	0.10	0.44	0.2	2	1
Safflower oil	24															
Dinner:																
Turkey breast, baked	82	168	28.0	6.1	1.5	3.0	1.0	0	10	1.1	62	0.03	0.32	8.6	0	10
Potato, baked, peeled	78	71	2.4	Tr.	----	----	----	16.6	7	0.6	Tr.	0.08	0.03	1.3	15	0
Asparagus, cooked	100	21	2.3	Tr.	----	----	----	3.4	19	1.0	1037	0.13	0.17	1.2	23	0
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Pudding, dry, instant	21	80	Tr.	0.1	----	----	----	20.3	0	0	0	0	0	0	0	0
Plums, no pits, canned	122	90	Tr.	Tr.	----	----	----	25.0	10	1.3	280	0.03	0.03	0.5	1	0
Milk, dry, nonfat	13	45	4.5	Tr.	----	----	----	6.5	149	0.1	5	0.05	0.22	0.1	1	1
Safflower oil	27															
Total, safflower oil, omitted		1175	74.3	15.7	4.7	7.2	1.3	192.8	813	10.8	3953	0.99	1.96	16.4	156	264
" " ", included		1742	74.3	78.7	10.0	15.9	50.6	192.8	813	10.8	3953	0.99	1.96	16.4	156	264
" " ", partially hydrogenated, included		1742	74.3	78.7	18.3	54.5	3.8	192.8	813	10.8	3953	0.99	1.96	16.4	156	264

[1] Quantities are for Subject No. 1

[2] Niacin equivalent, 29 mg.

Appendix Table 2c — Menu No. 3 of the Experimental Diet[1]

Food	Weight	Calories	Protein	Fat	Fatty acids: Saturated	Fatty acids: Monounsaturated	Fatty acids: Polyunsaturated	Carbohydrate	Calcium	Iron	Vitamin A Value	Thiamine	Riboflavin	Niacin[2]	Vitamin C	Cholesterol
	gm.		gm.	gm.	gm.	gm.	gm.	gm.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.	mg.
Breakfast:																
Prune juice	80	56	0.3	Tr.	----	----	-------	15.0	11	3.3	0	Tr.	0.01	0.4	1	0
Shredded wheat	23	85	2.2	0.3	----	----	----	18.3	13	0.8	0	0.04	0.03	1.0	0	0
Egg, raw	50	80	6.0	6.0	2.0	3.0	Tr.	Tr.	27	1.1	590	0.05	0.15	Tr.	0	248
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Butter	6	40	Tr.	4.9	2.4	1.6	0	0	1	0	186	0	0	0	0	16
Milk, dry, nonfat	13	45	4.5	Tr.	----	----	----	6.5	149	0.1	5	0.05	0.22	0.1	1	1
Safflower oil	12															
Lunch:																
Split pea soup	125	145	10.0	0.5	----	----	----	26.0	14	2.1	60	0.18	0.11	1.1	Tr.	0
Salad																
Cottage cheese, dry	38	33	6.3	Tr.	----	----	----	1.0	34	0.2	3	0.01	0.11	Tr.	0	0
Celery	50	10	0.5	Tr.	----	----	----	2.0	25	0.3	0	0.03	0.02	0.2	4	0
Tomato, raw	100	20	1.3	Tr.	----	----	----	4.0	11	0.6	1099	0.05	0.04	0.5	23	0
Vinegar	5	1	0	----	----	----	----	Tr.	Tr.	Tr.	----	----	----	----	---	---
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Orange, navel	146	49	1.6	Tr.	----	----	----	13.0	40	0.4	194	0.10	0.04	0.4	61	0
Milk, dry, nonfat	25	90	9.0	Tr.	----	----	----	13.0	298	0.1	10	0.10	0.44	0.2	2	1
Safflower oil	25															
Dinner:																
Shrimp, cooked	82	106	22.2	1.0	----	----	----	----	95	2.5	48	0.01	0.03	1.8	0	125
Catsup	17	15	Tr.	Tr.	----	----	----	4.0	2	0.1	320	0.02	0.01	0.4	2	0
Lemon juice	15	5	Tr.	Tr.	----	----	----	1.0	1	Tr.	Tr.	Tr.	Tr.	Tr.	7	0
Potato, baked, peeled	78	71	2.4	Tr.	----	----	----	16.6	7	0.6	Tr.	0.08	0.03	1.3	16	0
Broccoli spears, cooked	75	23	2.5	Tr.	----	----	----	4.0	98	1.0	2500	0.05	0.11	0.6	55	0
Bread, white, enriched	23	60	2.0	1.0	0.4	0.4	0.1	12.0	19	0.6	Tr.	0.06	0.05	0.6	Tr.	1
Pudding, dry, instant	21	80	Tr.	0.1	----	----	----	20.3	0	0	0	0	0	0	0	0
Banana	38	21	0.3	Tr.	----	----	----	5.7	2	0.2	48	0.01	0.02	0.2	3	0
Milk, dry, nonfat	10	36	3.6	Tr.	----	----	----	5.2	119	Tr.	4	0.04	0.18	0.1	1	Tr.
Safflower oil	27															
Total, safflower oil, omitted		1191	78.7	15.8	5.6	5.8	0.3	191.6	1004	15.2	5067	1.00	1.70	10.1	176	394
" " " , included		1767	78.7	79.8	11.0	14.6	50.1	191.6	1004	15.2	5067	1.00	1.70	10.1	176	394
" " " , partially hydrogenated, included		1767	78.7	79.8	19.3	53.6	2.8	191.6	1004	15.2	5067	1.00	1.70	10.1	176	394

[1] Quantities are for Subject No. 1

[2] Niacin equivalent, 23 mg.

Appendix Table 3 Average Nutrient Intake per Subject, Period II, Third and Sixth Weeks

Nutrient	Subject No. 1			Subject No. 2			Subject No. 3		
	Week 3rd	Week 6th	Av.	Week 3rd	Week 6th	Av.	Week 3rd	Week 6th	Av.
Calories	1534	1335	1435	1437	1434	1436	1917	1796	1857
Protein, gm.	90	57	74	69	65	67	78	74	76
Fat, gm.	51	60	56	61	56	58	90	80	85
Fatty acids, gm.									
Saturated	17	25	21	20	20	20	36	32	34
Monounsaturated	19	25	22	25	22	24	31	31	31
Polyunsaturated	6	4	5	7	6	7	8	8	8
Carbohydrate, gm.	187	137	162	168	172	170	214	213	214
Calcium, mg.	1061	570	816	591	506	549	891	835	863
Iron, mg.	10.1	8.1	9.1	11.9	11.4	11.7	11.4	10.1	10.8
Vitamin A, I.U.	7864	4161	6013	10300	12314	11307	5927	5720	5824
Thiamine, mg.	1.4	0.7	1.1	1.2	1.1	1.2	1.7	1.1	1.4
Riboflavin, mg.	2.4	1.4	1.9	2.0	1.9	2.0	2.1	1.9	2.0
Niacin, mg.	13	9	11	13	13	13	14	14	14
Ascorbic acid, mg.	113	67	90	99	142	121	151	118	135
Cholesterol, mg.	273	388	331	442	423	433	400	537	469

Appendix Table 4 Weekly Means of Serum Lipoprotein Fractions, Percentage Concentration

Fraction	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Subject No. 1										
Lipalbumin	I	20.2	22.9	21.7	21.8	23.1	19.4	22.1	21.0	
	II	23.7	20.8	21.5	22.0	22.2	20.6	22.2	23.1	20.9
	III	21.9	23.4	22.8	22.2	22.9	21.5	19.7	21.5	
$Alpha_1$	I	7.1	7.4	7.2	7.4	6.5	6.9	7.0	7.2	
	II	7.3	7.9	7.2	6.5	7.8	8.6	7.2	8.5	7.8
	III	6.4	7.1	6.6	5.5	4.0	5.8	6.9	6.3	
$Alpha_2$	I	11.7	10.2	11.5	11.6	11.7	13.0	12.4	10.4	
	II	9.0	9.7	11.7	10.8	11.1	11.2	10.5	11.5	11.5
	III	11.5	10.2	8.7	10.5	9.4	10.1	9.9	10.2	
Beta	I	39.4	41.2	38.2	39.2	39.3	40.5	38.4	41.4	
	II	40.0	44.1	40.1	41.9	38.8	40.7	38.3	37.5	42.2
	III	42.2	40.1	41.9	42.9	44.5	41.4	43.6	41.9	
Gamma + Neutral fat	I	21.5	18.2	21.4	19.4	19.5	20.2	20.2	20.0	
	II	20.0	17.6	19.5	18.8	20.0	18.9	21.8	19.3	17.6
	III	18.0	19.3	19.6	18.9	19.2	21.2	19.9	20.1	
Subject No. 2										
Lipalbumin	I	26.4	28.0	28.8	26.5	26.1	22.4	27.8	26.1	
	II	30.3	27.1	24.7	27.2	27.7	25.0	24.1	29.2	29.5
	III	26.5	26.2	26.9	26.3	26.8	24.9	21.6	23.3	
$Alpha_1$	I	7.7	6.4	7.1	7.3	8.4	7.8	7.5	7.1	
	II	8.0	10.0	9.5	7.4	8.0	9.7	8.7	8.1	7.9
	III	6.2	8.7	6.7	6.8	6.8	6.7	8.6	6.9	
$Alpha_2$	I	10.2	9.8	9.3	10.0	10.4	13.0	12.2	11.4	
	II	9.4	10.6	10.1	10.2	11.5	11.1	10.8	9.8	11.2
	III	11.3	10.4	9.1	10.0	8.8	10.0	11.1	10.6	
Beta	I	35.8	35.2	34.9	36.8	34.0	36.8	34.3	32.4	
	II	31.1	34.1	37.4	34.9	33.1	33.8	35.3	31.3	32.2
	III	34.5	35.7	36.8	37.9	38.4	38.6	38.0	40.2	
Gamma + Neutral fat	I	19.8	20.7	20.1	19.4	21.1	20.0	18.2	23.0	
	II	21.2	18.2	18.4	20.2	19.6	20.5	21.2	21.6	19.2
	III	21.5	19.1	20.6	18.4	1917	20.0	20.6	18.9	

Appendix Table 4 (cont.)

Fraction	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
					Subject No. 3					
Lipalbumin	I	30.1	29.4	29.0	26.7	28.0	24.1	26.8	27.2	
	II	28.8	27.5	24.6	27.3	27.8	30.5	27.1	24.9	27.7
	III	26.3	26.0	25.2	26.5	27.6	25.3	24.7	24.0	
$Alpha_1$	I	6.6	7.9	7.3	8.2	9.0	9.0	9.4	9.3	
	II	10.4	7.6	9.5	7.8	9.4	8.2	9.7	9.2	7.8
	III	7.2	8.8	7.1	7.0	7.1	7.7	11.1	9.1	
$Alpha_2$	I	12.2	10.4	11.1	10.7	11.4	12.9	12.3	12.0	
	II	11.3	10.8	12.0	11.9	12.7	11.5	11.2	12.2	12.4
	III	11.8	12.5	12.7	11.1	12.0	10.8	12.3	12.0	
Beta	I	27.4	29.7	30.8	32.7	30.3	32.2	28.3	27.8	
	II	30.1	34.2	33.1	32.6	28.5	29.8	27.9	30.4	31.7
	III	32.8	32.1	34.3	34.4	32.4	33.9	32.0	34.3	
Gamma +	I	23.6	22.5	21.8	21.7	21.3	21.9	23.2	23.8	
Neutral fat	II	19.5	19.8	20.8	20.4	21.6	20.0	24.1	23.4	20.5
	III	22.0	20.6	20.7	21.0	20.9	22.2	19.8	20.6	

Appendix Table 5 Ratios of Serum Lipoprotein Fractions, Weekly Means

Ratio	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Subject No. 1										
Beta/Alpha$_1$ + Alpha$_2$	I	2.10	2.38	2.05	2.11	2.18	2.04	1.99	2.36	
	II	2.46	2.52	2.13	2.43	2.07	2.06	2.16	1.88	2.20
	III	2.38	2.34	2.32	2.69	3.34	2.67	2.63	2.55	
Beta/Alpha$_1$ + Alpha$_2$ + Lipalbumin	I	1.01	1.03	0.95	0.98	0.96	1.03	0.93	1.08	
	II	1.00	1.15	1.00	1.07	0.95	1.01	0.96	0.87	1.05
	III	1.06	0.99	1.06	1.23	1.16	1.20	1.13	1.11	
Beta/Lip-albumin	I	1.95	1.81	1.76	1.83	1.71	2.09	1.74	1.98	
	II	1.69	2.14	1.89	1.92	1.75	1.97	1.74	1.63	2.03
	III	1.93	1.71	1.85	1.93	1.94	1.93	2.21	1.96	
Beta/Gamma + Neutral fat	I	1.95	2.29	1.79	2.05	2.03	2.01	1.93	2.12	
	II	2.00	2.52	2.07	2.23	1.95	2.15	1.78	1.95	2.40
	III	2.34	2.08	2.16	2.27	2.36	2.00	2.19	2.19	
Subject No. 2										
Beta/Alpha$_1$ + Alpha$_2$	I	2.00	2.22	2.13	2.15	1.81	1.80	1.74	1.76	
	II	1.87	1.67	1.92	1.98	1.71	1.63	1.81	1.75	1.71
	III	1.97	1.88	2.36	2.36	2.44	2.32	1.94	2.30	
Beta/Alpha$_1$ + Alpha$_2$ + Lipalbumin	I	0.81	0.80	0.77	0.84	0.76	0.86	0.72	0.73	
	II	0.66	0.72	0.85	0.78	0.70	0.74	0.81	0.67	0.66
	III	0.79	0.79	0.87	0.90	0.90	0.93	0.92	0.99	
Beta/Lip-albumin	I	1.36	1.26	1.21	1.39	1.30	1.64	1.24	1.24	
	II	1.03	1.29	1.52	1.29	1.20	1.36	1.48	1.08	1.10
	III	1.30	1.37	1.37	1.47	1.42	1.55	1.76	1.73	
Beta/Gamma + Neutral fat	I	1.85	1.71	1.75	1.90	1.62	1.84	1.89	1.41	
	II	1.49	1.89	2.04	1.74	1.71	1.66	1.67	1.45	1.72
	III	1.61	1.88	1.79	2.11	1.92	1.92	1.87	2.13	

Appendix Table 5 (cont.)

Ratio	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
					Subject No. 3					
Beta/Alpha$_1$ + Alpha$_2$	I	1.73	1.63	1.68	1.74	1.49	1.47	1.31	1.31	
	II	1.39	1.86	1.54	1.67	1.30	1.52	1.34	1.45	1.58
	III	1.74	1.52	1.74	1.92	1.73	1.84	1.37	1.64	
Beta/Alpha$_1$ + Alpha$_2$ + Lipalbumin	I	0.56	0.62	0.65	0.72	0.63	0.70	0.59	0.57	
	II	0.60	0.75	0.72	0.70	0.58	0.60	0.58	0.67	0.67
	III	0.73	0.68	0.77	0.78	0.70	0.78	0.67	0.76	
Beta/Lip-albumin	I	0.91	1.01	1.07	1.23	1.08	1.34	1.05	1.02	
	II	1.06	1.25	1.35	1.20	1.03	0.98	1.04	1.23	1.16
	III	1.25	1.24	1.36	1.30	1.18	1.34	1.30	1.44	
Beta/Gamma + Neutral fat	I	1.16	1.33	1.41	1.51	1.46	1.47	1.22	1.18	
	II	1.57	1.74	1.59	1.61	1.33	1.50	1.17	1.30	1.55
	III	1.50	1.57	1.67	1.66	1.56	1.53	1.62	1.68	

Appendix Table 6 Variability in Serum Lipoprotein Fractions

Fraction	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 1				
Lipalbumin	I	8	17	21.6	±1.41	±0.34	7
	II	9	18	21.9	±1.44	±0.34	7
	III	8	16	22.0	±1.33	±0.33	6
$Alpha_1$	I	8	17	7.1	±0.74	±0.18	10
	II	9	18	7.7	±0.90	±0.21	12
	III	8	16	6.1	±1.20	±0.30	20
$Alpha_2$	I	8	17	11.6	±1.22	±0.30	10
	II	9	18	10.8	±1.12	±0.26	10
	III	8	16	10.1	±1.10	±0.27	11
Beta	I	8	17	39.7	±2.19	±0.53	6
	II	9	18	40.4	±2.66	±0.63	7
	III	8	16	42.4	±2.19	±0.55	5
Gamma+ Neutral fat	I	8	17	20.0	±2.35	±0.57	12
	II	9	18	19.3	±1.57	±0.37	8
	III	8	16	19.5	±1.62	±0.40	8
			Subject No. 2				
Lipalbumin	I	8	17	26.6	±1.94	±0.47	7
	II	9	18	27.2	±2.69	±0.63	10
	III	8	16	25.3	±2.12	±0.53	8
$Alpha_1$	I	8	17	7.4	±0.97	±0.24	13
	II	9	18	8.6	±1.60	±0.38	19
	III	8	16	7.2	±1.09	±0.27	15
$Alpha_2$	I	8	17	10.8	±1.28	±0.31	12
	II	9	18	10.5	±0.85	±0.20	8
	III	8	16	10.1	±1.17	±0.29	12
Beta	I	8	17	34.8	±2.11	±0.51	6
	II	9	18	33.7	±2.60	±0.61	8
	III	8	16	37.5	±s.16	±0.54	6
Gamma + Neutral fat	I	8	17	20.3	±1.72	±0.42	8
	II	9	18	20.0	±1.85	±0.44	9
	III	8	16	19.9	±1.41	±0.35	7

Appendix Table 6 (cont.)

Fraction	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 3				
Lipalbumin	I	8	17	27.6	±1.91	±0.48	7
	II	9	18	27.3	±2.09	±0.49	8
	III	8	16	25.7	±1.48	±0.37	6
$Alpha_1$	I	8	17	8.4	±1.03	±0.26	12
	II	9	18	8.8	±1.22	±0.29	14
	III	8	16	8.1	±1.51	±0.38	19
$Alpha_2$	I	8	17	11.6	±1.01	±0.25	9
	II	9	18	11.8	±1.08	±0.25	9
	III	8	16	11.9	±1.04	±0.26	9
Beta	I	8	17	30.2	±2.11	±0.53	7
	II	9	18	30.9	±2.75	±0.65	9
	III	8	16	33.3	±1.75	±0.44	5
Gamma + Neutral fat	I	8	17	22.3	±1.64	±0.41	7
	II	9	18	21.1	±1.97	±0.46	9
	III	8	16	21.0	±1.32	±0.33	6

Appendix Table 7 Variability in Ratios of Serum Lipoprotein Fractions

Ratio	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 1				
Beta/Alpha$_1$ + Alpha$_2$	I	8	17	2.13	±0.26	±0.06	12
	II	9	18	2.21	±0.26	±0.06	12
	III	8	16	2.67	±0.42	±0.10	16
Beta/Alpha$_1$ + Alpha$_2$ + Lipalbumin	I	8	17	0.99	±0.08	±0.02	8
	II	9	18	1.01	±0.11	±0.03	11
	III	8	16	1.12	±0.10	±0.03	9
Beta/Lip-albumin	I	8	17	1.85	±0.15	±0.04	8
	II	9	18	1.86	±0.23	±0.05	12
	III	8	16	1.93	±0.16	±0.04	8
Beta/Gamma + Neutral fat	I	8	17	2.02	±0.31	±0.08	15
	II	9	18	2.11	±0.28	±0.07	13
	III	8	16	2.19	±0.26	±0.06	12
			Subject No. 2				
Beta/Alpha$_1$ + Alpha$_2$	I	8	17	1.94	±0.27	±0.07	14
	II	9	18	1.78	±0.24	±0.06	13
	III	8	16	2.19	±0.31	±0.08	14
Beta/Alpha$_1$ + Alpha$_2$ + Lipalbumin	I	8	17	0.78	±0.07	±0.02	9
	II	9	18	0.73	±0.08	±0.02	11
	III	8	16	0.88	±0.08	±0.02	9
Beta/Lip-albumin	I	8	17	1.32	±0.15	±0.04	11
	II	9	18	1.26	±0.20	±0.05	16
	III	8	16	1.50	±0.18	±0.05	12
Beta/Gamma + Neutral fat	I	8	17	1.73	±0.23	±0.06	13
	II	9	18	1.71	±0.26	±0.06	15
	III	8	16	1.90	±0.21	±0.05	11

Appendix Table 7 (cont.)

Ratio	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 3				
Beta/Alpha$_1$ + Alpha$_2$	I	8	17	1.53	±0.19	±0.05	12
	II	9	18	1.51	±0.21	±0.05	14
	III	8	16	1.68	±0.23	±0.06	14
Beta/Alpha$_1$ + Alpha$_2$ + Lipalbumin	I	8	17	0.64	±0.06	±0.01	9
	II	9	18	0.65	±0.08	±0.02	12
	III	8	16	0.73	±0.06	±0.01	8
Beta/Lip-albumin	I	8	17	1.10	±0.12	±0.03	11
	II	9	18	1.14	±0.12	±0.04	14
	III	8	16	1.30	±0.10	±0.02	8
Beta/Gamma + Neutral fat	I	8	17	1.37	±0.19	±0.05	14
	II	9	18	1.48	±0.24	±0.06	16
	III	8	16	1.60	±0.16	±0.04	10

Appendix Table 8 Weekly Means of Serum Protein Fractions, Percentage Concentration

Fraction	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
					Subject No. 1					
Albumin	I	67.0	66.9	69.4	68.1	66.3	64.0	66.2	67.7	
	II	66.8	65.9	64.1	66.6	66.5	66.7	67.4	66.9	63.7
	III	65.5	68.4	69.6	69.4	68.9	65.6	62.8	65.2	
$Alpha_1$	I	3.2	3.7	4.0	3.3	3.5	3.8	3.7	3.4	
	II	3.6	4.5	4.6	4.2	3.8	3.9	3.8	3.5	4.2
	III	3.9	2.7	3.2	2.9	3.1	3.6	4.0	3.6	
$Alpha_2$	I	7.9	8.0	7.7	8.3	9.2	8.9	8.8	8.4	
	II	8.1	8.4	9.2	8.6	8.7	8.2	8.2	7.8	9.7
	III	9.4	7.3	7.3	7.2	7.4	8.6	8.9	8.2	
Beta	I	10.4	9.4	8.3	9.3	9.2	10.6	9.5	9.4	
	II	10.6	9.0	10.1	10.0	9.2	9.3	9.6	9.6	9.9
	III	9.9	9.9	8.9	9.7	8.7	10.3	11.3	11.3	
Gamma	I	11.5	12.0	10.6	11.0	11.8	12.7	11.9	11.2	
	II	10.9	12.3	12.0	10.6	11.8	11.9	11.1	12.2	12.6
	III	11.3	11.7	10.9	10.9	12.0	11.9	12.9	11.8	
					Subject No. 2					
Albumin	I	68.5	68.3	70.8	70.2	79.5	66.6	68.3	71.4	
	II	71.0	70.0	66.5	68.9	67.4	68.7	68.5	69.2	64.9
	III	67.6	68.5	71.8	71.2		67.1	63.8	67.9	
$Alpha_1$	I	4.0	4.0	3.7	3.8	3.8	4.5	4.0	3.3	
	II	3.5	4.0	4.5	3.7	4.4	3.4	3.8	4.0	4.4
	III	4.1	4.4	2.5	2.9		3.6	3.6	3.5	
$Alpha_2$	I	7.2	6.9	7.2	6.5	6.4	7.3	6.4	6.2	
	II	6.5	7.0	6.4	7.0	7.2	7.3	7.2	6.4	8.2
	III	6.9	6.1	5.9	6.3		6.6	8.0	7.0	
Beta	I	8.8	8.9	7.6	8.1	8.3	9.5	9.6	8.3	
	II	8.4	8.5	9.9	8.5	8.8	8.4	8.5	7.8	9.6
	III	9.5	8.8	8.3	8.4		9.8	11.0	8.9	
Gamma	I	11.6	12.0	10.7	11.5	12.1	12.1	11.7	10.8	
	II	10.6	10.6	12.7	11.9	12.2	12.2	12.0	12.6	12.9
	III	11.9	12.2	11.5	11.2		12.9	13.6	12.6	

Appendix Table 8 (cont.)

Fraction	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
					Subject No. 3					
Albumin	I	67.8	67.9	69.5	68.9	67.0	66.0	68.0	71.6	
	II	70.3	68.0	65.8	69.0	68.7	68.3	69.7	68.6	66.3
	III	69.4	67.7	72.0	70.8	70.0	66.3	66.6	69.8	
$Alpha_1$	I	4.6	4.1	3.4	3.7	4.4	4.3	4.4	3.6	
	II	3.8	4.4	4.0	3.5	4.6	4.3	4.0	4.0	4.3
	III	3.6	4.3	2.9	3.4	3.6	3.9	4.2	3.6	
$Alpha_2$	I	6.8	7.0	7.2	7.3	7.9	7.0	7.1	6.6	
	II	6.2	7.1	7.3	7.5	6.6	7.0	7.0	5.7	8.6
	III	6.9	7.4	6.5	6.6	7.1	7.8	7.6	6.6	
Beta	I	8.2	8.3	7.2	7.8	8.1	9.8	9.2	8.0	
	II	8.5	8.3	9.6	8.5	8.2	8.5	7.9	9.1	8.9
	III	8.5	8.7	7.3	7.8	7.5	8.5	9.5	8.6	
Gamma	I	12.7	12.6	12.7	12.4	12.6	12.8	11.3	10.2	
	II	11.3	12.2	13.3	11.4	11.9	11.8	11.3	12.6	11.9
	III	11.6	12.0	11.3	11.5	12.2	13.6	12.2	11.4	
					STANDARD					
Albumin	I	63.0	65.9	65.5	65.4	64.4	61.8	63.6	65.7	
	II	67.7	66.2	63.4	64.0	63.9	65.9	63.8	66.4	61.3
	III	61.4	65.7	68.1	69.8	68.5	64.4	62.4	63.9	
$Alpha_1$	I	4.2	3.2	3.4	3.5	3.8	3.8	4.1	3.3	
	II	3.4	3.6	4.0	3.6	3.8	3.6	3.5	3.4	4.5
	III	4.0	3.5	2.8	2.7	3.1	3.5	3.7	3.7	
$Alpha_2$	I	8.2	7.9	7.8	7.7	8.2	8.3	7.2	7.6	
	II	6.9	7.7	7.8	8.1	7.9	7.6	8.6	6.5	8.1
	III	8.3	7.6	7.0	6.4	7.0	7.9	7.7	7.0	
Beta	I	9.5	9.3	9.3	9.0	8.6	9.7	10.1	9.2	
	II	9.2	8.9	9.7	9.6	9.4	9.0	9.3	9.7	9.6
	III	11.1	9.0	8.6	8.5	8.2	10.2	10.3	10.4	
Gamma	I	15.2	13.7	14.0	14.4	15.1	16.4	15.0	14.2	
	II	12.9	13.6	15.1	14.7	15.0	13.9	14.8	14.0	16.6
	III	15.3	14.3	13.6	12.6	13.2	14.1	15.8	14.9	

Appendix Table 9 Ratios of Serum Protein Fractions, Weekly Means

Ratio	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Subject No. 1										
Albumin/ Globulin	I	2.04	2.03	2.28	2.15	1.97	1.78	1.96	2.13	
	II	2.02	1.93	1.79	2.00	1.99	2.01	2.08	2.02	1.76
	III	1.90	2.19	2.30	2.28	2.22	1.91	1.69	1.88	
Beta/ Albumin	I	0.16	0.14	0.12	0.14	0.14	0.17	0.14	0.14	
	II	0.16	0.14	0.16	0.15	0.14	0.14	0.14	0.14	0.16
	III	0.15	0.15	0.13	0.14	0.13	0.16	0.18	0.18	
Beta/Alpha$_1$ + Alpha$_2$	I	0.95	0.81	0.71	0.81	0.73	0.83	0.76	0.80	
	II	0.91	0.70	0.74	0.79	0.74	0.77	0.81	0.85	0.72
	III	0.75	1.00	0.85	0.98	0.84	0.84	0.88	0.96	
Beta/Alpha$_1$ + Alpha$_2$ + Albumin	I	0.14	0.12	0.11	0.12	0.12	0.14	0.12	0.12	
	II	0.14	0.12	0.13	0.13	0.12	0.12	0.12	0.12	0.13
	III	0.13	0.13	0.11	0.13	0.11	0.14	0.15	0.15	
Subject No. 2										
Albumin/ Globulin	I	2.18	2.15	2.43	2.36	2.28	2.00	2.16	2.55	
	II	2.45	2.33	2.00	2.23	2.07	2.19	2.19	2.24	1.86
	III	2.10	2.18	2.54	2.49	2.34	2.05	1.77	2.14	
Beta/ Albumin	I	0.13	0.13	0.11	0.12	0.12	0.14	0.14	0.12	
	II	0.12	0.12	0.15	0.12	0.13	0.12	0.13	0.11	0.15
	III	0.14	0.13	0.12	0.12	0.12	0.15	0.18	0.13	
Beta/Alpha$_1$ + Alpha$_2$	I	0.79	0.83	0.70	0.79	0.82	0.81	0.93	0.88	
	II	0.84	0.77	0.90	0.80	0.76	0.79	0.78	0.74	0.77
	III	0.86	0.84	0.99	0.91	0.88	0.99	0.95	0.85	
Beta/Alpha$_1$ + Alpha$_2$ + Albumin	I	0.11	0.12	0.09	0.10	0.11	0.12	0.13	0.11	
	II	0.11	0.11	0.13	0.11	0.11	0.11	0.11	0.10	0.13
	III	0.12	0.11	0.11	0.11	0.10	0.13	0.15	0.12	

Appendix Table 9 (cont.)

Ratio	Period	Weeks 1st	2nd	3rd	4th	5th	6th	7th	8th	9th
					Subject No. 3					
Albumin/ Globulin	I	2.12	2.12	2.28	2.22	2.03	1.95	2.13	2.53	
	II	2.37	2.13	1.92	2.23	2.21	2.16	2.31	2.18	1.97
	III	2.27	2.10	2.58	2.45	2.29	1.97	1.99	2.32	
Beta/ Albumin	I	0.12	0.12	0.11	0.12	0.12	0.15	0.14	0.12	
	II	0.13	0.12	0.15	0.13	0.13	0.13	0.12	0.13	0.14
	III	0.12	0.13	0.10	0.11	0.11	0.13	0.15	0.13	
Beta/Alpha$_1$ + Alpha$_2$	I	0.73	0.75	0.68	0.71	0.66	0.87	0.80	0.79	
	II	0.85	0.73	0.85	0.78	0.74	0.76	0.72	0.94	0.70
	III	0.81	0.74	0.78	0.77	0.70	0.74	0.82	0.84	
Beta/Alpha$_1$ + Alpha$_2$ + Albumin	I	0.11	0.11	0.09	0.10	0.10	0.13	0.12	0.10	
	II	0.11	0.11	0.13	0.11	0.11	0.11	0.10	0.12	0.12
	III	0.11	0.11	0.09	0.10	0.10	0.11	0.12	0.11	
					Standard					
Albumin/ Globulin	I	1.72	1.94	1.91	1.90	1.81	1.62	1.75	1.94	
	II	2.10	1.97	1.73	1.79	1.77	1.93	1.77	1.98	1.58
	III	1.59	1.92	2.14	2.31	2.18	1.81	1.68	1.79	
Beta/ Albumin	I	0.16	0.14	0.14	0.14	0.14	0.16	0.16	0.16	
	II	0.14	0.14	0.16	0.15	0.15	0.14	0.15	0.15	0.16
	III	0.18	0.14	0.13	0.12	0.12	0.16	0.17	0.17	
Beta/Alpha$_1$ +Alpha$_2$	I	0.79	0.84	0.84	0.81	0.72	0.80	0.90	0.85	
	II	0.90	0.80	0.83	0.83	0.81	0.80	0.77	0.98	0.76
	III	0.90	0.82	0.88	0.94	0.81	0.90	0.90	0.97	
Beta/Alpha$_1$ + Alpha$_2$ + Albumin	I	0.13	0.12	0.12	0.12	0.11	0.14	0.14	0.12	
	II	0.12	0.12	0.13	0.13	0.13	0.12	0.13	0.13	0.13
	III	0.15	0.12	0.11	0.11	0.11	0.14	0.14	0.14	

Appendix Table 10 Variability in Serum Protein Fractions, Percentage Concentration

Fraction	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 1				
Albumin	I	8	17	67.2	±2.11	±0.51	3
	II	9	17	66.0	±1.55	±0.38	2
	III	8	16	66.9	±2.82	±0.71	4
$Alpha_1$	I	8	17	3.6	±0.44	±0.11	12
	II	9	17	4.0	±0.47	±0.11	12
	III	8	16	3.4	±0.59	±0.15	17
$Alpha_2$	I	8	17	8.3	±0.77	±0.19	9
	II	9	17	8.6	±0.67	±0.16	8
	III	8	16	8.0	±0.94	±0.24	12
Beta	I	8	17	9.4	±0.90	±0.22	10
	II	9	17	9.7	±0.63	±0.15	6
	III	8	16	10.0	±0.99	±0.25	10
Gamma	I	8	17	11.5	±0.83	±0.20	7
	II	9	17	11.7	±0.83	±0.20	7
	III	8	16	11.7	±0.91	±0.23	8
			Subject No. 2				
Albumin	I	8	17	69.4	±2.16	±0.52	3
	II	9	17	68.3	±2.16	±0.52	3
	III	8	15	68.4	±2.93	±0.76	4
$Alpha_1$	I	8	17	3.9	±0.43	±0.10	11
	II	9	17	4.0	±0.50	±0.12	13
	III	8	15	3.5	±0.73	±0.19	21
$Alpha_2$	I	8	17	6.7	±0.69	±0.17	10
	II	9	17	7.1	±0.60	±0.15	8
	III	8	15	6.6	±0.88	±0.23	13
Beta	I	8	17	8.6	±0.95	±0.23	11
	II	9	17	8.8	±0.83	±0.20	9
	III	8	15	9.2	±1.06	±0.27	12
Gamma	I	8	17	11.5	±0.80	±0.19	7
	II	9	17	11.9	±1.16	±0.28	10
	III	8	15	12.3	±1.20	±0.31	10

Appendix Table 10 (cont.)

Fractions	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 3				
Albumin	I	8	17	68.5	±2.01	±0.49	3
	II	9	17	68.3	±1.66	±0.40	2
	III	8	16	69.0	±2.36	±0.59	3
$Alpha_1$	I	8	17	4.0	±0.51	±0.12	13
	II	9	17	4.1	±0.42	±0.10	10
	III	8	16	3.7	±0.67	±0.17	18
$Alpha_2$	I	8	17	7.1	±0.61	±0.15	9
	II	9	17	7.1	±0.79	±0.19	11
	III	8	16	7.1	±0.59	±0.15	8
Beta	I	8	17	8.3	±0.93	±0.23	11
	II	9	17	8.6	±0.61	±0.15	7
	III	8	16	8.3	±0.97	±0.24	12
Gamma	I	8	17	12.1	±1.06	±0.26	9
	II	9	17	11.9	±0.86	±0.21	7
	III	8	16	12.0	±0.87	±0.22	7
			Standard				
Albumin	I	8	17	64.6	±2.33	±0.57	4
	III	8	16	65.5	±3.38	±0.84	5
$Alpha_1$	I	8	17	3.6	±0.57	±0.14	16
	III	8	16	3.4	±0.59	±0.15	17
$Alpha_2$	I	8	17	7.8	±0.86	±0.21	11
	III	8	16	7.3	±0.74	±0.18	10
Beta	I	8	17	9.2	±0.80	±0.19	9
	III	8	16	9.5	±1.25	±0.31	13
Gamma	I	8	17	14.7	±1.06	±0.26	7
	III	8	16	14.2	±1.24	±0.31	9

Appendix Table 11 Variability in Ratios of Serum Protein Fractions

Ratio	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 1				
Albumin/ Globulin	I	8	17	2.06	±0.20	±0.05	10
	II	9	17	1.95	±0.13	±0.03	7
	III	8	16	2.05	±0.27	±0.07	13
Beta/ Albumin	I	8	17	0.14	±0.02	±0.004	13
	II	9	17	0.15	±0.01	±0.003	9
	III	8	16	0.15	±0.02	±0.005	14
Beta/$Alpha_1$ + $Alpha_2$	I	8	17	0.79	±0.08	±0.02	10
	II	9	17	0.77	±0.08	±0.02	10
	III	8	16	0.89	±0.09	±0.02	11
Beta/$Alpha_1$ + $Alpha_2$ + Albumin	I	8	17	0.12	±0.01	±0.003	12
	II	9	17	0.12	±0.01	±0.002	8
	III	8	16	0.13	±0.02	±0.004	12
			Subject No. 2				
Albumin/ Globulin	I	8	17	2.28	±0.25	±0.06	11
	II	9	17	2.17	±0.21	±0.05	10
	III	8	15	2.19	±0.30	±0.08	14
Beta/ Albumin	I	8	17	0.12	±0.02	±0.004	13
	II	9	17	0.13	±0.02	±0.004	13
	III	8	15	0.14	±0.02	±0.006	16
Beta/$Alpha_1$ +$Alpha_2$	I	8	17	0.82	±0.08	±0.02	9
	II	9	17	0.80	±0.06	±0.02	8
	III	8	15	0.91	±0.10	±0.03	11
Beta/$Alpha_1$ +$Alpha_2$ + Albumin	I	8	17	0.11	±0.01	±0.004	14
	II	9	17	0.11	±0.01	±0.003	11
	III	8	15	0.12	±0.02	±0.004	14

Appendix Table 11 (cont.)

Ratio	Period	Weeks	N	Mean	S.D.	S.E.	C.V.
			Subject No. 3				
Albumin/ Globulin	I	8	17	2.19	±0.21	±0.05	9
	II	9	17	2.16	±0.17	±0.04	8
	III	8	16	2.24	±0.25	±0.06	11
Beta/ Albumin	I	8	17	0.12	±0.02	±0.004	13
	II	9	17	0.13	±0.01	±0.002	8
	III	8	16	0.12	±0.02	±0.004	14
Beta/$Alpha_1$ + $Alpha_2$	I	8	17	0.75	±0.07	±0.02	10
	II	9	17	0.77	±0.08	±0.02	11
	III	8	16	0.77	±0.08	±0.02	11
Beta/$Alpha_1$ + $Alpha_2$ + Albumin	I	8	17	0.10	±0.01	±0.003	13
	II	9	17	0.11	±0.01	±0.003	9
	III	8	16	0.11	±0.01	±0.003	13
			Standard				
Albumin/ Globulin	I	8	17	1.84	±0.19	±0.05	10
	II	9	17	1.84	±0.19	±0.04	10
	III	8	16	1.93	±0.28	±0.07	15
Beta/ Albumin	I	8	17	0.14	±0.02	±0.004	12
	II	9	17	0.15	±0.01	±0.003	9
	III	8	16	0.15	±0.02	±0.007	18
Beta/$Alpha_1$ + $Alpha_2$	I	8	17	0.82	±0.08	±0.02	10
	II	9	17	0.82	±0.08	±0.02	10
	III	8	16	0.89	±0.07	±0.02	8
Beta/$Alpha_1$ + $Alpha_2$ + Albumin	I	8	17	0.12	±0.01	±0.004	12
	II	9	17	0.12	±0.01	±0.002	8
	III	8	16	0.13	±0.02	±0.005	16