Serum Cholesterol Response in Man to Oral Ingestion of Arachidonic Acid

ANCEL KEYS, PH.D.,* JOSEPH T. ANDERSON, PH.D.,† AND FRANCISCO GRANDE, M.D.‡

IT IS NOW CLEAR that fats in the diet of man have important effects on the concentration of cholesterol and related lipids in the blood. and that the effects are related to the character as well as amount of the fatty acids in the diet. In general, when one fat is isocalorically substituted for another fat or for carbohydrate in the diet of man, the serum cholesterol response is the resultant of the fact that the common saturated fatty acids tend to raise the serum cholesterol, linoleic acid (or polyenė in general?) tends to lower it, while oleic acid has substantially no effect.[1,2] The depressant effect of polyene or of linoleic acid upon serum cholesterol has been discussed frequently as being related to the "essentiality" of these fatty acids as demonstrated by other effects in experiments on animals receiving highly artificial diets extremely low in polyunsaturated fats.[3,4]

In view of the fact that arachidonic acid is widely believed to be the most active or effective of the fatty acids in regard to "essentiality," it is interesting to examine its effect, when provided in the diet, on the serum cholesterol level in man. The present paper is a report of a controlled experiment on men receiving a dietary supplement of arachidonic acid. By inference from the discussions in some of the recent literature, it might be expected that arachidonic acid would have some remarkable capacity to lower the serum cholesterol level.[5] The findings reported here do not correspond to such expectations.

From The Laboratory of Physiological Hygiene, University of Minnesota, and The Hastings State Hospital, Minneapolis, Minnesota.

* Professor and Director, Laboratory of Physiological Hygiene, University of Minnesota School of Public Health; † Associate Professor of Physiological Hygiene, University of Minnesota School of Public Health; ‡ Professor of Physiological Hygiene, University of Minnesota School of Public Health and Physiologist, Jay Phillips Research Laboratory, Mt. Sinai Hospital, Minneapolis, Minnesota.

This work was aided by a research grant from The Upjohn Company, Kalamazoo, Michigan.

SUBJECTS, DIET AND PROGRAM

Nine men, aged 40 to 58 years, long domiciled at the Hastings State Hospital, were selected as subjects for this study on the basis of stability and cooperativeness demonstrated in previous studies, and the absence of signs of physiologic or biochemical abnormality. After preliminary study while the men subsisted on a standardized "house" diet, they were assigned to three groups of three men each, matched in age, relative body weight, and serum total cholesterol concentration on this standard diet. Thereafter for 70 days all men were maintained on a constant diet consisting of only two alternating daily menus. Each man was weighed, nude, before breakfast every day and, when necessary to maintain weight constancy, individual adjustments were made in the amounts of bread, jelly, and potatoes served, the rest of the diet being constant. The men were maintained under 24-hour daily supervision in a locked building, and during outside recreation regulated so as to provide a constant level of physical activity. All meals were prepared, measured, and served in a special dietary kitchen and the men had no opportunity to obtain other food.

The total experimental period was divided into five periods, the diet being the same in all but during periods II and IV each man ingested capsules identical in appearance providing either 8 g of arachidonic acid concentrate or 8 g of oleic acid daily. The experimental design is summarized in Table I. Venous blood

TABLE I

Experimental Design for Groups 1, 2, 3 for 70 Consecutive Days of Constant Diet

Group	I (21 days)	II (11 days)	III (14 days)	IV (10 days)	V (14 days)
1	—	A	—	C	—
2	—	C	—	A	—
3	—	C	—	C	—

NOTE: Daily dietary supplement of arachidonic acid (A) or control of oleic acid (C) provided during periods II and IV as indicated.

samples were drawn from each man on appropriately spaced occasions during the five periods.

Average characteristics of the three groups of men at the start and the changes in body weight during the five consecutive experimental periods are shown in Table II. The matching of the groups and the constancy of body weight are evident. The mean actual daily nutrient intakes for the five periods are shown in Table III.

THE ARACHIDONIC ACID PREPARATION

The arachidonic acid concentrate was generously supplied by The Upjohn Company (their preparation No. U-9519). It was prepared from liver and brain tissue, with 2 per cent each of lecithin and tocopherol added as stabilizers. Absence of *trans* double bonds was indicated by infrared spectroscopy and the nuclear magnetic resonance (NMR) spectrum. The NMR spectrum was fully in agreement with the structure of arachidonic acid and showed no extraneous bands. Ultraviolet absorption spectroscopy showed no absorption ascribable to conjugated diene, triene, or tetraene. In the assay, the octabromoarachidonic acid obtained proved to have an infrared spectrum identical with published data.[6] Assay indicated a maximum of 80 per cent arachidonic acid (by bromination) and a minimum of 40 per cent (from NMR data, assuming the material to be a mixture of arachidonic and oleic acids).

This arachidonic acid preparation was put up in opaque brown gelatin capsules, each containing 0.5 g of the preparation and estimated to contain not less than 0.25 g of arachidonic acid. The capsules were stored under refrigeration in an atmosphere of carbon dioxide. During the arachidonic supplementation period each subject ingested 16 capsules daily, thereby receiving not less than 4 g and probably about 5 g of arachidonic acid per day.

As control, capsules identical in appearance but containing 0.5 g. each of commercial grade of oleic acid were used.

TABLE II

Average Characteristics for the Three Groups of Men at the Start of the Experiment and the Changes in Average Body Weight (in kg) from the Starting Weight During the Five Experimental Periods

Group	Age	Relative weight	Δ Body weight (kg) from start				
			I	II	III	IV	V
1	47.3	95.0	−0.3	0	−0.2	−0.4	−0.5
2	46.0	95.7	−0.2	+0.1	−0.1	−0.2	+0.1
3	47.0	95.0	−0.5	+0.1	−0.1	−0.2	+0.2

NOTE: Relative weight computed from the standard U. S. Medico-Actuarial tables for height, weight, and age.

TABLE III

Mean Daily Nutrient Intakes for the Five Consecutive Dietary Periods

Nutrient	I	II	III	IV	V
Calories	3880	3970	3840	3840	3820
Proteins (g)	111.4	112.1	110.9	111.1	110.3
% calories from total fats	35.3	35.0	35.8	35.8	35.8
Saturated fatty acids (g)	70.2	70.0	69.3	69.3	69.0
Mono-ene fatty acids (g)	67.3	68.2	67.8	67.8	67.3
Poly-ene fatty acids (g)	10.4	11.2	11.2	11.1	11.2
Cholesterol (mg)	769	752	747	747	740

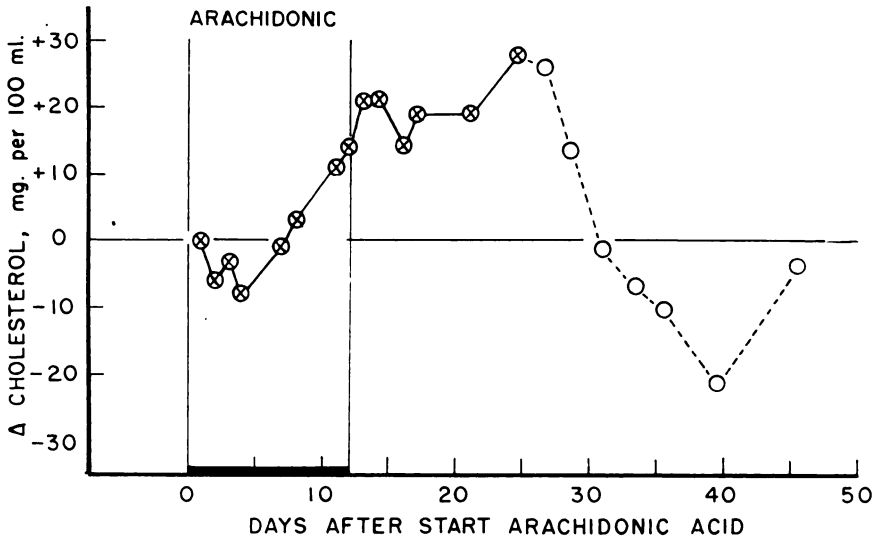

Fig. 1. Mean changes in concentration of serum total cholesterol during and after administration of arachidonic acid. Groups 1 and 2 combined (six men) until day 25; group 1 only (three men) thereafter.

METHODS

Blood serum was analyzed for total cholesterol by our modification of the method of Abell *et al.*[7] Cholesterol in the alpha and beta lipoprotein fractions, separated by fractionation with cold ethanol,[7] was measured in the same samples. All samples were analyzed in duplicate.

RESULTS

The principal experimental results in groups 1 and 2 are summarized in Figure 1. After a trivial initial decline from previous control values, the serum cholesterol rose during the last days of the administration of arachidonic acid and rose still further and continued to stay high for about two weeks after cessation of the administration.

The experimental design involved a cross over or switchback arrangement of group 1 versus group 2, i.e., arachidonic acid versus control and vice versa, with group 3 being a continuing control. It was expected that any effects of the arachidonic acid would disappear within 14 days after the cessation of its administration so that groups 1 and 3 would serve as controls when group 2 received arachidonic acid (period IV), but the unexpected persistence of an effect of the arachidonic acid after its withdrawal from the diet made it impossible to use group 1 as a control for group 2.

Fortunately, no large general time trend appeared in the controls (group 3 throughout, group 2 in periods I, II, and III). The mean values in groups 2 and 3 were 200.3, 207.3, and 202.1 in periods I, II, and III, respectively, none of these values being statistically different from their grand mean of 203.2 mg per 100 ml; the mean values in group 3, the only valid control for time trends in periods IV and V, were 191.1, 187.3, and 187.7 for periods I, IV, and V, respectively.

In the absence of a general time trend it was possible to take the average of the cholesterol values for each man during period I as his own control and to express all of his subsequent cholesterol values as deviations from that base. The averages of these individual deviations are shown in Figure 1, in which each point is the average from six blood samples. Until day 24 the six samples are from the six men in groups 1 and 2; thereafter only data for the three men in group 1 are available and each point in Figure 1 is the average of values on two consecutive days for each of the three men.

In order to obtain numbers more satisfactory for statistical analysis the data from successive time periods were grouped as shown in Table

TABLE IV

Mean Concentration of Serum Total Cholesterol (Δ Chol.), Expressed in mg per 100 ml as Difference from the Mean in Period I, at Different Times (Days) after Start of Administration of Arachidonic Acid to the Men in Groups 1 and 2

Groups 1 and 2					Group 3				
Days	Regimen	N	Δ Chol.	S.E.	Days	Regimen	N	Δ Chol.	S.E.
1–4	A	21	− 5.2	±3.3	1–11	C	21	−1.4	±2.5
7–11	A	24	+10.6	±3.6					
12–17	C	24	+19.7	±4.3	12–25	C	18	−1.3	±2.5
18–25	C	18	+24.4	±5.0					
26–33	C	15	+16.5	±4.1	26–39	C	18	−7.8	±2.1
35–39	C	15	− 6.0	±2.7					
40–49	C	15	− 9.9	±2.8	40–49	C	18	−1.2	±2.4

NOTE: Six men in groups 1 and 2 except after day 25 when only the three men in group 1 were available. Regimen A = standard diet plus capsules of arachidonic acid; Regimen C = standard diet plus capsules of oleic acid N = number of blood samples analyzed; S.E. = standard error of the mean.

IV. The average serum total cholesterol value did not change significantly during the first four days after starting the administration of arachidonic acid. During days 7 through 11 of administration, however, the cholesterol level tended to rise. This trend increased during the subsequent two weeks following cessation of administration and thereafter fell to values slightly below the means during the preadministration control period. In the control group (group 3), which received no arachidonic acid supplement at any time, there was no significant change in the serum cholesterol value at any time from period I except for a trivial fall, averaging 7.8 mg per 100 ml., during the period from day 26 to day 39.

Analyses of beta lipoprotein cholesterol as well as total cholesterol were made on the control (period I) samples from the men in groups 1 and 2 and on the samples from these same six

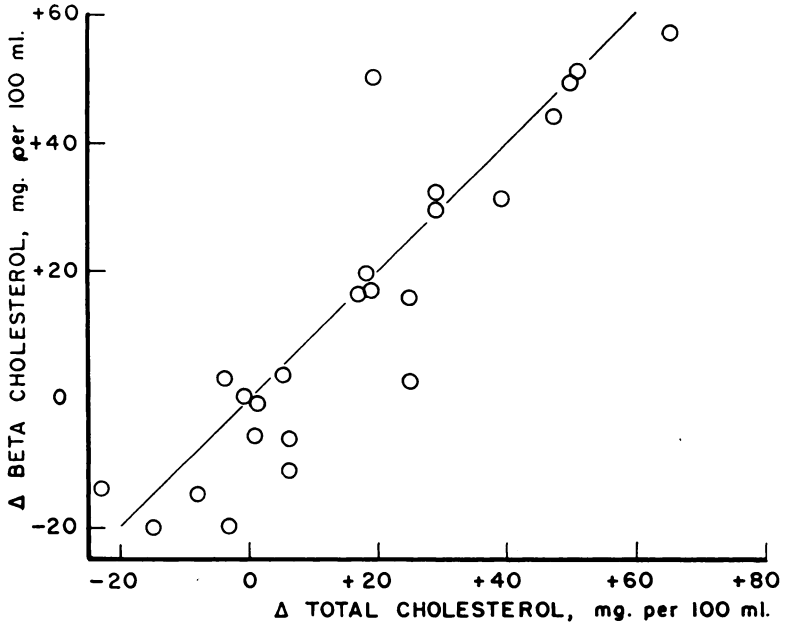

Fig. 2. Relationship between changes from individual control values for concentration of total cholesterol and beta lipoprotein cholesterol in the blood of the men in groups 1 and 2 on days 10, 11, 24, and 25 after the start of arachidonic acid administration.

men on days 10, 11, 24, and 25 after the start of the arachidonic acid administration. The grand mean difference of these 6 × 4 = 24 samples from the corresponding individual control averages was + 17.9 (S.E. = ±4.9) for total cholesterol and +14.6 (S.E. = ±4.8) for beta lipoprotein cholesterol, both values being in milligrams per 100 milliliters of serum. Figure 2 shows the individual values in this material. The relationship between Δ beta lipoprotein cholesterol and Δ total cholesterol is approximated reasonably well by the 45 degree linear trend line. Disregarding sign, the mean discrepancy between Δ beta and Δ total cholesterol is 7.9 mg per 100 ml, with a standard deviation of ±7.7. It appears that all the observed changes in total cholesterol may be accounted for by changes in beta lipoprotein cholesterol. This is in agreement with all other studies in which changes in serum cholesterol have been induced by dietary changes.[8,9]

DISCUSSION

The arrangements adopted in this experiment were dictated by the limited amount of arachidonic acid concentrate available. The dose used, of the order of 1.5 mg per calorie of diet or 70 mg per kg of body weight, is a large amount, many times that which could be obtained in any ordinary diet, but it is possible that a larger daily dosage would have a different effect.

Similarly, a longer period of arachidonic acid feeding might have produced effects not indicated in the present experiment. The duration of feeding chosen, 11 days, was selected because a great deal of experience in feeding various fats to men under controlled conditions indicates that much of the ultimate cholesterol response to dietary fats is usually obtained in a week to ten days, subsequent changes being merely an extension, in the same direction, of the trend usually evident in the averages of groups of subjects within a very few days.

The necessarily small number of men in the present experiment limits the security of concluding that the numerical results, as averages, apply precisely to men in general. From the present evidence it is not possible to rule out the possibility of a true slight average decline in serum cholesterol concentration in the first few days of administration of the arachidonic acid. It is clear, however, that the evidence offers no support for the idea that supplementing an ordinary diet with arachidonic acid will produce any important depression of the serum cholesterol level at any time.

Kinsell *et al.*[5] reported results in a single patient from which they concluded that arachidonic acid feeding depresses the serum cholesterol level. The preparation they used was stated to be "a phosphatide mixture derived from mammalian liver, with a fatty acid composition of 12.5 per cent of tetranoic (probably cis-arachidonic) acid, saturated acids 75 per cent, oleic acid 5 per cent, and conjugated dienes 2.4 per cent." The daily dose, for 18 days, was 75 g of this mixture, from which we estimate that the intake of arachidonic acid (or rather tetraenoic acid) was of the order of 7 g daily. The serum cholesterol data on their patient do not indicate statistically a very impressive effect and, in any case, there is no reason to believe that any effects obtained may not have been produced by the massive dosage of phosphatides rather than by the small part of the supplement accounted for by tetraenoic acid. It is interesting, also, to note that the feeding of this preparation was attended by a marked steatorrhea, with a rise in fecal fat to an average level 6.9 times the average for this patient on all other diets. No steatorrhea was observed in any of the six men studied in the present experiment, suggesting that either the patient studied by Kinsell *et al.* was peculiar or that the preparation they used produced steatorrhea because of ingredients in it other than arachidonic acid. It should be noted that, in general, steatorrhea from any cause tends to result in a decrease in the plasma cholesterol concentration.

The present data indicate that when the body has been flooded with 4 or 5 g of arachidonic acid daily for a week or so, the serum cholesterol concentration rises and remains moderately elevated for several weeks, as if the effect were produced by an excessive accumulation of arachidonic acid in the body which persists until the excess had been disposed of. Since there

is no reason to suggest that this finding does not have general validity, it is necessary to consider possible explanations.

One consequence of a glut of arachidonic acid in the body should be to inhibit the normal synthesis of arachidonic acid in the body. It is widely believed that linoleic acid is the precursor of arachidonic acid and the "linoleate is somewhat inefficiently converted to arachidonate."[10] The expectation, then, would be that flooding the body with exogenous arachidonic acid would spare the use of linoleic acid for the synthesis, thereby creating a relative surplus of linoleic acid that would be available for other uses.

Linoleic acid in the human body has three obvious uses. Besides its use in the synthesis of arachidonic acid and as a simple fuel for combustion, linoleic acid is clearly an important ingredient of lipoproteins, in which it normally comprises some 40 to 50 per cent of the fatty acids in the cholesterol esters, the remainder being largely oleic acid.[11,12] Accordingly, the release of linoleic acid from its use in arachidonic acid synthesis would be to provide an unusual abundance of a fatty acid which seems to be a preferred ingredient in the synthesis of lipoproteins. Since the liver has a large capacity to synthesize cholesterol, and there is no evidence that other ingredients of lipoproteins are normally limiting in its synthesis, the result should be to accelerate the formation of lipoprotein, including the usual and necessary complement of cholesterol in it.

This explanation of the observed phenomenon hinges on the idea that a relative surplus of linoleic acid is made available in the liver. This concept is not in conflict with the general observation that the addition of linoleic acid to the human diet tends to depress the serum cholesterol level and that of the beta lipoprotein of which it is a major constituent. It is necessary to distinguish between effects dependent on events in the liver and those that reflect events in the intestine.

From the literature[13] and from our own experiments to be reported elsewhere,[14] it appears that the major result of dietary linoleic acid on the serum cholesterol level in man is caused by the effect of dietary linoleic acid in producing an increased fecal excretion of steroids—cholesterol itself and the bile acids made from cholesterol. Apparently, linoleic acid in the diet stimulates the flow of bile, or inhibits the absorption of cholesterol and bile acids in the intestine, or both, while saturated fatty acids have the opposite effect. The indications are that the rate of cholesterol synthesis in the liver is actually increased on linoleic acid feeding, and decreased on feeding saturated fatty acids, but that these changes in the rate of synthesis are insufficient to compensate fully for the changes in the removal, via the feces, of cholesterol and the steroids derived from it. In other words, the primary effects of the fatty acids in the diet would seem to be on the bile flow and reabsorption of bile constituents in the intestine, changes in the synthesis in the liver being perhaps a secondary and imperfect homeostatic response.

There is some evidence that there is competition for the use of linoleic acid for synthetic purposes in the liver. When animals are maintained on a diet severely restricted in linoleic acid, the addition of large amounts of cholesterol to the diet precipitates or enhances the development of signs of essential fatty acid deficiency.[15] The explanation may be that there is competition for the linoleic acid for the synthesis of arachidonic acid on the one hand and for the synthesis of plasma lipoprotein on the other; an overabundance of exogenous cholesterol would favor the route of use to make lipoproteins. It is interesting to note also that on extremely low fat diets, in which the supply of linoleic acid is necessarily very small, the serum cholesterol, and presumably also the associated beta lipoproteins, does not rise but may fall to very low levels.[16] A deficiency of linoleic acid for the esterification of cholesterol to go into lipoproteins would seem to be a reasonable explanation; it is unnecessary to suggest that there is any defect in the ability of liver to synthesize cholesterol in this situation.

SUMMARY

In a rigidly controlled experiment with the subjects on a constant diet of two alternating daily menus, six middle-aged men ingested capsules containing a concentrate of arachi-

donic acid or, during control periods, of oleic acid. Three other men, matched in age and other characteristics with those in the experimental group, subsisted on the same diet but ingested only oleic acid capsules. The arachidonic acid dosage was 4 to 5 g per man per day for 11 days.

The concentration of total cholesterol in the blood serum showed no significant change during the first few days of supplementation with arachidonic acid but thereafter it tended to rise, and it remained elevated for several weeks following the withdrawal of the arachidonic acid supplement before returning to approximately the presupplement level. The changes in the serum total cholesterol concentration were accounted for by changes in the cholesterol in the beta lipoprotein fraction of the serum. The significance of these findings is discussed.

ACKNOWLEDGMENT

Mrs. Helen Williams supervised the diet preparation and measurements. Mrs. Nedra Foster supervised the blood cholesterol measurements.

REFERENCES

1. Keys, A., Anderson, J. T., and Grande, F.: Prediction of serum cholesterol responses of man to changes in fats in the diet. *Lancet* 2: 959, 1957.
2. Keys, A., Anderson, J. T., and Grande, F.: Effect of serum cholesterol in man of mono-ene fatty acid (oleic acid) in the diet. *Proc. Soc. Exper. Biol. & Med.* 98: 387, 1958.
3. Sinclair, H. M.: Deficiency of essential fatty acids and atherosclerosis. *Lancet* 1: 381, 1956.
4. Bronte-Stewart, B., Antonis, A., Eales, L., and Brock, J. F.: Effects of feeding different fats on serum cholesterol level. *Lancet* 1: 521, 1956.
5. Kinsell, L. W., Friskey, R. W., Michaels, G. D., and Splitter, S.: Essential fatty acids, lipid metabolism and atherosclerosis. *Lancet* 1: 334, 1958.
6. Sinclair, R. G., McKay, A. F., Myers, G. S., and Jones, R. N.: Infrared absorption spectra of unsaturated fatty acids and esters. *J. Am. Chem. Soc.* 74: 2578, 1952.
7. Anderson, J. T. and Keys, A.: Cholesterol in serum and lipoprotein fractions, its measurement and stability. *Clin. Chem.* 2: 145, 1956.
8. Anderson, J. T., Keys, A., and Grande, F.: The effects of different food fats on serum cholesterol concentration in man. *J. Nutrition* 62: 421, 1957.
9. Keys, A., Kimura, N., Kusukawa, A., and Yoshitomi, M.: Serum cholesterol in Japanese coal miners. Am. J. Clin. Nutrition 5: 245, 1957.
10. Deuel, H. J., Jr.: *The Lipids*, Vol. III, Interscience, New York, 1957, pp. 183–185.
11. Tuna, N., Reckers, L., and Frantz, I. D.: The fatty acids of normal plasma and of arteriosclerotic plaques. *J. Clin. Invest.* 36: 932, 1957.
12. Luddy, F. E., Barford, R. A., Riemenschneider, R. W., and Evans, J. D.: Fatty acid composition of component lipides from human plasma and atheromas. *J. Biol. Chem.* 232: 843, 1958.
13. Gordon, H., Lewis, B., Eales, L., and Brock, J. F.: Dietary fat and cholesterol metabolism. Faecal elimination of bile acids and other lipids. *Lancet* 2: 1299, 1957.
14. Kaleta, Z., Anderson, J. T., Grande, F., and Keys, A.: The balance of neutral and acid steroids in men on butter and safflower oil diets. *Fed. Proc.* 18: 531, 1959.
15. Pfeiffer, J. J. and Holman, R. T.: Essential fatty acids, diabetes, and cholesterol. *Arch. Biochem.* 57: 520, 1955.
16. Keys, A., Mickelsen, O., Miller, E. V. O., and Chapman, C. B.: The relation in man between cholesterol levels in the diet and in the blood. *Science* 112: 79, 1950.