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Authors

Nichols, Alex V.
Dobbin, Virginia
Gofman, John W.

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Alex V. Nichols, Virginia Dobbin, and John W. Gofman

Donner Laboratory of Biophysics and Medical Physics,
Radiation Laboratory, and
Dietetic Department of the E. V. Cowell Memorial Hospital
University of California, Berkeley, California

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ABSTRACT

1. In a controlled study of five individuals on isocaloric diets, the Standard $s_f^o 0-12$ and Standard $s_f^o 12-20$ lipoproteins are much higher in a diet high in fat of animal origin than with either a diet equally high in fat of vegetable origin or a diet low in fat, but supplemented by carbohydrate. With respect to $s_f^o 0-12$ and $s_f^o 12-20$ lipoproteins, no significant difference was apparent between the diet low in fat and the diet high in vegetable fat. The difference between animal and vegetable fat appears to be due to an unfavorable effect of animal fat rather than a favorable effect of vegetable fat.

2. In the same study, the level of the Standard $s_f^o 20-400$ group of lipoproteins was essentially similar on the diet high in vegetable fat to the level on the diet high in animal fat, but distinctly higher on the low-fat, high-carbohydrate diet. The changes in $s_f^o 20-400$ lipoproteins appear to have been the result of the elevation in carbohydrate intake on the low-fat diet. Calories per se appear unimportant, with respect to this effect, since all diets were isocaloric.

3. A group of subjects in a weight-reduction study ingested a low-fat, low-carbohydrate diet. The $s_f^o 0-12$ and $s_f^o 20-400$ levels fell on this regimen, suggesting again that the effect on $s_f^o 20-400$ lipoproteins observed in the isocaloric diets had been due to the carbohydrate supplementation, rather than to any fat deficiency.

4. An egg-yolk supplement equivalent in egg yolk and cholesterol to the high-animal-fat diet in the isocaloric experiment produced minor elevation in the $s_f^o 0-12$ lipoproteins in a group of persons already on a moderately high animal-fat intake. Whether egg yold or cholesterol would produce a larger effect on a low-fat diet or a high-vegetable-fat diet remains unanswered from these data.

5. A dissociation exists between the effects of various dietary measures on the $s_f^o 0-20$ lipoproteins and the $s_f^o 20-400$ lipoproteins. On certain types of diets both classes change in the same direction; on others, the two classes may change in opposite directions. Since both classes are cholesterol bearers, the dissociation could be obscured if the only analytical measurement available were the serum cholesterol level.

THE INFLUENCE OF DIETARY FACTORS UPON HUMAN SERUM LIPOPROTEIN CONCENTRATIONS

Alex V. Nichols,* Virginia Dobbin,** and John W. Gofman

Donner Laboratory of Biophysics and Medical Physics,
Radiation Laboratory, and
Dietetic Department of the E. V. Cowell Memorial Hospital
University of California, Berkeley, California

May 8, 1956

INTRODUCTION

The significant position of lipoproteins in the series of variables generally considered pertinent to the study of atherosclerosis has prompted an evaluation of the effect of the dietary composition on human serum lipoprotein concentrations. The specific groups of lipoproteins significantly related to clinical atherosclerosis are the Standard $s_f^0 0-12$, $s_f^0 12-20$, $s_f^0 20-100$, and $s_f^0 100-400$ classes. Evidence has been presented that arteriosclerosis accumulates with increasing duration of elevated levels of these groups of lipoproteins[1] in the blood stream. Particular attention is given in this study to such variables as total dietary fat intake, source of dietary fat, carbohydrate intake, and caloric intake in the evaluation of the role of diet in the control of the serum levels of these particular lipoproteins.

The guiding principles of the dietary evaluation of total fat intake, source of fat, and carbohydrate intake were as follows:

(a) All dietary periods were approximately isocaloric. Thus, after initial stabilization, weight change was not a variable in the study.

(b) Calories derived from protein were kept approximately constant throughout the study.

(c) Only the calories derived from fat and carbohydrate were varied within the isocaloric periods.

(d) Sources of fat (i.e., animal or vegetable) were quantitatively recorded for each period.

The role of a combined regimen of weight decrease and fat restriction was assessed in a separate study of obese women, and its marked influence on serum lipoproteins is included in this report. Also, as a consequence of observations made during the dietary evaluation, an investigation was made of the effect on serum lipoproteins of a daily supplement of eggs to the routine diet of presumably normal individuals.

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* Research Fellow of the San Joaquin County Heart Association, an affiliate of the American Heart Association.

** Senior dietitian at Cowell Memorial Hospital.

METHODS

In the major dietary evaluation five male subjects ranging in age from 20 to 49 years of age (LH, 20; JM, 35; ES, 35; HS, 42; and WE, 49) ate their noon and evening meals at a diet table conducted by Mrs. Virginia Dobbin, Senior Dietitian, at the Cowell Memorial Hospital, University of California. Breakfast usually consisted of fruit juice, toast or cereal, skim milk, and (or) coffee, and was eaten at home.* All food prepared for each subject at the diet table was to be eaten; otherwise notation was to be made listing the amount not eaten at that meal.

Blood samples (30 cc) were ordinarily drawn at weekly intervals and the serum was analyzed for the concentration of the low-density lipoprotein classes ($s_f^0 0-12$, $s_f^0 12-20$, $s_f^0 20-100$, and $s_f^0 100-400$) by ultracentrifugal techniques described elsewhere.[2]

In profile the consecutive periods were of the duration and dietary composition presented in Tables I and II.

The investigation of the influence of weight reduction achieved on a diet restricted in total fat and carbohydrate was performed with the cooperation of the Herrick Memorial Hospital, Department of Research, Berkeley, California. This study sought to evaluate the nature of the response of obese individuals to a group situation organized to motivate weight reduction. A standard (1000 calories) reduction diet schedule (see Table III) was prepared and was available for the participants to use at home. The diet schedule emphasized substitution for foods high in fat and cholesterol. Twenty-eight subjects lost 10 or more pounds in a 2-month evaluation period. Lipoprotein determinations were made for these subjects before institution of the diet and again at the end of the 2-month period.

The egg-supplement experiment was performed with the cooperation of seven members of the Donner Laboratory staff** and Mrs. Virginia Dobbin. Following a 3-week control period, five of the above subjects added to their routine diet an egg preparation which contained three egg yolks per serving. Two servings were consumed during each day for a 5- to 6-week period.*** These subjects were followed for another 2 to 3 weeks after conclusion of this elevated egg-yolk intake. Two of the total of seven subjects served as controls for a 10-week period. Blood samples were drawn weekly and the serum analyzed for lipoprotein concentrations.

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* The average composition of breakfast was as follows: calories, 271; protein, 13 grams; fat, 2 grams; carbohydrates, 54 grams; cholesterol negligible.

** None of these seven was identical with any of the five subjects of the Cowell Hospital study.

*** Composition of average daily intake: egg yolks, 6; protein, 26 g; fat, 34 g, carbohydrate, 22 g; calories, 498. The egg supplement was provided in three different preparations: custard, skim milk "ice cream," and egg sandwiches. Each subject was allowed to choose, ad lib, the egg preparation for any particular day.

Table I

Profile of the dietary study periods

Period	Duration (weeks)	Average total calories/day	Average grams per day		
			Protein	Fat	Carbohydrate
I	2	2018	94	18	370
II	11	2088	78	100	219
III	10-1/2	2075	84	103	203
IV	5	2215	99	19	412
V	6	Interruption of diet table due to influenza epidemic			
VI	17	2034	97	18	371

	Percentage composition of total caloric intake		
	Protein	Fat	Carbohydrate
I	19	8	73
II	15	43	42
III	16	45	39
IV	18	8	74
V	6-week interruption		
VI	19	8	73

Period	Origin of fat (expressed as percentage of total fat intake per day)		
	Animal origin	Vegetable origin	Total fat (grams)
I	31	69	18
II	15	85	100
III	93	7	103
IV	60	40	19
V	6-week interruption		
VI	56	44	18

Table II

Detailed composition of the dietary in the several study periods

Distribution of protein sources

Period	Approximate percentage composition of total protein intake: Meat	Dairy	Egg (white or yolk)	Vegetable	Total protein (grams)
I	15	25	8	52	94
II	26	26	4	44	78
III	37	24	26	13	84
IV	18	32	2	48	99
V			(interruption)		
VI	17	30	-	53	97

Distribution of fat sources

Period	Approximate percentage composition of total fat intake: Meat	Dairy	Egg (yolk)	Vegetable	Total fat (grams)
I	26	5	-	69	18
II	14	1	-	85[a]	100
III	31	24	38	7	103
IV	50	10	-	40	19
V			(interruption)		
VI	50	6	-	44	18

Period	Daily cholesterol intake	Daily egg-yolk intake
I	Less than 0.5 g	None
II	Less than 0.5 g	None
III	Average of 2.2 g	Approximately 7/day
IV and VI	Less than 0.5 g	None

[a] Most of the vegetable fat utilized was a nonhydrogenated cottonseed oil.

Table III

Composition of the 1000-calorie reduction diet (Herrick Hospital)

	Calories	Protein (grams)	Fat (grams)	Carbohydrate (grams)
Breakfast	307	13	6	54
Lunch	319	30	10	29
Dinner	343	31	11	33
Totals	969	74	27	116

RESULTS

In the evaluation of total dietary fat, source of dietary fat, and carbohydrate intake, periods I, IV, and VI have been combined, since they represented similar dietary composition and since no statistically significant differences can be shown to exist for the group of subjects in these periods. The mean concentrations of the four classes of lipoproteins, s_f^o-12, s_f^o-20, $s_f^o20-100$, and $s_f^o100-400$, together with the standard error of the means, are presented in Table IV. The differences in lipoprotein levels between various dietary periods and the significance tests upon such differences (expressed as the probability that such differences may have arisen by chance) are presented in Table V.

No separation of so-called transition periods from one diet to the next was made. What transition effects there may be are included in the dietary period of which they represent a part. By having dietary periods sufficiently long, transition effects are minimized and it would seem reasonable that, if anything, the observed differences are conservatively stated.

The results of the study of 28 women who lost 10 pounds or more on the prescribed 1000-calorie reduction diet are presented in Table VI.

The results of the egg-supplement study are presented in Table VII. In the presentation of these data the control period represents the combined observations of the period before and after the egg-yolk-supplement period.

In consideration of the results, the various dietary periods of the Cowell Hospital experiment may be characterized by the following summary designations:

Periods I, IV, VI, -- Low total fat, low cholesterol, high carbohydrate.
Period II -- High vegetable fat, low cholesterol, moderate carbohydrate (some animal fat present)
Period III -- High animal fat, high cholesterol, moderate carbohydrate (some vegetable fat present).

From the nature of the above dietary regimes it should be possible to assess the effects of (a) variation in the origin of fat consumed, i.e., animal or vegetable origin, and (b) substitution of carbohydrate for fat in an isocaloric diet schedule on serum lipoprotein concentrations.

Table IV

Mean lipoprotein levels in the various dietary study periods
(Expressed in mg/100 ml)

Period	Number of Determinations[a]	$s_f^o 0-12$	$s_f^o 12-20$	$s_f^o 20-100$	$s_f^o 100-400$
Subject: WE					
I, IV, VI	27	335±4[b]	56±3	169±3	308±21
II	10	290±11	47±4	142±8	135±16
III	7	394±20	54±4	126±4	125±13
Subject: ES					
I, IV, VI	30	333±6	62±2	150±3	160±11
II	11	316±21	70±5	127±11	109±12
III	8	463±10	100±3	141±10	100±7
Subject: HS					
I, IV, VI	27	295±9	63±3	132±5	102±8
II	11	284±13	62±5	97±8	60±7
III	9	466±28	105±7	129±11	72±8
Subject: LH					
I, IV, VI	19	260±25	30±7	56±18	33±16
II	4	269±17	23±4	30±9	18±7
III	8	341±26	32±3	40±5	17±3
Subject: JM					
I, IV, VI	18	273±8	44±2	142±5	73±8
II	5	299±25	53±3	147±21	61±13
III	6	365±18	65±4	137±16	56±13

[a] Generally the interval between blood samples was one week.

[b] All standard errors are calculated as follows: $SE = SD/\sqrt{n-1}$, where SE = standard error of mean, SD = standard deviation of mean, and n = number of determinations.

Table V

Differences in lipoprotein levels between various dietary periods

Subject	Mean lipoprotein levels for dietary Period II minus mean for Dietary Periods I, IV, VI		Mean lipoprotein levels for dietary Period III minus mean for Periods I,IV, VI		Mean lipoprotein levels for dietary Period III minus mean for Period II	
	s_f^o0–12 lipoprotein					
	Δ Lipoprotein Level	Significance Test	Δ Lipoprotein Level	Significance Test	Δ Lipoprotein Level	Significance Test
WE	-45	p<0.01	+59	p<0.01	+104	p<0.01
ES	-17	NS	+130	p<0.01	+147	p<0.01
HS	-12	NS	+171	p<0.01	+183	p<0.01
LH	+9	NS	+81	0.02<p<0.05	+73	0.02<p<0.05
JM	+26	NS	+92	p<0.01	+66	0.05<p<0.1
	s_f^o12–20 lipoprotein					
WE	-9	0.05<p<0.1	-2	NS	+7	NS
ES	+7	NS	+38	p<0.01	+30	p<0.01
HS	-1	NS	+42	p<0.01	+43	p<0.01
LH	-7	NS	+2	NS	+8	NS
JM	+10	0.01<p<0.02	+22	p<0.01	+12	0.02<p<0.05
	s_f^o20–100 lipoprotein					
WE	-27	p<0.01	-43	p<0.01	-16	0.05<p<0.10
ES	-24	0.02<p<0.05	-9	NS	+15	NS
HS	-35	p<0.01	-3	NS	+33	0.02<p<0.05
LH	-26	NS	-16	NS	+10	NS
JM	+5	NS	-5	NS	-10	NS
	s_f^o100–400 lipoprotein					
WE	-173	p<0.01	-183	p<0.01	-10	NS
ES	-52	p<0.01	-60	p<0.01	-9	NS
HS	-42	p<0.01	-30	0.01<p<0.02	+10	NS
LH	-15	NS	-16	NS	0	NS
JM	-13	NS	-17	NS	-5	NS

Probability values of 0.1 or less are given; probability values greater than 0.1 are designated as NS (not significant)

Table VI

Changes in lipoprotein concentrations on 1000-calorie weight-reduction program (28 subjects)

	$s_f^o 0-12$ (mg/100 ml)	$s_f^o 12-20$ (mg/100 ml)	$s_f^o 20-100$ (mg/100 ml)	$s_f^o 100-400$ (mg/100 ml)
Initial mean lipoprotein levels	372	93	94	64 Mean wt. = 212 lbs.
Mean lipoprotein levels after 2 months on diet	326	61	72	27 Mean wt. = 198 lbs.
Δ Lipoprotein levels	46	32	22	37 Δ weight = 14 lbs.
Significance test for lipoprotein changes	$0.05<p<0.1$	$p<0.01$	$p<0.01$	$p<0.01$

Table VII

The effect of an egg-yolk supplement on serum lipoprotein levels

Subjects receiving egg-yolk supplement	s_f^0 0–12 (mg/100 ml)	s_f^0 12–20 (mg/100 ml)	s_f^0 20–100 (mg/100 ml)	s_f^0 100–400 (mg/100 ml)
FG(Male, 26 years)				
Lipoprotein level during egg supplement	277	41	37	4
Control lipoprotein level	243	30	34	7
Δ Lipoproteins	34	11	3	-3
Significance test	0.01<p<0.02	NS	NS	NS
AH(Female, 36 years)				
Lipoprotein level during egg supplement	449	19	7	1
Control lipoprotein level	430	26	15	1
Δ Lipoproteins	19	-7	-8	0
Significance test	NS	NS	NS	NS
DP(Female, 34 years)				
Lipoprotein level during egg supplement	295	19	11	4
Control lipoprotein level	243	19	19	4
Δ Lipoproteins	52	0	-8	0
Significance test	0.02<p<0.05	NS	NS	NS
DR(Male, 32 years)				
Lipoprotein level during egg supplement	494	52	41	4
Control lipoprotein level	479	49	56	4
Δ Lipoproteins	15	3	-15	0
Significance test	NS	NS	NS	NS
AT(Male, 27 years)				
Lipoprotein level during egg supplement	239	30	37	7
Control lipoprotein level	224	26	22	4
Δ Lipoproteins	15	4	15	3
Significance test	NS	NS	NS	NS

From the data in Tables IV and V, contrasting the low-fat, low-cholesterol, high-carbohydrate intake (Periods I, IV, VI) with the high-vegetable fat, low-cholesterol, moderate-carbohydrate diet (Period II), the following results are noted:

(a) Lipoproteins $s_f^0 0-12$. There is no consistent direction of difference in the $s_f^0 0-12$ lipoprotein group. There was a trend toward increased values of $s_f^0 0-12$ lipoproteins in most of the subjects during the latter half of the high-vegetable-fat period. However, the $s_f^0 0-12$ concentration values in this latter half could not be shown to be significantly different from those in the first half of the same period.

(b) Lipoproteins $s_f^0 12-20$. There is no consistent direction of difference in the $s_f^0 12-20$ lipoprotein group in the five subjects.

(c) Lipoproteins $s_f^0 20-100$. There is a lower level of $s_f^0 20-100$ lipoproteins in four of the five subjects in the high-vegetable-fat, moderate-carbohydrate period. The magnitude of the difference for the subjects covers a range of from 16% to 47% of the mean $s_f^0 20-100$ level that was present in the low-fat, high-carbohydrate period. The average difference for these four subjects is approximately 27%.

(d) Lipoproteins $s_f^0 100-400$. There is a markedly lower level of $s_f^0 100-400$ lipoproteins in all five subjects in the high-vegetable-fat, moderate carbohydrate period. The magnitude of the difference covers a range of from 17% to 56% of the mean $s_f^0 100-400$ level that was present in the low-fat, high-carbohydrate-period. The average difference for all subjects is approximately 38%.

Contrasting the low-fat, low-cholesterol, high-carbohydrate period (Periods I, IV, VI) with the high-animal-fat, high-cholesterol, moderate-carbohydrate period (Period III), we can make the following observations:

(a) Lipoproteins $s_f^0 0-12$. There is a markedly elevated level of $s_f^0 0-12$ lipoproteins in all five subjects in the high-animal-fat, moderate-carbohydrate period. The magnitude of the difference covers a range of from 17% to 58% of the mean $s_f^0 0-12$ level in the low-fat, high-carbohydrate period. The average difference for all subjects is approximately 36%.

(b) Lipoproteins $s_f^0 12-20$. There is a higher level of $s_f^0 12-20$ lipoproteins in four of the five subjects in the high-animal-fat, moderate-carbohydrate period. The magnitude of the difference covers a range of from 6% to 67% of the mean $s_f^0 12-20$ level in the low-fat, high-carbohydrate period. The average difference for these four subjects is approximately 46%.

(c) Lipoproteins $s_f^0 20-100$. There is a lower level of $s_f^0 20-100$ lipoproteins in all five of the subjects in the high-animal-fat, moderate-carbohydrate period. The magnitude of the difference covers a range of from 2% to 29% of the mean $s_f^0 20-100$ level in the low-fat, high-carbohydrate period. The average difference for all subjects is approximately 13%.

(d) Lipoproteins $s_f^0 100-400$. There is a definitely lower level of $s_f^0 100-400$ lipoproteins in all five of the subjects in the high-animal-fat, moderate-carbohydrate period. The magnitude of the difference covers a range of from 23% to 60% of the mean $s_f^0 100-400$ level in the low-fat, high-carbohydrate period. The average difference for all subjects is approximately 40%.

When the high-vegetable-fat, moderate-carbohydrate period (Period II) is contrasted with the high-animal-fat, moderate-carbohydrate period (Period III), the following observations can be made:

(a) Lipoproteins $s_f^o 0-12$. There is a markedly higher level of $s_f^o 0-12$ lipoproteins in all five of the subjects in the high-animal-fat period. The magnitude of the difference covers a range of from 22% to 64% of the mean $s_f^o 0-12$ level in the high-vegetable-fat period. The average difference for all subjects is approximately 39%.

(b) Lipoproteins $s_f^o 12-20$. There is a higher level of $s_f^o 12-20$ lipoproteins in all five subjects in the high-animal-fat period. The magnitude of the difference covers a range of from 15% to 70% of the mean $s_f^o 12-20$ level in the high-vegetable-fat period. The average difference for all subjects is approximately 38%.

(c) Lipoproteins $s_f^o 20-100$. There is no consistent direction of difference in the $s_f^o 20-100$ lipoproteins in the five subjects.

(d) Lipoproteins $s_f^o 100-400$. There is a slightly lower measured level of $s_f^o 100-400$ lipoproteins in four of the five subjects in the high-animal-fat period, but the changes cannot be proven significant. The magnitude of the difference covers a range of from 2% to 8% of the mean $s_f^o 100-400$ level in the high-vegetable-fat period, for each subject. The average difference for these four subjects is approximately 6% (not provably significant either for individuals or for the group).

DISCUSSION

One of the most striking features of these studies of the relationship of dietary factors to serum lipid levels is the dissociation between the effects produced by a particular diet on one segment of the lipoprotein spectrum and the effects produced on another segment of that spectrum. Broadly, it may be stated from the current data that the Standard $s_f^o 0-12$ and Standard $s_f^o 12-20$ lipoproteins are markedly affected by certain dietary modifications, with essentially no effect on the Standard $s_f^o 20-100$ and Standard $s_f^o 100-400$ lipoproteins, whereas other dietary modifications produce little or no effect on the Standard $s_f^o 0-12$ and Standard $s_f^o 12-20$ lipoproteins, but do produce marked effects upon the Standard $s_f^o 20-100$ and Standard $s_f^o 100-400$ lipoproteins. Since cholesterol is a chemical component in all these lipoprotein classes, it is possible that the serum cholesterol may rise, may remain constant, or may fall with certain dietary alterations, depending upon the direction of change and the magnitude of change of the various parts of the low-density lipoprotein spectrum. A constant serum cholesterol level could be expected even in the face of major alterations in serum lipoprotein transport if changes in one segment approximately balanced those in another. Under such circumstances the constancy of the serum cholesterol would falsely indicate no dietary effect.

"Animal" versus "Vegetable" Fat in the Diet

There exists considerable contradictory opinion in the literature as to the relative roles of fats of animal and of vegetable origin in the control of serum lipid levels. Most studies employed serum cholesterol levels as a guide in assessing responses. Furthermore, the composition

of either the animal fat or the vegetable fat varied from study to study. Hildreth[3] and Keys[4] found that addition of vegetable fat to a low-fat diet caused a rise in serum cholesterol levels, both indicating that fat of either animal or vegetable origin was equivalent in effect upon serum cholesterol levels. Kinsell,[5] Groen,[6] Ahrens,[7] and Hardinge[8] concluded that animal fat in the dietary was associated with higher serum cholesterol levels than was vegetable fat. In the studies, presented here, with diets specified in Tables I and II, the following conclusions are quite clearly indicated:

(a) There is a marked difference with respect to effect upon serum lipid levels between isocaloric diets high in vegetable fat and those high in animal fat.

(b) The Standard $s_f^o 0-12$ and Standard $s_f^o 12-20$ lipoprotein levels* are both markedly higher when fat of animal origin is utilized in the diet than when fat of vegetable origin is used. The Standard $s_f^o 20-100$ and Standard $s_f^o 100-400$ lipoprotein levels are virtually independent of whether the fat ingested is of animal or vegetable origin.

(c) The question arose as to whether the cholesterol of the animal fat regimen was responsible for the lipoprotein effects observed, since the animal-fat diet was also very high in cholesterol, primarily from egg yolks.[9] From the data obtained (see Table VII) in the egg-yolk-supplement study, it appears that cholesterol per se or any constituent of egg yolk cannot provoke a large rise in $s_f^o 0-12$ lipoproteins when that supplement is added to an average American diet (moderately high in animal fat). Whether the marked rise in $s_f^o 0-12$ lipoprotein observed in going from the low-fat or high-vegetable-fat diet to the high-animal fat, high-cholesterol diet was the result of cholesterol (or other constituent of egg yolk) or of some other agent, such as animal fat itself, cannot be answered from the studies reported herein.

(d) The observations raise the question whether diets high in fat of animal origin exert a noxious effect upon serum lipid levels or whether, alternatively, there is some positively favorable effect of some constituent of diets high in fat of vegetable origin. These studies lead to the conclusion that a noxious effect of animal-fat sources, rather than a protective effect of vegetable-fat sources, explains the observed results. This conclusion is based upon the observation that, with isocaloric diets, the $s_f^o 0-12$ and $s_f^o 12-20$ lipoprotein levels are not significantly different in the period of high-vegetable-fat intake from what they are in the period of low total fat intake. Were the vegetable fat protective, this result would not be expected.

(e) From the lipoprotein alterations observed and from independent studies of the chemical composition of serum lipoproteins, the expected differences in serum cholesterol levels between the animal-fat and vegetable-fat dietary periods can be estimated, if the assumption is made that there is no alteration in high-density lipoprotein.[10]

For Standard $s_f^o 0-20$ lipoproteins, approximately 34% of the lipoprotein is cholesterol. For Standard $s_f^o 20-400$ lipoproteins, approximately 13% of the lipoprotein is cholesterol. For the five study cases in

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* These lipoprotein classes are the major cholesterol bearers in serum.

this series, the lipoprotein and estimated cholesterol alterations between animal- and vegetable-fat diets are presented as follows:

	Case					Mean
	LH	JM	HS	WE	ES	
Δs_f^0-20 (animal-fat period minus vegetable-fat period)(mg/100 ml)	81	78	226	111	178	135
$\Delta s_f^{20}-400$(animal-fat period minus vegetable-fat period)(mg/100 ml)	10	-15	45	-26	6	4
Δ cholesterol from s_f^0-20	27	22	76	37	60	45
Δ cholesterol from $s_f^{20}-400$	1	-2	6	-3	1	1
estimated Δtotal serum cholesterol	28	20	82	34	61	46

From independent serum cholesterol determinations on these five subjects during the high-animal-fat period, the mean serum cholesterol was 297 mg/100 ml. With an estimated drop of 46 mg/100 ml (from the lipoprotein alterations), this would represent a 15.5% fall in cholesterol in the shift to high vegetable fat from high animal fat. The order of magnitude of this fall is quite comparable with the 24% observed by Ahrens in his six subjects on formula diets, where transition periods were excluded from his analysis.[7]

(f) It should be emphasized that caloric intake per se played no role in the observed changes between high-vegetable-fat and high-animal-fat diets, since in both periods the caloric intake was maintained constant.

High-Fat versus Low-Fat Diets (at Isocaloric Levels)

In addition to the above-described observations on the relative effects of fats of animal and of vegetable origins, the current studies allow certain conclusions to be drawn concerning the effects of high-fat versus low-fat diets. In maintenance of isocaloric composition of the diet when shifting from high fat to low fat, two possible choices of supplementation of calories are possible--replacement of the fat with either carbohydrate or protein. Only the replacement with carbohydrate was investigated in this experiment. From these studies with a carbohydrate replacement for fat in the low-fat but isocaloric diet, the following conclusions may be drawn:

(a) The low-fat diet, maintained isocaloric with carbohydrate supplementation, produces effects upon the serum lipoproteins different from either the high-vegetable fat or high-animal-fat diet discussed above.

(b) The s_f^0-12 and $s_f^{12}-20$ lipoproteins showed no consistent differences for the high-vegetable-fat diet in comparison with the low-fat isocaloric diet. The s_f^0-12 and $s_f^{12}-20$ lipoproteins were markedly lower on the low-fat diet than on the diet high in fat of animal origin. Since the s_f^0-12 and $s_f^{12}-20$ lipoprotein concentrations were essentially identical on the

low-fat and the high-vegetable-fat diets, at isocaloric levels, there would appear to be no reason to suspect any effect of vegetable fat in keeping the levels of these lipoprotein classes low; rather, the evidence suggests that animal fat is associated with their elevation.

(c) The $s_f^o 20-100$ and $s_f^o 100-400$ lipoprotein classes, considered as a group, showed a consistent and signficant elevation during the low-fat dietary period in contrast with either the high-vegetable-fat period or the high-animal-fat period. Of especial interest is the fact that although the $s_f^o 20-100$ and $s_f^o 100-400$ lipoproteins, from chemical composition studies, are high in triglyceride content (approximately 50% of the lipoprotein is triglyceride), the serum levels of these lipoprotein classes are elevated on a diet low in triglyceride content. It is of prime importance to understand what specific dietary factor may account for the observed elevation of $s_f^o 20-100$ and $s_f^o 100-400$ lipoproteins on the low-fat high-carbohydrate diet. Calories, per se, should be considered, but it is evident that calories per se cannot possibly be responsible for the observed lipoprotein findings, since all three dietary periods were isocaloric. The effect on $s_f^o 20-400$ lipoproteins was the same for animal and for vegetable fat, both of which are equivalent in carbohydrate content. The one outstanding difference in the low-fat period was the high carbohydrate content of the diet, which was necessary to maintain the diets isocaloric. It appears that the most reasonable explanation of the observations is that carbohydrate itself in excess can result in elevation of the $s_f^o 20-400$ lipoprotein classes. Good evidence from the work of Hatch, Abell, and Kendall, already in the literature,[11] is in agreement with this conclusion. In their studies of the rice diet, it was shown that the replacement of dietary fat by carbohydrate in the form of rice (at isocaloric levels) resulted, in many patients, in an increase in the serum concentration of neutral fat and, where measurements were available, in the $s_f 20-100$ lipoprotein levels. Although their lipoprotein measurements were not corrected to standard flotation conditions, there is a high correlation between the older $s_f 20-100$ measurements and the Standard $s_f^o 20-400$ measurements. The elevation of neutral fat would in general bespeak a probable elevation in $s_f^o 20-400$ lipoproteins, since these are ordinarily the major neutral fat bearers of serum. Whether or not the replacement of dietary fat by protein instead of carbohydrate, in the effort to maintain isocaloricity, would have resulted in analogous $s_f^o 20-400$ lipoprotein elevations cannot be answered from the studies, here reported, since such protein supplementation was not tried.

A possible alternative explanation of either the results reported here or those of Hatch and co-workers is that fat deficiency rather than carbohydrate supplementation is responsible for the observed elevation in $s_f^o 20-400$ lipoprotein levels. We would doubt the validity of such an explanation from our observations on simultaneously fat- and carbohydrate-restricted diets (see discussion below).

(d) Consideration should be given to the expected observations with a serum cholesterol measurement in the shift from high-fat moderate-carbohydrate to low-fat high-carbohydrate diets, where the major changes are in the $s_f^o 20-400$ lipoprotein classes. In the five subjects studied the $s_f^o 20-400$ was 80 mb/100 ml higher in the low-fat high-carbohydrate diet than in the high-vegetable-fat moderate-carbohydrate period, and was 76 mg/100 ml higher in the low-fat high-carbohydrate diet than in the high-animal-fat moderate-carbohydrate diet. Since the cholesterol represents

approximately 13% of the $s_f^0 20-400$ lipoproteins, the expected cholesterol changes would be only 10 mg/100 ml, a change which would be difficultly observable in comparison of the low-fat period with the high-vegetable- or high-animal-fat periods. Furthermore, in comparison of a high-animal-fat moderate-carbohydrate diet with a low-fat high-carbohydrate diet, the $s_f^0 0-12$ alterations are shifting the serum cholesterol level in a direction opposite to that produced by the $s_f^0 20-400$ lipoprotein alterations.

(e) It should be emphasized that the findings reported here are for five individuals carefully studied, none of whom was characterized by extremes in lipoprotein distributions. Thus no cases were included who showed the massive elevation of $s_f^0 0-12$ as seen in xanthoma tendinosum, nor were there any with the massive elevations in $s_f^0 20-100$ or $s_f^0 100-400$ as seen in xanthoma tuberosum or idiopathic hyperlipemia. Whether or not such cases demonstrating extremes of lipoprotein transport defects would respond to the various diets in a manner similar to the cases reported here cannot be discerned from the data presently at hand. Thus, for example, Keys[4] reported on one case of xanthoma tuberosum whose serum cholesterol level fell from 900 mg/100 ml to 250 mg/100 ml on a low-fat diet and rose back essentially to the initial level when vegetable fat was added to the diet. Since xanthoma tuberosum is characterized by massive elevations in $s_f^0 20-400$, it would appear that in this one case the $s_f^0 20-400$ levels must have been raised by vegetable fat. Yet in these studies replacement of carbohydrate by vegetable fat resulted in a lowering of the $s_f^0 20-400$ levels. Most likely the results reflect no inconsistency, but rather a difference in responsiveness in individuals with differences in lipoprotein transport patterns.

Low-Fat Diets without Caloric Restriction versus Low-Fat Diets with Caloric Restriction

In the foregoing discussion it was pointed out that when a shift is made from a high-fat diet (with fat of either vegetable or animal origin) to a low-fat diet, isocalorically equivalent by the supplementation with carbohydrate, there is an elevation in the lipoproteins of the $s_f^0 20-400$ classes. It was indicated that carbohydrate was responsible for this change. Further evidence to support this view is available from the weight-reduction study reported in Table VI. In that study fat was restricted to approximately the same extent as in the other low-fat studies reported here. However, carbohydrate supplementation was not used, since an effort was being made to have the patients lose weight. Indeed the advised carbohydrate intake in the weight-reduction study was approximately half that in the high-fat period of the controlled isocaloric experiment and approximately one-fourth that of the low-fat, carbohydrate-supplemented period. In the weight-reduction experiment, for those persons who demonstrated probable adherence to the diet by having lost 10 or more pounds in a 2-month period, there was a fall in the level of both the $s_f^0 0-20$ lipoprotein and the $s_f^0 20-400$ lipoprotein concentrations. The actual data were the following:

For 28 individuals,
mean weight loss, 14 pounds,
mean fall in $s_f^0 0-20$ lipoproteins, 78 mg/100 ml,
mean fall in $s_f^0 20-400$ lipoproteins, 59 mg/100 ml.

The fall in $s_f^o 0-20$ lipoprotein concentration is of the order that would be anticipated from the data above for the restriction of fat of animal origin in the diet. The fall in the $s_f^o 20-400$ lipoprotein concentration is marked and significant and is opposite to the results obtained with the low-fat diet supplemented by carbohydrate, where the $s_f^o 20-400$ lipoproteins actually increased. Since both the isocaloric diet low in fat and the low-caloric diet low in fat are comparable with respect to diminution of fat intake, it is extremely difficult to visualize that fat per se can be responsible for the opposite behavior of the $s_f^o 20-400$ lipoproteins. However, the isocaloric diet is high in carbohydrate whereas the low-calorie diet is very low in carbohydrate, and the results are quite consistent with the concept that dietary carbohydrate increases are on the average accompanied by an elevation in $s_f^o 20-400$ lipoprotein level. Calories per se would seem not to be of prime importance in this effect, because as shown above the $s_f^o 20-400$ lipoprotein elevation accompanied carbohydrate replacement of fat even at isocaloric levels. The lowering of $s_f^o 20-400$ lipoprotein concentrations observed in these experiments on caloric restriction is consistent with the lowering of $s_f 20-100$ lipoproteins (uncorrected to standard flotation conditions) reported by Walker et al. and with the observed association of lipoproteins of the $s_f^o 20-400$ classes with relative weight.[1, 12]

Long-Term versus Short-Term Dietary Effects

Consideration of the potential application of dietary measures in the control of serum lipoprotein levels--as, for example, in efforts to reduce the rate of accumulation of arteriosclerosis--necessarily brings up the question of whether or not dietary effects observed over a relatively short period can be generalized to those which might be expected over a period of many years. In these studies of high-animal-fat diets, high-vegetable-fat diets, and low-fat diets, all at isocaloric levels, the following durations were involved:

Total period of high animal fat intake	10-1/2 weeks
Total period of high-vegetable-fat intake	11 weeks
Total period of low-fat high-carbohydrate intake . .	24 weeks

These are relatively long periods in an experimental sense, although short compared with the periods that might be involved in therapeutic or prophylactic studies. Whether the observed findings would be altered if any period were extended greatly cannot be determined from these data, but it would appear that the burden of proof that different results would be obtained over "lifetime" periods would rest with those who might suspect such differences.

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