Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat

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ABSTRACT Elderly institutionalized men were assigned at random to two groups, one of which received a conventional diet while the other was fed a diet in which the major modification was substitution of unsaturated for saturated fat. Changes in serum lipids and in adipose tissue over periods up to 5 years are described.

In control subjects, mean serum cholesterol rose 4% over the first 20 months, then fell during the next 40 months to a level 10% below the starting concentration. In the experimental group there was an immediate drop, followed by further changes roughly parallel to those in the control subjects. The mean difference between the control and experimental groups was 14.0% of the starting level. Changes in serum total lipid were similar, but the percentage difference between control and experimental groups was only 6.8% of the baseline level.

All major esterified serum lipid fractions of experimental subjects contained increased concentrations of linoleic acid. This was most marked in triglyceride, which at 3 years had a composition similar to that of the dietary fat in both groups of subjects.

Adipose tissue linoleic acid rose in men on the experimental diet from 11% of total fatty acid at time zero to 32% at 5 years. The rate of rise during the 1st year was correlated negatively with initial body weight and positively with weight gain; the influence of adherence to the diet was much less pronounced.

KEY WORDS dietary · unsaturated fat · long-term · serum · lipids · cholesterol · adipose tissue · turnover · linoleic acid · correlation · adiposity · weight change · adherence to diet · man

A WEALTH OF EVIDENCE suggests that dietary changes which lower serum cholesterol levels might be effective in preventing or retarding human atherosclerosis and its complications. In the past several years clinical experiments in this Center and elsewhere have been in progress for the purpose of testing this possibility (1-5). These studies all involve substitution of highly unsaturated for saturated fat; in some cases, other characteristics of the diet are also modified.

A major source of concern in regard to experimental design has been the difficult problem of evaluating adherence, particularly in programs dealing with free-living participants. One proposal has been to use the fatty acid composition of erythrocyte lipids, which reflects the influence of dietary fat over a period of many weeks (6). It has also been suggested that the fatty acid composition of adipose tissue may be used to assess the composition of dietary fat integrated over a period of many years (7), and this approach has been used to evaluate adherence to an experimental diet in at least one study (8). In the present program, which deals with men who are institutionalized but who have access to food sources other than the experimental diet, records of attendance in the dining room have been employed as the primary index of adherence. Data on changes in composition of subcutaneous fat have also been obtained from many of the subjects. These data have provided information of theoretical interest and have also made it possible to evaluate the usefulness of adipose tissue sampling as an index of adherence to the experimental diet. These findings are described in the present paper, together with some details of changes in serum lipid composition.

METHODS

Subjects

The participants in the study are elderly men living in a Veterans Administration Domiciliary unit. They were
TABLE I  COMPOSITION OF DIETS

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<th>Experimental</th>
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</table>

* Mean ±SD of 216 one week pooled collections.
† Mean of seven analyses, each representing a 4 to 9 week pool in 1960-61, weighted as to length of collection period.
‡ Mean of three 1 week pools in 1964-65. Analyses were performed through the courtesy of Dr. Edward H. Ahrens, Jr., and Dr. Richard J. Jones. The procedure used was that employed at The Rockefeller University (9).

Selected as voluntary participants from the entire population of the home after the experiment and its objectives had been explained to them. Upon entering the study each man was assigned, by use of a table of random numbers, either to the control group or to the experimental group. The control group was placed on a conventional diet containing 40% of its calories as fat. This diet differs in only minor respects from the regular institutional diet, which all subjects had been eating for 4 months or more prior to the study. The experimental group was placed on a diet also containing 40% of its calories as fat, but in which vegetable oil was substituted for most of the saturated fats provided by the natural foods. The subjects and experimental conditions are described in greater detail in earlier publications (1, 2). Subjects were 55 years of age and older on entering the study; mean age was 66 years in both groups. During the first 5 years of the program, 389 men began the control diet and 393 began the experimental diet. Subjects were weighed every 4 months.

Diets and Food Service

Diets and methods of diet analysis have been described previously (1). Details of diet composition are summarized in Table 1 and Fig. 1.

The entire Domiciliary population, including participants in this study, are fed in a single large building. Food is served ad libitum, cafeteria style. Body weight is not controlled. Since men living in the Domiciliary are free to leave during the day and even for more extended periods, it is not possible to enforce total adherence to the study diets. To monitor this and to prevent participants from obtaining unauthorized food within the dining room, each man in the institution receives a color-coded meal ticket which is punched at every meal. These are collected monthly, and a permanent record is made of the number of meals taken each day. These records do not provide a perfect index of adherence, but several observations suggest that they give a realistic one. Although the participants eat ad libitum, most accept a standard tray and consume almost everything served. Although some men skip

![Fig. 1. Mean composition of dietary fat. Relative abundance of fatty acids in fat of experimental and control diets, determined by analysis of extracted fat. The fatty acids are designated by chain length and number of double bonds. Results are means of seven analyses, each representing an 8 week pool. Reprinted by courtesy of the New England Journal of Medicine (1).](image-url)
meals, interviews have indicated that most absences from the dining room represent meals taken elsewhere. Interviews have also suggested that between-meal snacks are not a major source of food for most of the subjects. Some of the nonadherence results from extended absences from the Center. It is assumed that the participants of both groups consume a diet similar to the control diet during these periods.

Adherence percentages for the present report were calculated as

\[
\text{Adherence} = \frac{\text{Meals taken in dining room}}{\text{Days since starting study}} \times \frac{100}{3}
\]

Serum and Adipose Tissue Analysis

While each participant was on the regular institutional diet, his base line serum cholesterol and total lipid levels were determined on two fasting blood samples taken 2 weeks apart. After starting the control or experimental diet, samples were obtained at 4-month intervals. Serum cholesterol was determined on each sample by the method of Abell, Levy, Brodie, and Kendall (10) and total lipid gravimetrically by the method of Folch, Lecs, and Sloane Stanley (11), generally within 1 week. Fatty acid content of major lipid fractions was determined on sera of selected subjects by fractionation on silicic acid columns, followed by preparation of methyl esters and gas-liquid chromatography; details of these procedures are described elsewhere (12).

As a check on analytical precision, a large pool of serum has been stored in small sealed ampoules under purified nitrogen at \(-20^\circ\) since the beginning of the study and used as a reference standard. Aliquots of this serum were included with each batch of cholesterol analyses. Mean and standard deviation of 276 values for cholesterol concentration in this pooled serum over a 5\(1/2\) year period were 204.5 \pm 6.3 mg/100 ml. Reproducibility of total lipid determination was checked by 14 analyses of a separate serum pool over a 2 month period; mean and standard deviation were 599 \pm 30 mg/100 ml.

Subcutaneous fat was aspirated from the buttocks by the method of Hirsch, Farquhar, Ahrens, Peterson, and Stoffel (7). Methyl esters of the component fatty acids were obtained either by methanolation in acidified methanol (13) or by the use of boron trifluoride-methanol (14), and were analyzed by gas-liquid chromatography. Details of chromatographic conditions and reproducibility of results have been published (12, 15). Fatty acids were identified only by chromatographic retention time.

Samples from control and experimental subjects were intermingled for all analyses, and were identified by code number only.

Data Analysis

An exponential expression describing changes in adipose tissue linoleic acid as a function of time was computed by means of the asymptotic regression program, BMD06R, of the Health Sciences Computing Facility, University of California at Los Angeles. Stepwise multiple regression analysis employed program BMD02R. Other statistical computations were performed using appropriate programs from the BMD manual (16).

RESULTS AND DISCUSSION

Body Weight

Mean body weight of the control subjects underwent a small decline, while there was a rise of similar magnitude in weight of the experimental group (Fig. 2). The reasons for these changes are not clear.

Serum Cholesterol and Total Lipid

The mean base line level for serum cholesterol was 232 mg/100 ml for the control subjects and 231 in the experimental group; for total lipid, 845 mg/100 ml for the controls and 850 for the experimental group.

Data on changes in serum cholesterol and total lipid are shown in Fig. 2. In order to provide a concise presentation of all analytical values, regardless of changes in composition of the population, each individual value was calculated as a percentage of the mean base line value for that subject. Figure 2 shows changes in the means of these percentages. The data are pooled regardless of degree of adherence. The only values omitted are base line values of subjects who left the Center or withdrew from the study during the first 4 months.

Changes in serum cholesterol in the control subjects are striking. While some fluctuations occurred in the values obtained on the pooled serum sample (also shown in Fig. 2), these were small and did not parallel the changes seen in the subjects. The occurrence of an approximately parallel change of at least equal magnitude in serum total lipid helps further to exclude laboratory errors and indicates clearly that the fluctuations in the control group reflect changes of biological significance. It can also be seen in Fig. 2 that the major dietary variable, degree of saturation of fat, did not fluctuate sufficiently to account for the changes in serum lipids.

It is possible to demonstrate that the major trends in serum cholesterol shown in Fig. 2 are not due to changes in composition of the study population. In Fig. 3 are plotted serum cholesterol data in the form of serial values on cohorts of subjects, each cohort consisting of...
men with the same period of uninterrupted follow-up (but not necessarily with the same starting date). The curves for these cohorts, of unchanging composition, are similar to those in Fig. 2 which represent the study population as a whole. It is inferred that the changes in the control subjects, and presumably part of the change in the experimental group, are related in some manner to advancing age, to prolonged residence in the Domiciliary, or to an uncontrolled and unidentified environmental variable.

Published studies of changes in serum cholesterol with aging in elderly men have yielded varying results, probably in part because most studies have not involved serial measurements on the same individuals (see, for example, references 17-23). Of these, the data most clearly suggestive of a decline in serum cholesterol level are those of Keys, Mickelsen, Miller, Hayes, and Todd (17), which suggest an approximately linear fall of about 3 mg/100 ml per year from age 60 to 75. In the control subjects of the present study, the rate of decrease from month 20 to month 60 is much greater, about 10 mg/100 ml per year. Reasons for the initial rise and subsequent fall of serum cholesterol in these men are the subject of continuing study.

The parallel changes in serum cholesterol levels of the control and experimental groups after the first 4 months (Fig. 2) suggest that these later changes were due to some factor other than the known dietary variables. The difference between the control group and the experimental group automatically includes corrections.
for aging, for possible uncontrolled environmental variables, and for variation within the laboratory. Therefore this difference, rather than change of the experimental group from its baseline, is assumed to reflect the net influence of the experimental diet. Although there is some fluctuation in this difference, no sustained long-term trends are apparent with regard to the effect on serum cholesterol level. Over-all mean difference between the control and experimental groups, weighted by the number of cases at each time interval, is 14.0% of the base line level. It is possible that this difference would be slightly greater if body weight had remained constant in both groups.

Percentage difference between control and experimental groups in the serum total lipid (over-all mean: 6.8%) is half that in serum cholesterol. That more than half of the decrease in total lipid can be accounted for by the fall in cholesterol indicates that the percentage change in other components was smaller. Here too the long term trends seem to be roughly parallel for the control and experimental groups. There is some tendency for the two curves to converge after the first 2 years, owing perhaps in part to the body weight changes.

It is of interest to relate serum cholesterol changes in these subjects to the regression equation derived by Keys, Anderson, and Grande, which expresses change of serum cholesterol as a function of several dietary variables (24). The result predicted from the most recent form of the equation, using the data of Table 1 and Fig. 1 and assuming 100% adherence, is -39.3 mg/100 ml. A corrected prediction, based on actual mean adherence of 83%, is -32.6 mg/100 ml. The actual mean value for the portion of the change in serum cholesterol attributable to the experimental diet (i.e., difference between control and experimental groups) is -32.5 mg/100 ml, in striking agreement with the formulation of Keys et al.

**Fatty Acids of Serum Lipids**

Fatty acids in the major lipid fractions, at the end of 3 years of the study, are shown in Fig. 4. All fractions contained increased percentages of linoleic acid. This is particularly marked in the triglycerides, which in both groups of subjects had a composition almost identical with that of the dietary fat.

**Adipose Tissue**

As shown in Fig. 5, subcutaneous fat of control subjects has shown no change. In the subjects on the experimental diet, there has been a progressive rise in the linoleic acid content of adipose tissue, at the expense of all other major fatty acids with the possible exception of stearic (Fig. 5). By the end of 5 years the mean linoleic acid content had risen to 32%, as compared to 11% at the start of the study.

\[ \Delta C = 2.4 \Delta S' - 1.2 \Delta P + 1.5 \Delta Z, \]

in which **ΔC** = change in serum cholesterol in mg/100 ml, **ΔS'** = change in saturated fatty acids of 12-16 carbons, as percentage of total calories, **ΔP** = change in polyunsaturated fatty acids as percentage of total calories, and **ΔZ** = change in

\[ \sqrt{\text{mg of cholesterol}/1,000 \text{ calories of diet}} \]
The percentage rise in adipose tissue linoleic acid is examined in further detail in Fig. 6. Data in this figure are limited to samples preceded by records of 80% adherence or better. The curve was fitted, as indicated under Methods, to the general form \( L = a + be^{-kt} \), in which \( L \) = linoleic acid percentage and \( t \) = time on the diet in days. The computed equation is \( L = 35.6 - 24.2e^{-0.0013t} \), which may be rearranged to

\[
\ln(35.6 - L) = 3.19 - 1.02 \times 10^{-3}t. \tag{I}
\]

The constant 35.6 represents the computed value of \( L_{\infty} \), i.e., the asymptotic value of \( L \), and the daily fractional turnover is \( 1.02 \times 10^{-3} \).

The half-time of this function is 680 days. If one assumes that linoleic acid and the other major fatty acids stored in depot fat are handled as a homogeneous pool, and that the linoleic acid content of the precursor pool(s) is constant, then this figure is an estimate of the mean half-time of fatty acid turnover in stored depot fat.\(^3\) Hirsch et al. arrived at a similar estimate, 350–750 days, for the half-time of depot fat in the human adult.\(^7\)

\(^3\) There is evidence that adipose tissue contains at least two pools of triglyceride\(^(25)\); presumably, the component with the slower turnover represents triglyceride of the storage droplet and the much smaller, rapidly exchanging component represents mainly cytoplasmic triglyceride actively involved in metabolic exchange. In vitro studies suggest that different fatty acids may not be mobilized from adipose tissue at identical rates\(^(26)\). However, the reported differences do not appear great enough to invalidate the use of linoleic acid as a tracer.

\(^7\) This type of analysis presupposes a steady state, which in this instance implies constant body weight. As indicated below, individual body weights were not constant, but mean changes were small. Mean values for weight change in the subjects included in Fig. 6 are, as percentages of initial weights, -0.2% at 1 year, +2.1% at 2 years, +1.3% at 3 years, +0.3% at 4 years, and +2.8% at 5 years. Even at constant body weight, the state of adipose tissue is only relatively steady, because of the fasting-refeeding pattern of human alimentation.
Concern about possible reasons for the wide dispersion of values in Fig. 6 led to a search for uncontrolled variables which might have influenced the rate of change in depot fat composition. Nonadherence to the experimental diet seemed an inadequate explanation, since the data charted in Fig. 6 were obtained from subjects with meal attendance records from 80 to 100%. It seemed probable that differences in adiposity, and weight changes during the study, would affect the rate of change in linoleic acid content of adipose tissue. This possibility was tested by further analysis of the 1 year data. There were 19 subjects on the experimental diet whose subcutaneous fat had been analyzed both at zero time and at 1 year (347–390 days), with adherence records ranging from 44 to 93%. Relevant information on these subjects is shown in Table 2. Simple scrutiny suggested that the change in linoleic acid content of adipose tissue over the 1st year was rather poorly correlated with adherence, but that there was a negative correlation with weight, estimated by body weight, and a positive correlation with weight gain. Correlation coefficients are shown in Table 2.

These relationships were investigated further by stepwise multiple regression analysis of the data in Table 2. The independent variable which best predicts change in adipose tissue linoleic acid (ΔL) is initial weight (W), with a correlation coefficient of −0.58. Addition of change in body weight (ΔW) as a second independent variable gives a multiple r of 0.71. Beyond that, addition of adherence (A) and height as additional independent variables has no appreciable effect on the multiple correlation coefficient. The multiple regression equation computed on the basis of the first two independent variables alone is ΔL = −0.114 W + 0.282 ΔW + 27.1. If adherence is included, one obtains ΔL = −0.113 W + 0.276 ΔW + 0.066 A + 22.1.

It is recognized that body weight is only a rough measure of adiposity. Skinfold thicknesses were measured, using a constant-pressure caliper, but the reproducibility of this technique was so poor that the additional information was not useful.
It seems likely that most of the residual variance in ΔL can be attributed to analytical error, to inaccuracy in use of dining room attendance as the index of adherence, to inadequacy of body weight as an estimate of adiposity, and to the likelihood that weight changes in some subjects were complex (e.g., intermittent or fluctuating). Some unexplained variance may also result from the fact that a linear function was fitted to the data, whereas the relationship to body weight and to weight change must certainly be nonlinear. It is clear from these relationships that the turnover rate of depot fat in individual subjects covers a wide range above and below the estimated mean value given above.

The relationship of ΔL to W and to ΔW is one which might have been predicted. If the rate of exchange between depot fat and plasma is more or less independent of pool size in the depot, then large pool size (i.e., a large value for W) will result in a slow fractional turnover rate—hence the negative correlation between W and ΔL. The positive correlation with ΔW results, presumably, from relatively rapid deposition of dietary fat in adipose tissue during weight gain [see rapid changes during weight gain in infants (27,28)] and from very limited movement of fatty acid into the triglyceride storage compartment of adipose tissue during periods of negative caloric balance.

Since dietary fat is diluted by fat synthesized in vivo from carbohydrate, which is free from linoleic acid, one would expect the asymptotic value for linoleic acid in adipose tissue to be lower than its concentration in dietary fat. Figure 6 bears this out. The fact that the extrapolated asymptote is close to the dietary level confirms the assumption that total food intake in these men has a linoleic acid content similar to that of the food samples submitted for analysis, and helps to validate the use of the meal attendance figure as the primary index of adherence.

Determining the adherence of individual subjects is an important problem in studies dealing with free-living participants. However, the usefulness of subcutaneous fat aspiration for this purpose has distinct limitations under experimental conditions where adiposity is variable and caloric balance cannot be controlled. For the adherence range covered by our data, Equation 1 could, theoretically, be used by solving for A and estimating its value from the experimental values of W, ΔW, and ΔL. But as a practical matter, this computed relationship would be applicable only for dietary conditions nearly identical with those of this study, and only at the end of 1 year of participation. It is possible that analyses obtained after several years, and thus closer to the asymptotic level, would show a better correlation with adherence and less effect from other variables. Unfortunately we cannot test this hypothesis with the present data, since the later samples include only men with good adherence records.
TABLE 2  INCREMENT IN ADIPOSE TISSUE LINOLEIC ACID IN FIRST YEAR, RELATED TO OTHER VARIABLES

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<th>Subject No.</th>
<th>1 Yr Change in Adipose Tissue 18:2 (mol)</th>
<th>% of total fatty acid</th>
<th>Adherence in 1st Yr, (A)</th>
<th>Initial Weight, (W)</th>
<th>Weight Change in 1st Yr, (ΔW)</th>
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Coefficient of correlation with change in adipose tissue 18:2

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