Serum Lipids of Normal Subjects With Aging

Studies in a Single Cohort

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The relative roles of environmental factors, of inheritance and of the inexorable effects of aging in the etiology of atherosclerosis and its related lipid disorders continue to be debated. An increase in serum lipids with age has been noted in many studies (1, 2, 3, 4). These studies, however, have involved measurements of people of different ages at one point in time rather than of one group at different ages. To conclude from such studies that a rise of serum lipids is a necessary accompaniment of age is perilous. Until a cohort can be studied through life conclusions regarding the effect of age on serum lipids are speculative only.

The present study compares the serum lipids of a group of 28 persons studied first in the early 1930's and again in the 1960's. The results suggest that a rise in serum lipids in men is not a relentless effect of time but may be related to weight gain during adult life. While the group is small; it is unique in the long span of time between the initial and the follow-up study.

SUBJECTS AND METHODS

The subjects were for the most part physicians, medical students, and other professional personnel, their spouses, and other persons associated with John P. Peters, M.D., Yale University Medical School. Dr. Peters was a pioneer in investigating the role of triglycerides in health and disease (6).

Initial blood samples were drawn after an overnight fast in the early 1930's. In almost half the subjects more than one such sample was obtained in connection

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with other studies on the influence of body build, body weight, food intake and seasonal variation (5, 6). In these instances, the initial value was represented by a mean. Some of the subjects were restudied in 1952 and 1953 and these findings have been reported (7).

Between 1963 and 1967 blood for follow-up examination was obtained from as many of the original group as could be located. In the morning after an overnight fast the bloods were drawn by one of us (P.L.) or in a few instances of those living in distant places, by the subject's own physician. In the latter event, serum was sent to West Virginia via air mail. Otherwise, all sera was stored and transported in the frozen state. A single serum sample was examined in duplicate for each subject of the 1960 series. A brief questionnaire requested information regarding weight at the time of blood drawing.

Analytical methods. The determinations of the 1930's and 1950's were carried out in the laboratory of one of the authors (E.B.M.); those in the 1960's in the laboratory of another of the authors (M.J.A.). Agreement between old and new methods was as satisfactory as if the same methods had been in continuous use when the following corrections for gravimetric cholesterol values were applied:

In the 1930's and in the 1950's total fatty acids were determined by titration after saponification of an alcohol–ether extract of serum (5–7). In the 1960's total fatty acids were determined by microtitration of a chloroform–methanol extract of serum (8). In 50 different sera, obtained during more than a year, total fatty acids were compared by old and new methods throughout a range from 9.3 to 34.4 mmole/liter. Agreement was excellent with mean total fatty acids by old method 15.5 and by new method 15.9 mmole/liter. The correlation coefficient between both methods was +0.96 with a regression equation: new = old (1.13) — 1.62. Corrections of old values are insignificant in this series; for example: total fatty acids of the weight-gaining men would change from 11.7 to 11.6 mmole/liter.

In the 1930's and 1950's cholesterol was precipitated by digitonin from the alcohol–ether extract and measured gravimetrically (5–7). In the 1960's cholesterol was determined colorimetrically by the method of Abell et al. (9) except that serum was extracted with chloroform and methanol initially (8). As reported previously, in 66 sera analyzed by both methods, Abell's method yielded cholesterols about 10% higher than the gravimetric values (10). The correlation coefficient between both methods was +0.96 with the regression equation: cholesterol Abell = 1.02 (cholesterol gravimetric) + 13. A correction factor of 1.10 was therefore applied to all gravimetric cholesterol values (10). Values so corrected agree with those in the literature using the Abell method (10).

Phospholipids were determined as lipid phosphorus in a lipid extract of serum. The phosphorus was analyzed by a modification of the method of Fiske and Subbarow (11) which has not changed substantially over the years except for replacement of the optical colorimeter used in the 1930's by a spectrophotometer for the later determinations.

Triglycerides were determined indirectly from the total fatty acids, total chole-

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1 From a list of 45 subjects in the original group known to one or more of the authors 28 were available for retesting. Of the 17 not obtained, four were agreeable, but problems of distance were insurmountable. Four were dead (one of ruptured abdominal aneurysm, one of an airplane accident, one of myocardial infarction, one of post streptococcal nephritis). Eight did not respond to a letter. One could not be located.
terol and lipid phosphorus (6, 8). Triglycerides were expressed as triglyceride fatty acids. The upper limit of normal is considered to be 5.5 mmole/liter (10), equivalent to 155 mg per 100 ml.

RESULTS

The means and standard deviations for all the lipids measured in the 1930’s and again in the same individuals in the 1960’s are shown divided according to sex in Table 1. The paired one-tailed t-test was used to test for significant differences. Small increases in all lipid fractions, not statistically significant, were observed for men. For women there were increases in all lipid fractions statistically significant, except for triglycerides. The 1960 values are open to the criticism that only one serum was collected on each subject, and lipids may vary in any one individual during a few weeks (5). This objection is partially answered by data obtained in the 1950’s on ten men and six of the women observed also in 1960. The mean of each of the lipid fractions of men and of women in 1950’s agreed within one standard deviation with the respective mean for the 1960’s. However for the women the 1960 means were all slightly higher than the 1950 means.

The men and women were further divided into those who had gained less than 10 pounds since the initial determination or had lost weight (weight-stable group) and those who had gained 10 pounds or more (weight gainers). The results are shown in Table 2. There were no appreciable changes in the serum lipids of the 11 weight-stable men. As seen by the standard deviations, the variability for this latter group was considerably less than for the weight gaining group.

Only five of the sixteen men fell into the weight-gaining category. For the weight gainers, all lipid values were higher in the 1960’s than in the 1930’s. Despite the smallness of the numbers, the difference was significant for total fatty acids, cholesterol and lipid phosphorus. The per cent change in cholesterol for the weight-gainers (+24%) was significantly different from the per cent change for the weight-stable group (−4%, $p < 0.02$). The changes in triglycerides were not significant, possibly because of their greater variability. Of the twelve women the rise in all lipid categories was evident for the eight weight-stable women but not for the four weight-gaining persons, possibly because the group was so small. The ini-

### Table 1

**Means and Standard Deviations for Weight and Serum Lipids of Persons Studied in the 1930’s and Again in the 1960’s**

| Year | N  | Age (yr) | Weight (lb) | TFA (mmole/liter) | TC (mg%) | LP (mg%) | TGFA (mmole/liter) |
|------|----|----------|-------------|------------------|---------|---------|------------------|
| Males |    |          |             |                  |         |         |                  |
| 1930’s | 16 | 31 ± 6   | 159 ± 18    | 12.1 ± 2.2       | 221 ± 41| 9.1 ± 1.1 | 3.0 ± 1.6        |
| 1960’s | 16 | 60 ± 6   | 168 ± 24    | 13.6 ± 2.9       | 227 ± 40| 9.5 ± 1.1 | 3.8 ± 2.1        |
| P    |    |          |             |                  |         |         |                  |
| Females |   |          |             |                  |         |         |                  |
| 1930’s | 12 | 27 ± 6   | 131 ± 28    | 11.8 ± 2.1       | 211 ± 38| 9.4 ± .8  | 2.4 ± 1.2        |
| 1960’s | 12 | 55 ± 7   | 141 ± 26    | 13.8 ± 2.8       | 232 ± 36| 10.9 ± 1.7 | 3.1 ± 1.0        |
| P    | 0.03 | 0.004 | 0.03 | 0.004 |

*a The differences for lipids and weight of females are indicated as the p values, calculated by paired one-tailed t-test. Unless a p value is shown, the differences were not significant.

*b TFA = total fatty acids; TC = total cholesterol; LP = phospholipids as lipid phosphorus; TGFA = triglycerides expressed as triglyceride fatty acids.
TABLE 2
Means and Standard Deviations for Weight and Serum Lipids of Persons Studied in the 1930’s and Again in the 1960’s, Given According to Weight Gains

| Year | N  | Age (yr) | Weight (lb) | TFAa | TC | LP | TGFA |
|------|----|----------|-------------|------|----|----|------|
|      | Males, weight-stable (less than 10 lb gained) |
| 1930’s | 11 | 32 ± 7   | 158 ± 19    | 12.3 ± 2.5 | 226 ± 38 | 9.3 ± 1.1 | 2.8 ± 1.6 |
| 1960’s | 11 | 60 ± 7   | 162 ± 22    | 12.7 ± 1.7 | 217 ± 36 | 9.9 ± 1.1 | 3.2 ± 1.7 |
| P    |     |          |             | 0.001 | 0.05 | 0.005 |       |
|      | Males, weight-gaining (10 lb or more gained) |
| 1930’s | 5  | 30 ± 2   | 161 ± 18    | 11.7 ± 1.7 | 200 ± 42 | 8.6 ± 1.0 | 3.5 ± 1.2 |
| 1960’s | 5  | 59 ± 3   | 182 ± 22    | 15.3 ± 4.1 | 248 ± 48 | 9.6 ± 1.1 | 5.1 ± 3.3 |
|      | Females, weight-stable |
| 1930’s | 8  | 25 ± 5   | 136 ± 31    | 10.9 ± 1.5 | 199 ± 28 | 9.1 ± 0.9 | 1.92 ± 1.7 |
| 1960’s | 8  | 52 ± 6   | 137 ± 30    | 13.5 ± 1.8 | 218 ± 18 | 10.9 ± 1.9 | 3.12 ± 1.1 |
| P    |     |          |             | 0.005 | 0.04 | 0.005 | 0.001 |
|      | Females, weight-gaining |
| 1930’s | 4  | 32 ± 7   | 116 ± 8     | 13.6 ± 3.8 | 237 ± 39 | 10.9 ± 0.6 | 3.1 ± 0.8 |
| 1960’s | 4  | 61 ± 7   | 149 ± 18    | 14.3 ± 2.0 | 261 ± 52 | 10.9 ± 1.4 | 3.0 ± 0.8 |

* The mean percent change in cholesterol, for the male weight-gainers, +24%, was significantly different from the mean percent change in cholesterol, for the weight-stable males, −4%, p < 0.02. Correction was made for unequal variances (21).

a See Table 1 for symbols.

tial cholesterol concentration of the women destined to gain weight was higher than for the weight-stable group (Table 2).

Not only was the mean cholesterol concentration of the weight-stable men virtually unchanged after 30 years but each individual weight-stable person tended to maintain his original rank. The weight-gaining men and women on the other hand largely lost their rank position. Figure 1 shows the mean of the first and second cholesterol determinations of the weight-stable men and women and the weight gainers ranked into subgroups according to the first cholesterol concentration. The mean of each weight-stable subgroup shows the same rank as initially.

Bivariate correlation coefficients were calculated individually for the pairs second cholesterol and first cholesterol, and second cholesterol and weight change between the 1930’s and 1960’s. In addition, to show the interaction of the effects of the first cholesterol and of weight change in determining the second cholesterol concentration, multiple correlation analysis was carried out with the second cholesterol as the dependent variable and with the first cholesterol and weight change as a subset of independent variables. Both the bivariate and multiple correlation coefficients (r) and the per cent of variability of the second cholesterol accounted for by each correlation (r2 × 100) are shown in Table 3. For both men and women the cholesterol of the 1930’s combined with intervening weight gain provided a more accurate prediction of cholesterol in the 1960’s than either alone, and accounted for about 50% of the variability of the second cholesterol. Multiple regression analyses revealed the following formulae for predicting the second cholesterol:

men: cholesterol 2 = 0.54 (cholesterol 1) + 1.63 (Δ wt) + 103
women: cholesterol 2 = 0.51 (cholesterol 1) + 1.09 (Δ wt) + 115
all: cholesterol 2 = 0.49 (cholesterol 1) + 1.42 (Δ wt) + 114

in which Δ wt = second weight (in pounds) minus first weight.
Fig. 1. Mean and SEM of cholesterol concentrations and mean ages at time of first determination (left) and second determination (right) of the 28 subjects divided into subgroups according to the first cholesterol concentration. The weight-stable men (upper frame) and all weight-gainers (lower frame) are divided into tertiles, while the weight-stable women (middle frame) are divided into two groups. The numbers in each group are indicated in parenthesis.

TABLE 3
Correlation Coefficients ($r$) and Percent Contribution (%) of Cholesterol in 1930's and of Weight Change, Together and Separately, on Cholesterol in 1960's

| Cholesterol, 1960's | N  | r   | %  | p  |
|---------------------|----|-----|----|----|
| Cholesterol, 1930's |    |     |    |    |
| Men                 | 16 | 0.40| 16 | .12|
| Women               | 12 | 0.60| 43 | .02|
| All                 | 28 | 0.48| 23 | .009|
| Weight change between 1930's and 1960's |    |     |    |    |
| Men                 | 16 | 0.39| 15 | .13|
| Women               | 12 | 0.61| 37 | .03|
| All                 | 28 | 0.48| 23 | .009|
| Combined effect of cholesterol in 1930's and weight gain |    |     |    |    |
| Men                 | 16 | 0.66| 44 | .02|
| Women               | 12 | 0.73| 54 | .08|
| All                 | 28 | 0.68| 47 | .0006|

A similar influence of initial value and weight change on second value was also observed for total fatty acids, lipid phosphorus and triglycerides but significance was borderline or lacking, probably owing at least in part to greater technical and biological variability. There was no correlation between absolute weight and any lipid at either the first or second determination.
DISCUSSION

The present paper records serum lipids of a small group of men and women before and after an interval of nearly 30 years. The results show that no detectable change occurred in the 11 weight-stable men but that an increase in all lipid fractions occurred in the five men who had gained more than 10 pounds between the determinations. The increase was significant for total fatty acids, cholesterol and lipid phosphorus according to the one-tailed paired t-test. An increase in cholesterol and total fatty acids occurred in women regardless of weight gain or lack of it.

A rise in serum cholesterol with age has been reported in many studies. The mean cholesterol for men tends to rise until a plateau is reached between the ages of 40 and 65 with a subsequent decline. The values at the time of the peak are variably reported as 230–245 mg% (1, 2), and in some instances somewhat higher (3). A similar rise with age has been reported in the triglyceride fraction (3, 4). However, these studies are based on examinations of different people at one point in time rather than of a cohort followed through life. The conclusion that prevalence data reflect change in individuals through life is, of course, merely speculative. A few cohort studies have been reported. Man and Peters studied 16 men and women at a 10–20 yr interval. Some of the subjects appear in the present study. They found only an inconstant tendency for lipids to rise with age (7). Sperry and Webb reached similar conclusions (12). Page and Lewis reported relative constancy of cholesterol in two persons over many years (13). The various authors concluded that some factor other than age alone might cause the increase in certain persons.

Whether or not a rise in lipids is a consequence of normal aging is a matter of considerable importance. Unless one can define normal values with confidence, attempts to relate serum lipids to disease processes in aging subjects may be frustrating. Prevalence data may represent the resultant of many factors. Some of these might lead to changes with age due to the undetected presence among the normals of an abnormal population which in early life fails to demonstrate this abnormality. Thus, for example, gradual increase in mean cholesterol of a presumably normal population by inclusion of some persons with a nondetected abnormality of cholesterol metabolism, and reduction in the size of this fraction by increased mortality, could account for the peak and decline observed with aging in presumably normal prevalence groups. Even if stable cholesterol throughout life were characteristic of true normality, inclusion in the “normal” group of a fraction with an undetected disorder producing gradual rise in cholesterol with age and susceptibility to early death would produce a rise in mean cholesterol until the point where increasing mortality in this fraction resulted in an overall decrease of mean cholesterol in the subsequent prevalence groups.

Low-grade associations between obesity and serum triglycerides (14) have indicated that obesity may be such a factor. A similar association between obesity and serum cholesterol concentration has been doubted by some (3, 15) while others have supported a slight correlation (16, 17). Our previous studies have suggested that weight gain after maturity rather than obesity per se might be the age-associated factor which causes the rise in serum lipids with time. These, too, were prevalence data so that the association with weight gain was conjectural (14).

In contrast to these studies the present study is concerned with changes in weight
and lipids in individuals rather than with groups of differing degrees of obesity and shows that for a small group of men serum lipids remained constant when men who gained weight were excluded. The magnitude of increase in serum lipids of any group with age may therefore be related in part to the percent of the group undergoing significant weight gain during adult life. In the present study the lack of significant change in lipids of the males, taken as a whole, is probably a reflection of the fact that the majority had not gained weight during adult life. The men in the study were clinically oriented and unusually aware of the disadvantages of weight gain.

*Serum Lipid Changes in Women*

The results for the women were different from those of the men in that the women as a group showed rise in all lipids with time regardless of whether or not they gained weight. Other studies report that women have a continuous rise in cholesterol and triglyceride (3) with an accelerated rise between the ages of 45–55, at which time they exceed the cholesterol concentration of men. The present study is consistent with these reports.

*Cholesterol Rank*

While every effort was made to adjust for differences in methods it is also possible that the apparent lack of rise in cholesterol in weight-stable men reflected the interim change in method. However, quite apart from the question of absolute cholesterol values, the rank of weight-stable subjects with regard to cholesterol concentrations was essentially preserved three decades later. About 50% of the variability of cholesterol concentration at about age 60 was accounted for by the combined effect of cholesterol concentration at about age 30 and intervening weight change.

The variability of cholesterol concentration within the U.S. has not been explained by variations in diet (18). Hatch has postulated that if a cohort were followed through life each member would maintain his own cholesterol rank, a rank determined in part by heredity (19). The constancy of cholesterol rank through life in the weight-stable persons observed in the present study confirms his hypothesis of a genetic component to factors which determine cholesterol "set."

**CONCLUSIONS**

It was concluded that, in the absence of weight gain during adult life, little if any increase in serum lipids occurs with aging in men. Prevalence of obesity and patterns of weight change through life are factors of obvious importance in comparing one population with another with respect to serum lipids and related vascular disease. If elevation of serum cholesterol and triglyceride concentration is associated with increased risk of atherosclerotic vascular disease then avoidance of weight gain with increasing age in men might reduce significantly the incidence of atherosclerotic disease (20).

In order to assess change in serum lipids with time, 16 men and 12 women studied in the early 1930's at a mean age of 30 were studied again in the 1960's at a mean age of 58.

The men and women were divided according to weight gain of more than 10 pounds between the 1930's and 1960's. Mean serum lipids did not increase with time in the 11 men who were weight stable.

The five weight-gaining men had increases in cholesterol from a mean of 200 to one of 248 mg/100 ml and in triglycerides from 3.5 to 5.1 mmole/liter.
The women as a whole had increases in cholesterol and triglycerides without relation to weight gain.

Weight-stable men and women tended to occupy the same cholesterol rank in the 1960's as in the 1930's. This constancy suggests a genetic component to factors which determine cholesterol set.

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