Journal of Clinical Investigation
Vol. 43, No. 11, 1964

The Effect of Partial Hydrogenation of Dietary Fats, of the Ratio of Polyunsaturated to Saturated Fatty Acids, and of Dietary Cholesterol upon Plasma Lipids in Man*

B. ARTHUR ERICKSON, ROBERT H. COOTS, FRED H. MATTSON, AND ALBERT M. KLIGMAN

(From the Miami Valley Laboratories of the Procter & Gamble Company, Cincinnati, Ohio, and the University of Pennsylvania Medical School, Philadelphia, Pa.)

The effect of alterations in dietary fat on the serum cholesterol level has been extensively studied during recent years. Numerous investigations have shown reductions in the serum lipid level of man after the dietary substitution of many vegetable oils for common animal fats or certain tropical oils such as coconut oil (1–11). The relatively high degree of saturation of coconut oil and fats of animal origin has been associated with their cholesterol-elevating effect (8–12) and has led to the concept that saturated fatty acids per se are hypercholesterolemic (13). In this regard, Keys, Anderson, and Grande (12) reported that saturated fatty acids were approximately twice as active in raising serum cholesterol as polyunsaturated fatty acids were in reducing it. A reduction in the total unsaturation of the dietary fat, as measured by iodine value, has been reported to elevate serum cholesterol (9). Jolliffe (13) proposed that the effect of dietary fats on the serum cholesterol concentration was a function of their ratio of polyunsaturated to saturated fatty acids (P/S). Although this concept that saturated fatty acids, or dietary fats with low P/S ratios, are hypercholesterolemic is widely accepted, it has not been uniformly demonstrated. Hashim, Arteaga, and Van Itallie (14), for instance, reported that a triglyceride consisting of saturated fatty acids with chain lengths ranging from 6 to 12 carbon atoms was hypocholesterolemic relative to dietary butterfat. In an earlier study, Hashim, Clancy, Hegsted, and Stare (15) observed that formula diets containing safflower oil or an equal mixture of safflower and coconut oil produced a marked and approximately equal reduction in the serum cholesterol level of hypercholesterolemic subjects. The replacement of a low-fat diet or one containing ethyl linoleate with one in which ethyl stearate was the principal dietary fat was shown to have no elevating effect on the serum cholesterol level by Horlick and Craig (16). In a subsequent study, Horlick (17) observed a decline in the serum cholesterol of two subjects when ethyl stearate or a sterol-free, partially hydrogenated soybean oil was added to a low-fat diet. Further evidence that a relatively saturated fat does not always result in elevated serum cholesterol values is provided in the findings of Malmros (18). A net reduction was observed in the serum cholesterol level of two subjects after substituting a low-fat diet supplemented with cocoa butter for a free-choice diet. Thus, not all reports in the literature agree as to the effect of relative saturation on the ability of a dietary fat to maintain or lower the serum cholesterol level.

The effect of hydrogenation on the hypocholesterolemic action of dietary fats has been a subject of considerable interest and is of practical importance, since hydrogenated fats normally constitute about 7% of the total dietary fat. Results obtained in this laboratory (19), in addition to those of other investigators (20–24), have indicated that partial hydrogenation has little effect on the hypocholesterolemic action of dietary fats. There are, however, numerous reports (8, 9, 13, 25, 26) in disagreement with this conclusion. The effectiveness of partially hydrogenated fats in maintaining or lowering the plasma cholesterol level has been questioned not only because of the reduction in polyunsaturated fatty acids, but also because of their isomeric unsaturated fatty acids.

* Submitted for publication March 23, 1964; accepted July 1, 1964.

Presented in part at the annual meeting of the Council on Arteriosclerosis, American Heart Association, Los Angeles, Calif., October 24, 1963.

TABLE I

Composition of the dietary fats

Analysis	Dietary fats							
	A	B	C	D	E	F	Egg yolk	G*
Iodine value	108.5	104.7	83.8	81.4	38.0	66.8		59.2
Saponification value	191.3	192.2	191.4	193.4	193.9	194.9		203.0
Total fatty acid, %	95.0	95.8	95.6	95.0	96.1	95.4		94.2
Unsaponifiable, %	0.7	0.8	0.8	0.8	0.8	0.7		0.5
Tocopherol, %	0.16	0.03	0.11	0.03	0.03	0.03		0.03
Fatty acid composition, *% of total weight*†								
Palmitic	10.6	11.9	15.9	15.8	24.6	26.0	25.9	23.2
Palmitoleic	0	0.9	0.2	0.4	0.6	0.7	4.1	2.6
Stearic	5.5	5.8	15.9	16.2	35.4	11.1	11.3	13.0
Oleic	45.7	45.1	43.1	42.9	34.9	47.2	45.5	43.5
Cis	38.0	45.1	37.1	42.9	34.9	47.2	45.5	38.3
Trans	7.7	0	6.0	0	0	0	0	5.2
Linoleic	33.6	34.7	21.9	24.6	3.1	12.8	12.1	10.1
Cis, cis	30.7	34.7	19.5	24.6	3.1	12.8	12.1	9.3
Cis, trans	2.9	0	2.4	0	0	0	0	0.8
Linolenic	3.1	0.9	2.3	0	0	0.7	0	1.3
Total trans fatty acids	10.6	0	8.4	0	0	0		6.0
Conjugated diene	0.4	0.2	0.3	0.2	0	0.2		.3

* In addition, Fat G contained the following short-chain fatty acids: caprylic, 0.1; capric, 0.5; lauric, 0.8; myristic, 3.9; myristoleic, 1.1.

† Determined by gas-liquid chromatography and infrared analysis after fractionation of the mercuric acetate derivatives.

Since the process of hydrogenation, as customarily used in the preparation of edible fats, causes little or no increase in the content of saturated fatty acids, any question would be limited to the effects produced by the conversion of polyunsaturated fatty acids to monounsaturated acids or by isomerization of the unsaturated fatty acids. In brief, there is disagreement concerning the effect of hydrogenated fats on the serum cholesterol level.

In view of the contradictory reports concerning the effects of various types of dietary fats on the plasma cholesterol level, the following study was conducted. The role of long-chain saturated and polyunsaturated fatty acids and the ratio of these were investigated by feeding vegetable fats of different fatty acid compositions. The effect of hydrogenation was studied by comparing the plasma lipid response of subjects to a partially hydrogenated fat and to a blend of unhydrogenated fats having the same fatty acid content but containing no isomeric fatty acids. The quantity of trans isomers provided by the partially hydrogenated fat was slightly greater than that found in the average American diet.[1]

[1] A laboratory blend of animal and vegetable fats (Fat G) similar in composition to that of all fats found in the average American diet (27) was analyzed for trans fatty acids and served as a basis for estimating the average intake of trans fatty acids.

For several years dietary cholesterol was held to have little effect on the serum cholesterol level. However, recent reports have demonstrated significant elevations in the serum cholesterol and phospholipid levels (28, 29) of subjects receiving cholesterol in the form of egg yolk. In the present study, the partially hydrogenated fat and the blend of unhydrogenated fats were fed with and without dried egg yolk powder in order to determine whether there is a difference in the plasma lipid response to these fats when fed in diets containing none or a substantial quantity of dietary cholesterol.

Methods

Subjects. The subjects were male inmates of the Philadelphia County Prison at Holmesburg, Pennsylvania, ranging in age from 29 to 45 years. The median age was 35 years. All of the subjects were examined before the study and found to be in good health with no known metabolic disorders.

Diet. The composition of the formula diets and the method of their preparation have been described (19). The fats and the P/S ratios of diets containing these fats were as follows: Fat A (P/S = 1.6), a partially hydrogenated soybean oil; Fat B (P/S = 1.5), a vegetable fat mixture composed of 52.5% olive oil, 39.5% safflower oil, and 8.0% cocoa butter (this fat was similar to Fat A in saturated and mono- and polyunsaturated fatty acid content but contained no hydrogenated fats); Fat C (P/S = 0.7), a mixture composed of Fat A and cocoa butter; Fat D (P/S = 0.7), a mixture composed of Fat

TABLE II

Composition of the complete dry mixes

Ingredient	Dry Mix No. 1				Dry Mix No. 2			
	Amount	Protein*	Carbohydrate*	Fat*	Amount	Protein*	Carbohydrate*	Fat*
	g/100 g				*g/100 g*			
Dried egg white†	18.4	15.2			21.0	17.3		
Dextrose	57.5		52.1		57.5		52.1	
Fat	16.8			16.8	16.8			16.8
Dried egg yolk powder‡	6.4	2.1		3.8				
Fat F§					3.8			3.8
Sodium chloride‖	0.5				0.5			
Imitation vanilla	0.4				0.4			
Total	100.0	17.3	52.1	20.6	100.0	17.3	52.1	20.6
Calories supplied		69	208	194		69	208	194
% of total calories		15	44	41		15	44	41

* Expressed on a moisture-free basis.
† Biotin added at a level of 0.1 mg per 100 g of diet to inactivate avidin in the egg white.
‡ Provided 144 mg cholesterol per 100 g of dry mix.
§ Fat F in Dry Mix 2 simulated the fatty acid composition of egg yolk lipid present in Dry Mix 1.
‖ Contained 0.01% potassium iodide.

B and cocoa butter; and Fat E (P/S = 0.1), cocoa butter. Fats A and B were fed both with and without dietary cholesterol, added in the form of dried egg yolk powder. To compensate for the fatty acids provided by the dried egg yolk powder, a fat mixture (Fat F) having a fatty acid composition similar to that of all the lipids of egg yolk was included in all of the diets not containing dried egg yolk powder. Fat F consisted of 36.0% olive oil, 31.0% palm oil, 25.0% cocoa butter, and 8.0% cottonseed oil. The fatty acids provided by the egg yolk or Fat F were included in calculating the P/S ratios of the dietary fats. In all cases the experimental fats contributed 81.6% of the total dietary fat and thus accounted for the main bulk of fatty acids. Fat G was a mixture of animal and vegetable fats and was fed to all groups during the transition from a mixed diet to a formula diet. Analytical data obtained on the experimental fats and the fat isolated from the dried egg yolk powder are given in Table I. Fatty acid composition was determined by gas-liquid chromatography (30). The level of geometric isomers in the experimental fats was determined by infrared spectroscopy (31) of the fractions obtained after column chromatography of the mercuric acetate addition complexes (32). The remaining analytical values were obtained by the usual methods (31).

The compositions of the dry mixes, which were used for preparation of the formula diets, are shown in Table II. Dried egg white was used as a source of protein.[2] Since two of the diets contained egg yolk powder, it was necessary to use two different dry mixes so that the level of carbohydrate, fat, and protein would be the same in all diets. The complete Dry Mix No. 1 represents the composition of diets containing 6.4% of dried egg yolk powder. Subjects ingesting the diets containing egg yolk received an average of 742 mg of cholesterol per day. The composition of diets from which dried egg yolk powder was omitted is represented by the complete Dry Mix No. 2. Fat F was included in this mix to compensate for the egg yolk lipid fatty acids present in Dry Mix No. 1. The total fat in the diets supplied 41% of the total calories.

The preparation of dry mixes and their ultimate use in emulsion diets were similar to those described by McOsker, Mattson, Sweringen, and Kligman (19).

Dietary supplement and feeding procedure. The subjects were fed four times daily. At each feeding each subject received one-fourth of his daily allotment of emulsion. Initially, the emulsion diets were fed at a level of 35 calories per kg of body weight. However, some individual adjustment in caloric intake was necessary to maintain constant body weight. Subjects registered slight gains and losses in body weight; the average weight change was an increase of 2.6 pounds per subject.

As described previously (19), the subjects also received vitamin-mineral capsules, salt tablets, methylcellulose wafers, and one small serving of a low-fat, low-calorie food item per day.

Blood samples and plasma lipid analyses. Blood samples were drawn twice weekly before the morning meal. The blood was collected in oxalated Vacutainer[3] tubes and the plasma obtained after centrifugation. Duplicate portions of each plasma sample were analyzed for total cholesterol (33) and triglyceride (34) content. The triglyceride values are expressed in terms of mg of tripalmitin per 100 ml of plasma. In addition, a single phospholipid determination (35) was made on each plasma sample. To establish the reliability of the analytical procedures, replicate samples of a desiccated serum

[2] At the suggestion of Dr. E. H. Ahrens of the Rockefeller Institute.

[3] Becton, Dickinson and Company, Rutherford, N. J.

sample were analyzed in conjunction with the experimental plasma samples during the entire study. The standard deviations for the analytical methods, in mg per 100 ml of plasma, were: 7.3 for cholesterol, 3.4 for phospholipid, and 7.9 for triglyceride.

Experimental design. A standard seven-treatment, four-replication, incomplete Latin square design was used. This design made it possible to feed all of the diets in each of the four periods. Any period or time effects were thus self-compensating. The experimental design did not, however, permit the feeding of all diets to all groups, and since all groups do not respond identically to a given dietary fat, statistical adjustments in the calculated treatment means were necessary (36). The design of the experiment is shown in Table III.

During an initial 25-day pre-experimental period, all 42 subjects received the normal prison diet. On this regimen, the average plasma lipid levels, in mg per 100 ml, were as follows: cholesterol, 218.5; phospholipid, 216.3; and triglyceride, 101.8. At the end of this period the 42 subjects were divided into seven groups of six subjects each. The subjects were distributed so that the average body weight of each group was similar. In addition, the average cholesterol level of each group was essentially equal, and the range of plasma cholesterol levels was similar within each group. During the next 10 days the diet was gradually changed from solid food to the liquid formula. This changeover period was necessary to prevent gastrointestinal discomfort. During this changeover, all of the subjects received a formula diet containing a blend (Fat G) of animal and vegetable fats similar in composition to that of all fats, both visible and invisible, that are present in the average American diet (27).

The 20-week experiment was divided into four periods of 5 weeks each. The first 2 weeks of each period served as an equilibration period during which the subjects attained their new plasma lipid levels. Data obtained previously (19), and confirmed here, indicate that 2 weeks is sufficient time for the serum cholesterol to reach a steady state after a change in the dietary fat. The data collected during the final 3 weeks of each period were used to determine the effect of each diet on the plasma lipid levels.

Of the original 42 subjects, 36 completed the study. The six who failed to complete the experiment dropped out for reasons unrelated to the study. The number of subjects completing the study in each group is shown in Table III.

The data obtained during the final 3 weeks of each experimental period were analyzed for statistically significant differences by an analysis of variance technique described by Cochran and Cox (36). Only the values obtained on the subjects who completed the entire study were used. The analysis of variance indicated that significant differences did exist. Therefore, the Newman-Keuls technique for multiple comparisons (37) was used to establish which of the adjusted treatment means were significantly different from one another.

One restriction inherent in the incomplete Latin square design is that a valid comparison of treatment effects cannot be made before the completion of the experiment. Thus, the only cholesterol values that are comparable are the adjusted cholesterol levels that are calculated from the values obtained for each fat over the entire study.

Results

The combined, adjusted, average plasma cholesterol, phospholipid, and triglyceride values for the final 3 weeks of each experimental period are shown in Table IV. Those values not significantly different from each other at the 95% probability level are mutually underlined.

The addition of egg yolk cholesterol to diets containing the partially hydrogenated soybean oil (Fat A) and the blend of unhydrogenated fats (Fat B) significantly increased the plasma cholesterol level, 23.8 and 26.6 mg per 100 ml, respectively. The plasma cholesterol levels obtained when Fat A or Fat B was fed together with dietary cholesterol were also significantly higher than those produced by the cholesterol-free diets containing fats with lower P/S ratios (Fats C, D, and E).

TABLE III

Feeding sequence of the various dietary fats

Experimental period	Length of period	Dietary fat received by each group*						
		1	2	3	4	5	6	7
Pre-experimental	25 days	Mixed†	Mixed	Mixed	Mixed	Mixed	Mixed	Mixed
Changeover	10 days	G	G	G	G	G	G	G
1	5 weeks	A	A+	B	D	E	C	B+
2	5 weeks	B+	A	A+	C	D	B	E
3	5 weeks	D	E	B+	A+	B	A	C
4	5 weeks	E	B	D	A	C	B+	A+
No. of subjects‡		4	6	5	6	5	5	5

* A plus symbol indicates that the diet contained cholesterol.
† Normal prison diet.
‡ Initially, each group contained six subjects.

TABLE IV

Adjusted average plasma lipid levels produced by the dietary fats and egg yolk cholesterol

Dietary fat*	**A+**	B+	E	**A**	**C**	**B**	**D**
P/S	1.6	1.5	0.1	1.6	0.7	1.5	0.7
Adjusted average plasma cholesterol, *mg/100 ml*†	217	215	195	193	190	188	188
Dietary fat	B+	**A+**	E	**D**	**C**	**A**	**B**
P/S	1.5	1.6	0.1	0.7	0.7	1.6	1.5
Adjusted average plasma phospholipid, *mg/100 ml*†	211	210	201	194	192	192	189
Dietary fat	B+	**A+**	E	**A**	**C**	**D**	**B**
P/S	1.5	1.6	0.1	1.6	0.7	0.7	1.5
Adjusted average plasma triglyceride, *mg/100 ml*†	110	110	107	106	101	99	96

* A plus symbol indicates that egg yolk cholesterol was fed in conjunction with the dietary fat. The boldfaced fats contained a partially hydrogenated soybean oil.

† Those values not mutually underlined are significantly different from each other at the 95% confidence level ($p = 0.05$ or less); no proof of difference exists for those values mutually underlined.

This elevating effect was caused by an average daily intake of 742 mg of cholesterol per subject.

The difference between the plasma cholesterol levels obtained with the partially hydrogenated fat (Fat A) and the blend of unhydrogenated vegetable fats (Fat B) was not significant whether or not cholesterol was present in the diet. Furthermore, a reduction in the P/S ratio of the partially hydrogenated fat and blend of unhydrogenated vegetable fats (Fats C and D), by the addition of cocoa butter, had no significant effect on the plasma cholesterol response in comparison to that obtained with Fat A or Fat B alone; the fats involved in these latter comparisons were fed only in the cholesterol-free diets.

A comparison of the results obtained with all of the cholesterol-free diets shows that variations in the P/S ratio of the dietary fats had no effect on the plasma cholesterol response. The diet containing Fat E (cocoa butter) with a P/S ratio of 0.1, for instance, produced a plasma cholesterol level of 195.0 mg per 100 ml. This value is not significantly different from those produced by the fats with higher P/S ratios such as the diet containing Fat A, which had a P/S ratio of 1.6, and resulted in a plasma cholesterol level of 192.8 mg per 100 ml.

The plasma phospholipid response was similar to that of the cholesterol. The adjusted plasma phospholipid levels were increased, 18.4 and 22.1 mg per 100 ml, respectively, by the addition of cholesterol to diets containing Fats A and B. As in the case of the plasma cholesterol, this elevation was statistically significant. The differences in the plasma phospholipid values obtained with the partially hydrogenated soybean oil (Fat A) and unhydrogenated blend (Fat B) were not significant whether or not cholesterol was present in the diet. Relative to the cholesterol-free diets, however, the plasma phospholipid level produced by Fat E (cocoa butter) was significantly greater than that resulting from the four fats with higher P/S ratios. There were no significant differences among the plasma phospholipid values produced by the diets containing Fats A, B, C, and D even though the P/S ratios ranged from 1.6 to 0.7.

The plasma triglyceride response to the various dietary treatments did not follow a definite pattern as did the response of plasma cholesterol and phospholipid. This is shown by the underlying brackets indicating statistical significance in Table IV. The inconsistencies can be seen in a comparison of the effects of the hydrogenated and unhydrogenated fats. When the partially hydrogenated fat (Fat A) and the blend of unhydrogenated vegetable fats (Fat B) were fed in the presence of dietary cholesterol or in conjunction with cocoa butter (Fats C and D), there was no significant difference between the effects of the hydrogenated fat and unhydrogenated blend on the plasma triglyceride level. In the cholesterol-free diets, however, the plasma triglyceride response was slightly but significantly higher with the partially hydrogenated fat (Fat A) than with the blend of untreated vegetable fat (Fat B).

Discussion

The change from the normal prison diet to the cholesterol-free liquid diets resulted in a decrease in the plasma cholesterol level. This type of change has been observed previously (9, 14, 19) and might be regarded as evidence that formula diets per se are hypocholesterolemic. However, the plasma cholesterol levels produced by the cholesterol-containing formula diets were not greatly different from those produced by the normal prison diet. Thus, the reduced plasma cholesterol level is probably due to the removal of cholesterol from the diet rather than the change from a solid to a liquid diet.

The reports in the literature do not agree as to whether hydrogenation of a vegetable oil alters its hypocholesterolemic action (8, 9, 17, 20, 26, 38, 39). Since the process of hydrogenation, as normally used in the preparation of edible fats, results in the conversion of polyunsaturated to monounsaturated fatty acids and in the formation of some isomeric unsaturated fatty acids, either one or both types of change might possibly modify the plasma cholesterol response. In many of the experiments, already reported, it is impossible to determine which of these changes in composition is responsible for the observed alterations in the serum cholesterol level. To measure the effects of the isomeric acids, it is necessary to feed pairs of hydrogenated and unhydrogenated fats, each similar with regard to content of saturated and mono- and polyunsaturated fatty acids, but differing in level of isomeric acids. Any difference between the cholesterol levels caused by the two fats would then be a function of the isomeric unsaturated fatty acids present in the partially hydrogenated fat. This was done in the present study.

The results obtained with the cholesterol-containing diets revealed that no interaction occurred between dietary cholesterol and the isomeric unsaturated fatty acids present in the partially hydrogenated fat. The quantity of trans fatty acids provided by the partially hydrogenated fat was slightly greater than that calculated to be present in the average American diet. These data suggest, therefore, that the present level of trans fatty acids in the average American diet does not alter the plasma cholesterol level relative to that obtained by a corresponding intake of the cis isomers.

A lightly hydrogenated soybean oil and a blend of unhydrogenated vegetable oils were also compared with regard to their effect on the plasma lipid response in human subjects by Grasso and co-workers (24). The fats were approximately equal in saturated and mono- and polyunsaturated fatty acid content but differed in that the hydrogenated fat contained 20% of trans fatty acids. In comparison to an equal intake of coconut oil, both fats produced a marked depression in the plasma cholesterol and phospholipid levels. Although the data are qualitatively similar to those presented here, direct comparisons are not possible, since the two subjects studied by Grasso and associates differed quantitatively in their response to the pair of hydrogenated and unhydrogenated fats.

Anderson, Grande, and Keys (26) have also studied the effect of hydrogenation on the hypocholesterolemic action of dietary fats. The serum cholesterol levels obtained in two experiments, when examined by their prediction equation, led to the conclusion that trans fatty acids in the partially hydrogenated fats caused either a decrease or no change in the serum cholesterol level. The plasma cholesterol response to a pair of hydrogenated and unhydrogenated fats, each similar in fatty acid content, was studied in a third experiment. In this case the isomeric unsaturated fatty acids of the partially hydrogenated fat were reported to have a hypercholesterolemic effect. Although in two instances the effect of trans fatty acids was determined indirectly, the conflicting observations suggest that the trans fatty acids had no consistent effect on the serum cholesterol level.

In an earlier study conducted in this laboratory, McOsker and co-workers (19) showed that the hypocholesterolemic action of several partially hydrogenated vegetable fats was equal to that of unhydrogenated cottonseed oil. It might be asked if the same would be true of other hydrogenated and unhydrogenated fats, if the system were stressed by the presence of a relatively large amount of saturated fatty acids. In the present experiment this aspect of the problem was studied by reducing the P/S ratios of diets containing the partially hydrogenated soybean oil or blend of unhydrogenated vegetable fats by the addition of cocoa butter. We observed that even in the presence of the relatively large amount of saturated

fatty acids there was no significant difference between the effects of the partially hydrogenated and unhydrogenated fats on the plasma cholesterol response.

The diets used in this study afford a rigorous test of the concept that blood cholesterol is a function of the P/S ratio of the dietary fat, since comparisons were made among P/S ratios of 0.1, 0.7, 1.5, and 1.6. We found that a high ratio, 1.6, or an extremely low ratio, 0.1, resulted in essentially identical plasma cholesterol levels. These results are thus incompatible with such a concept. Jolliffe's recommendation (13) of a P/S ratio in excess of 1.25 was based on experiments in which not only the dietary fats were varied but other dietary components as well. The effectiveness of a P/S ratio of 1.25 thus may be fortuitous or the result of an interaction of this factor with the other dietary components not controlled in his experiments.

In many experiments the dietary fats producing elevations in serum cholesterol have been composed principally of coconut oil or animal fats such as butter, beef tallow, and lard. Although these fats are relatively saturated, their hypercholesterolemic effects are complicated by the presence of variable amounts of cholesterol or saturated fatty acids of short and intermediate chain length. Verification in this regard is provided in the observations of Ahrens and co-workers (9), who found that dietary butterfat in comparison to cocoa butter had a significant elevating effect on the serum cholesterol level. In the present study fats with various P/S ratios were fed in the absence of dietary cholesterol, and since none of the fats contained cholesterol or any appreciable quantity of short or intermediate chain length fatty acids, the final results were not complicated by either of these factors. These findings thus indicate that long-chain saturated fatty acids per se are not hypercholesterolemic. However, the effect of ingesting cholesterol in conjunction with variations in the P/S ratio of dietary fats on the plasma cholesterol response is unknown.

The plasma phospholipid response to variations in the diet followed a pattern similar to that of the cholesterol. Hence, the plasma phospholipid level was responsive to the presence or absence of cholesterol in the diet but was not significantly affected by partial hydrogenation of the dietary fat. The plasma phospholipid level was apparently more sensitive than the plasma cholesterol to variations in the P/S ratio, because the dietary fat with the lowest P/S ratio, 0.1, caused a significantly higher level of plasma phospholipid than those produced by the fats with higher P/S ratios.

The complexity of the plasma triglyceride response to the various dietary treatments makes it impossible to ascertain accurately the relative importance of dietary cholesterol, partial hydrogenation of the dietary fat, or variations in the P/S ratio on the plasma triglyceride level.

On the basis of studies previously reported in the literature and the one described above, it appears that with cholesterol-free formula diets the plasma cholesterol is not affected by variations in the P/S ratio of the dietary fat. The results produced by partial hydrogenation indicate that fats prepared by this process have an effect on the plasma cholesterol level similar to that of unhydrogenated fats containing equal quantities of saturated and mono- and polyunsaturated fatty acids. Furthermore, in the present study the efficacy of a partially hydrogenated vegetable fat to maintain or lower the plasma cholesterol level was not affected by either dietary cholesterol or the simultaneous consumption of a relatively saturated fat.

Summary

Forty-two healthy men were divided into seven groups and maintained for 20 weeks on formula diets. During this time the effects of the following dietary fats on plasma lipid levels were determined: Fat A, a partially hydrogenated soybean oil; Fat B, a blend of vegetable fats similar to Fat A in saturated and mono- and polyunsaturated fatty acid content but containing no hydrogenated fat; Fat C, a mixture composed of Fat A and cocoa butter; Fat D, a mixture composed of Fat B and cocoa butter; and Fat E, cocoa butter. The diets containing these fats had P/S ratios of 1.6, 1.5, 0.7, 0.7, and 0.1, respectively. Fats A and B were fed both with and without dietary cholesterol in the form of dried egg yolk powder. All of the other fats were fed only in cholesterol-free diets.

Addition of cholesterol (average of 742 mg daily per subject) to diets containing Fat A or Fat B significantly increased the plasma cholesterol levels, 23.8 and 26.6 mg per 100 ml, respectively.

The plasma cholesterol response to the partially hydrogenated fat (Fat A) was identical to that of the unhydrogenated fat (Fat B), whether or not cholesterol was included in the diet. A reduction in the P/S ratios of cholesterol-free diets containing Fats A and B from 1.6 and 1.5, respectively, to 0.7 by the addition of cocoa butter had no effect on the plasma cholesterol response.

In the cholesterol-free diets, cocoa butter produced an average plasma cholesterol level not significantly different from that obtained with the fat having the highest P/S ratio. We conclude that with a cholesterol-free formula diet the plasma cholesterol level is unaffected by variation in the P/S ratio of the diet from 1.6 to 0.1.

The plasma phospholipid values, like the values for cholesterol, were highest when the diet contained cholesterol. With the cholesterol-free diets the plasma phospholipid values produced by the ingestion of cocoa butter were somewhat higher than those obtained with the other fats. There were no significant differences among the other fats in their effect on the plasma phospholipid level.

Acknowledgments

We are indebted to the inmates of Holmesburg Prison for serving as volunteers and to the administration (Edward Hendrick, Superintendent) for use of the facilities. In addition, we gratefully acknowledge the technical assistance of Mr. Gordon S. Collins, Jr., and Mr. K. L. Harbaum of the Procter & Gamble Statistical Department, who aided in designing the experiment and made the statistical analyses of the data.

References

1. Kinsell, L. W., J. Partridge, L. Boling, S. Margen, and G. Michaels. Dietary modification of serum cholesterol and phospholipid levels. J. clin. Endocr. 1952, **12**, 909.
2. Groen, J., B. K. Tjiong, C. E. Kamminga, and A. F. Willebrands. The influence of nutrition, individuality and some other factors, including various forms of stress, on the serum cholesterol: an experiment of 9 months duration in 60 normal human volunteers. Voeding 1952, **13**, 556.
3. Kinsell, L. W., G. D. Michaels, J. W. Partridge, L. A. Boling, H. E. Balch, and G. C. Cochrane. Effect upon serum cholesterol and phospholipids of diets containing large amounts of vegetable fat. J. clin. Nutr. 1953, **1**, 224.
4. Ahrens, E. H., Jr., D. H. Blankenhorn, and T. T. Tsaltas. Effect on human serum lipids of substituting plant for animal fat in diet. Proc. Soc. exp. Biol. (N. Y.) 1954, **86**, 872.
5. Ahrens, E. H., Jr., T. T. Tsaltas, J. Hirsch, and W. Insull, Jr. Effects of dietary fats on the serum lipides of human subjects. J. clin. Invest. 1955, **34**, 918.
6. Beveridge, J. M. R., W. F. Connell, G. A. Mayer, J. B. Firstbrook, and M. S. DeWolfe. The effects of certain vegetable and animal fats on the plasma lipids of humans. J. Nutr. 1955, **56**, 311.
7. Beveridge, J. M. R., W. F. Connell, and G. A. Mayer. Dietary factors affecting the level of plasma cholesterol in humans: the role of fat. Canad. J. Biochem. 1956, **34**, 441.
8. Bronte-Stewart, B., A. Antonis, L. Eales, and J. F. Brock. Effects of feeding different fats on serum-cholesterol level. Lancet 1956, **1**, 521.
9. Ahrens, E. H., Jr., J. Hirsch, W. Insull, Jr., T. T. Tsaltas, R. Blomstrand, and M. L. Peterson. The influence of dietary fats on serum-lipid levels in man. Lancet 1957, **1**, 943.
10. Keys, A., J. T. Anderson, and F. Grande. "Essential" fatty acids, degree of unsaturation, and effect of corn (maize) oil on the serum-cholesterol level in man. Lancet 1957, **1**, 66.
11. Malmros, H., and G. Wigand. The effect on serum-cholesterol of diets containing different fats. Lancet 1957, **2**, 1.
12. Keys, A., J. T. Anderson, and F. Grande. Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet 1957, **2**, 959.
13. Jolliffe, N. Dietary factors regulating serum cholesterol. Metabolism 1961, **10**, 497.
14. Hashim, S. A., A. Arteaga, and T. B. Van Itallie. Effect of a saturated medium-chain triglyceride on serum-lipids in man. Lancet 1960, **1**, 1105.
15. Hashim, S. A., R. E. Clancy, D. M. Hegsted, and F. J. Stare. Effect of mixed fat formula feeding on serum cholesterol level in man. Amer. J. clin. Nutr. 1959, **7**, 30.
16. Horlick, L., and B. M. Craig. Effect of long-chain polyunsaturated and saturated fatty acids on the serum-lipids of man. Lancet 1957, **2**, 566.
17. Horlick, L. Studies on the regulation of serum cholesterol levels in man. The effects of corn oil, ethyl stearate, hydrogenated soybean oil, and nicotinic acid when added to a very low-fat basal diet. Lab. Invest. 1959, **8**, 723.
18. Malmros, H. The effect of dietary fats on serum cholesterol *in* Essential Fatty Acids. A Symposium, H. M. Sinclair, Ed. New York, Academic Press, 1958, p. 150.
19. McOsker, D. E., F. H. Mattson, H. B. Sweringen, and A. M. Kligman. The influence of partially hydrogenated dietary fats on serum cholesterol levels. J. Amer. med. Ass. 1962, **180**, 380.

20. Beveridge, J. M. R., W. F. Connell, G. A. Mayer, and H. L. Haust. Plant sterols, degree of unsaturation, and hypocholesterolemic action of certain fats. Canad. J. Biochem. 1958, **36**, 895.
21. Wilcox, E. B., and L. S. Galloway. Serum cholesterol and different dietary fats. J. Amer. diet. Ass. 1961, **38**, 227.
22. Morse, E. H., E. Bicknell, E. P. Lewis, S. B. Merrow, and C. A. Newhall. Relation of dietary fats to blood lipids in young men. J. Amer. diet. Ass. 1962, **41**, 323.
23. Beveridge, J. M. R., and W. F. Connell. The effect of commercial margarines on plasma cholesterol levels in man. Amer. J. clin. Nutr. 1962, **10**, 391.
24. Grasso, S., B. Gunning, K. Imaichi, G. Michaels, and L. Kinsell. Effects of natural and hydrogenated fats of approximately equal dienoic acid content upon plasma lipids. Metabolism 1962, **11**, 920.
25. Horlick, L. The effect of artificial modification of food on the serum cholesterol level. Canad. med. Ass. J. 1960, **83**, 1186.
26. Anderson, J. T., F. Grande, and A. Keys. Hydrogenated fats in the diet and lipids in the serum of man. J. Nutr. 1961, **75**, 388.
27. Food Consumption and Dietary Levels in Households in the United States, ARS-62-6, U. S. Dept. of Agriculture, Agricultural Research Service, August 1957.
28. Connor, W. E., R. E. Hodges, and R. E. Bleiler. Effect of dietary cholesterol upon serum lipids in man. J. Lab. clin. Med. 1961, **57**, 331.
29. Connor, W. E., R. E. Hodges, and R. E. Bleiler. The serum lipids in men receiving high cholesterol and cholesterol-free diets. J. clin. Invest. 1961, **40**, 894.
30. Mattson, F. H., and R. A. Volpenhein. The specific distribution of fatty acids in the glycerides of vegetable fats. J. biol. Chem. 1961, **236**, 1891.
31. Mehlenbacher, V. C., and T. H. Hopper. Official and Tentative Methods of the American Oil Chemists' Society, 2nd ed. Chicago, American Oil Chemists' Society, 1958.
32. Kuemmel, D. F. A mercury derivative-chromatographic method for the separation of unsaturated fatty acid esters. Analyt. Chem. 1962, **34**, 1003.
33. Pearson, S., S. Stern, and T. H. McGavack. A rapid, accurate method for the determination of total cholesterol in serum. Analyt. Chem. 1953, **25**, 813.
34. Van Handel, E. Suggested modification of the micro determination of triglycerides. Clin. Chem. 1961, **7**, 249.
35. Zilversmit, D. B., and A. K. Davis. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J. Lab. clin. Med. 1950, **35**, 155.
36. Cochran, W. G., and G. M. Cox. Experimental Designs, 2nd ed. New York, John Wiley & Sons, 1957, p. 510.
37. Cochran, W. G., and G. M. Cox. Experimental Designs, 2nd ed. New York, John Wiley & Sons, 1957, p. 75.
38. Anderson, J. T., F. Grande, and A. Keys. Safflower oil, hydrogenated safflower oil and ascorbic acid effects on serum cholesterol in man. Fed. Proc. 1957, **16**, 380.
39. Malmros, H., and G. Wigand. Treatment of hypercholesteremia. Minn. Med. 1955, **38**, 864.