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## 2. Stoffwechsel und Arteriosklerose – Métabolisme et artério-sclérose – Metabolism and arteriosclerosis

### a) *Blutlipoide, Blutlipoproteide – Lipides et lipoprotéïdes sanguins – Bloodlipids, bloodlipoproteins*

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### **The Role of Lipoproteins in Coronary Disease**

By F. T. Lindgren, Ph.D., and J. W. Gofman, M.D.

Arteriosclerosis and its most frequent clinical entity, coronary artery disease, presents one of the major medical problems, particularly in the more prosperous areas of the world. Undoubtedly, there are many factors operating over the entire lifespan of an individual which participate in the actual development of the arteriosclerotic lesion, yet, at the present time, the exact sequence of events and relative importance of these factors is a matter of debate. Without definitively choosing between the relative importance of these factors one can study the relationship of several biochemical variables with the clinical presence or absence of arteriosclerosis. However, one of the major limitations in the quantitative evaluation of arteriosclerosis is that without an actual autopsy there is no way in which to accurately assess the amount of disease present in a given individual. Thus, in our efforts to study the disease, at least for the present, we must rely upon indirect methods to evaluate the amount of arteriosclerosis present in an individual or in a group of individuals.

For a long time the blood lipids have been suspected as a major contributing factor in the development of arteriosclerosis. The fact that the intimal deposits generally contain significant quantities of all the constituent lipids present in the blood stream has given wide support to this view. However, the actual evidence implicating the serum lipids is that elevated levels of certain of the serum lipids (such as serum cholesterol) are associated with the presence of more than average arteriosclerosis (1, 2). Recently it has been technically possible to study and characterize the actual molecular units which circulate in the blood stream and which carry essentially all of the known serum lipids. In these studies (3, 4, 5, 6) certain of the lipoproteins of the low density class (characterized

ultracentrifugally and electrophoretically) have been strongly associated with the presence of more than average arteriosclerosis of the coronary system.

Considering the foregoing evidence, however, there is no basis to conclude, or for that matter, to assume that lipids and lipoproteins are causally related to the development of arteriosclerosis. What is established is that in groups of individuals where there is known to be more disease than in control normal groups, there is observed elevations of certain serum lipids and lipoproteins. Unfortunately there is no way of selecting a group of adults who are free of all arteriosclerosis. By the age of thirty the almost universal presence, to some degree, of the disease must be definitely assumed (5). In addition to the presence of the disease in nearly all individuals a wide spectrum in the amount of arteriosclerosis from individual to individual must also be assumed.

Before discussing the relation of lipoproteins to arteriosclerosis and its most common sequela, coronary artery disease, let us consider the nature of the blood lipids. In the average individual approximately one

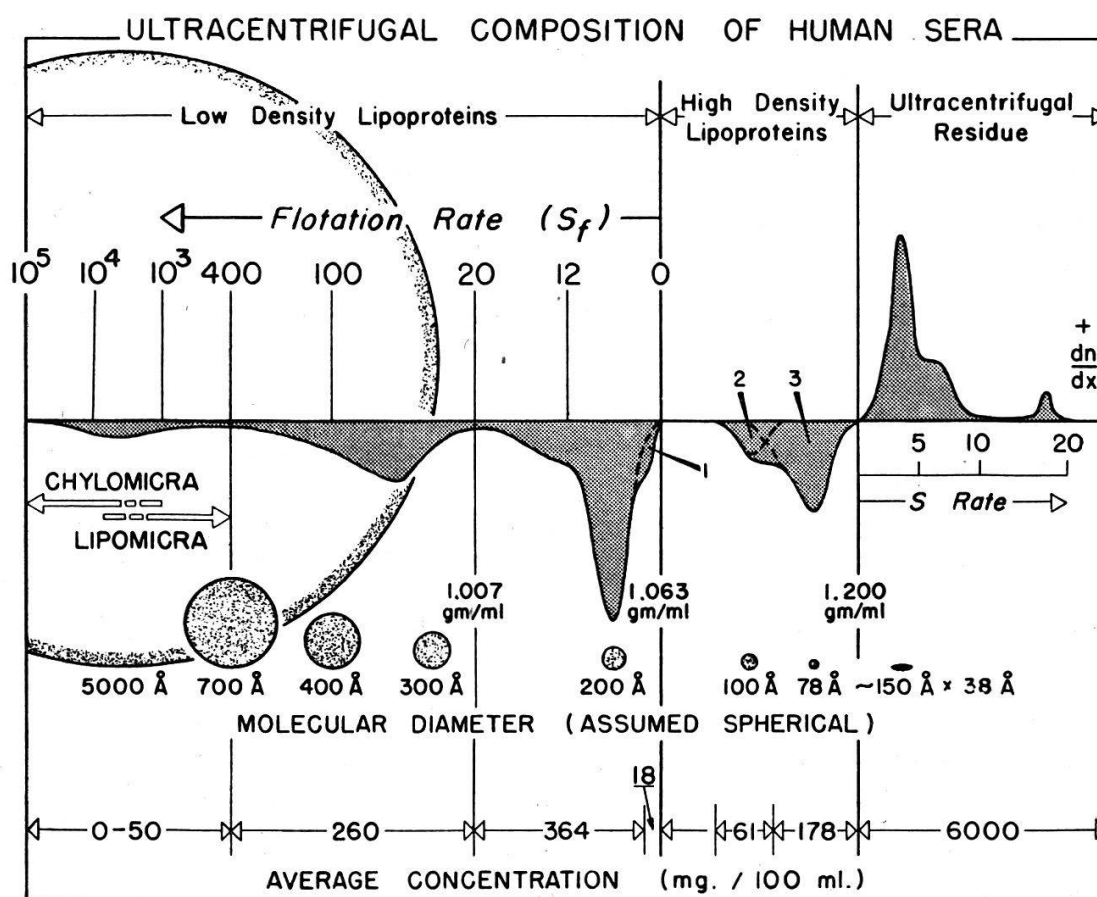


Fig. 1. The ultracentrifugal composition of human serum showing relative molecular sizes as well as average serum concentration present in 45 year old males. The ultracentrifugal residue plotted on a 30 fold reduced  $dn/dx$  scale contains the total ultracentrifugal albumin, globulin and "20" components.

tenth by weight of the proteins of the blood are lipoproteins. These lipoproteins (see Fig. 1) range from approximately 70–5000 Å in molecular diameter (assuming spheres). They exhibit a wide range of lipid composition and hydrated density. For instance, the hydrated densities vary from approximately 0.93 g/ml for the chylomicra-lipomicra class to 1.145 g/ml for the most dense lipoprotein (HDL<sub>3</sub>)<sup>1</sup> yet characterized. Because the blood lipoproteins possess such large differences in size and density they are particularly suited for isolation and characterization in the ultracentrifuge. Thus, we can ultracentrifugally isolate the major lipoprotein classes and characterize each class with regard to lipid chemical composition. The results of such procedures reveal that in addition to differing markedly in physical properties the serum lipoproteins are characterized by widely varying lipid compositions. Figs. 2–4 show the chemical lipid composition of the three major ultracentrifugal lipoprotein classes, which altogether account for 80% or more of the total serum lipoprotein content. In the order of increasing density and decreasing size these classes are the S<sub>f</sub> 20–400, the S<sub>f</sub> 0–20 and the major high density lipoprotein class (HDL<sub>2</sub> and HDL<sub>3</sub>). In all cases the lipid chemistry included analyses for cholesteryl ester, unesterified cholesterol, glyceride, phospholipid and unesterified fatty acid by a method (8) combining silicic acid chromatography and infra-red spectrophotometry.

Fig. 2 shows the lipid composition of the S<sub>f</sub> 20–400 lipoproteins. Glyceride (most of which is probably in the form of tri-glyceride) is the dominant lipid of this class, constituting from 46–64% of the lipid content by weight. Phospholipid accounts for approximately one-fifth of the total lipid. Unesterified fatty acid content is low. Within this lipoprotein class there exists considerable variability, particularly in the cholesteryl ester and unesterified cholesterol content. However, this variability may be related, at least in part, to the lipoprotein distribution of each of the S<sub>f</sub> 20–400 samples (there appears to be a higher cholesteryl ester content the closer the lipoprotein distribution is to S<sub>f</sub> 20).

Fig. 3 shows the S<sub>f</sub> 0–20 class lipoproteins. For this class the dominant lipid is cholesteryl ester which contributes 29–51% of the total lipid. Phospholipid content is approximately 25% and shows low variability. Unesterified cholesterol and glyceride are subordinate components showing considerable variability. Unesterified fatty acid content is low. Overall variability within this class, as with the S<sub>f</sub> 20–400, is large but may be associated, in part, with lipoprotein distributions closer to S<sub>f</sub> 20 than to S<sub>f</sub> 7.

Fig. 4 shows the data for the major high density lipoproteins (HDL<sub>2</sub> and HDL<sub>3</sub>). This class is characterized by the dominance of phospholipid and a high concentration of unesterified fatty acid relative to the other two major lipoprotein classes. Cholesteryl ester, unesterified cholesterol and glyceride are subordinate components showing marked variability. Some, but not all, the variability within this lipoprotein class, may be the result of differences in the relative HDL<sub>2</sub> and HDL<sub>3</sub> content of the total fraction studied.

For both normal and abnormal individuals the results of the lipid chemistry show that each of the three major lipoprotein classes is

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<sup>1</sup> HDL is an abbreviation for high density lipoprotein.

LIPID COMPOSITION S<sub>f</sub> 20-400

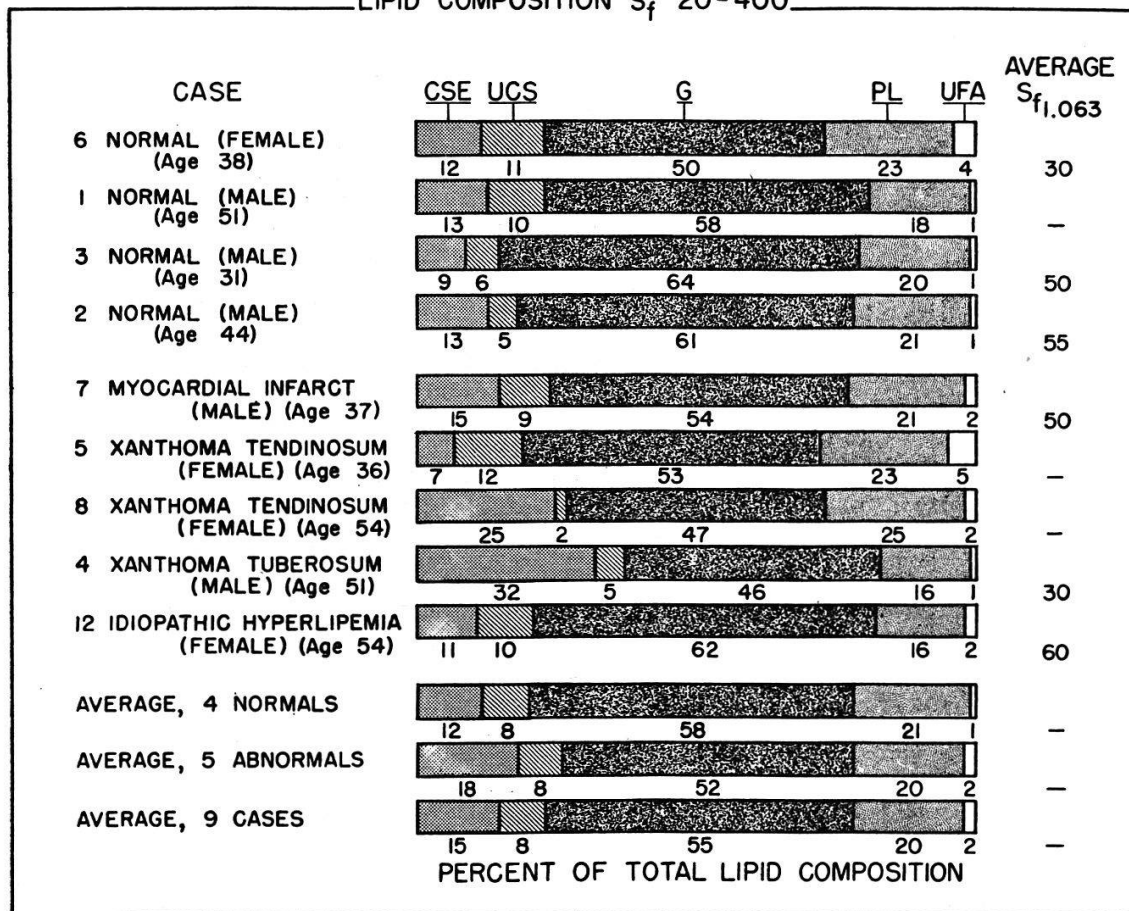


Fig. 2. Lipid chemical composition of S<sub>f</sub> 20-400 lipoproteins from fasting serum. Composition of constituent lipids are expressed as percent of total lipid. Where available, the average S<sub>f</sub> rate of the total S<sub>f</sub> 20-400 lipoprotein spectrum is given.

characterized by the dominance of one constituent lipid. However, in each of the lipoprotein classes all types of lipids analyzed are present. Variability, within each class is considerable, yet there does exist a relatively consistent lipid composition for each of the three major lipoprotein classes.

Our present view is that all three lipoprotein classes (as well as lipoproteins above S<sub>f</sub> 400) represent stages of fat transport that are constantly going on in the blood stream. The exact nature of this process is not known but it appears to be characterized by lipoprotein transformation of the chylomicra-lipomicra class down through the entire range of the low density lipoproteins (9). Chemically this process is characterized by a hydrolysis of glyceride with fatty acid release (10, 11) in which the role of a "clearing factor" (12) (heparin activated serum lipoprotein lipase) appears to be of prime importance.

Although all the major lipoprotein classes of serum have been studied, thus far only the low density lipoprotein class have been found to be *significantly* related to the presence of arteriosclerosis. However, high

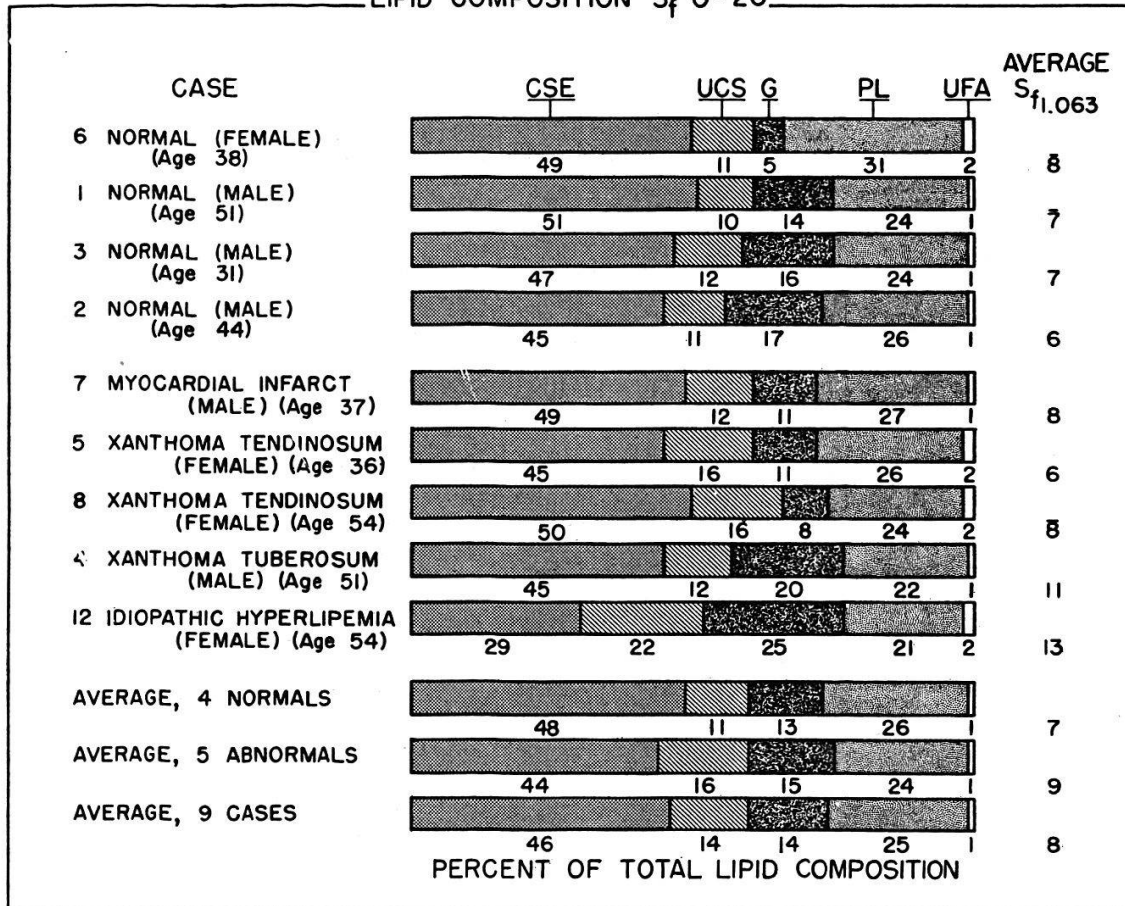
LIPID COMPOSITION S<sub>f</sub> 0-20

Fig. 3. Lipid chemical composition of S<sub>f</sub> 0-12 lipoproteins from fasting sera.

density lipoprotein 1 ( $\sigma = 1.05$  g/ml) present at very low abundance in the serum is definitely elevated in coronary disease (13). HDL<sub>1</sub> may be related to the removal of unesterified cholesterol from the blood to tissue compartments (14).

The technique for the low density lipoprotein isolation and study is presented in detail elsewhere (15). In addition to correction for self slowing, the lipoprotein concentrations are also corrected for effects of the type described by *Johnston* and *Ogston* (16). For the present discussion we will consider only the two major subgroups of the low density class, namely, the S<sub>f</sub><sup>0</sup> 0-12 and S<sub>f</sub><sup>0</sup> 12-400 class lipoproteins.

Individuals who have experienced a proven myocardial infarction probably best represent a group which on the average have more arteriosclerosis than a control group of the same age and sex. Here, of course, we are considering arteriosclerosis present only in the coronary arteries, yet it is reasonable to assume that in general the amount of arteriosclerosis in the coronary system also may be a good measure of total arteriosclerosis. Indeed, recent studies by *Young*, *Simon*, *Malamud*, and *Gofman* (17) show that a high degree of correlation exists between the

LIPID COMPOSITION HDL<sub>2+3</sub>

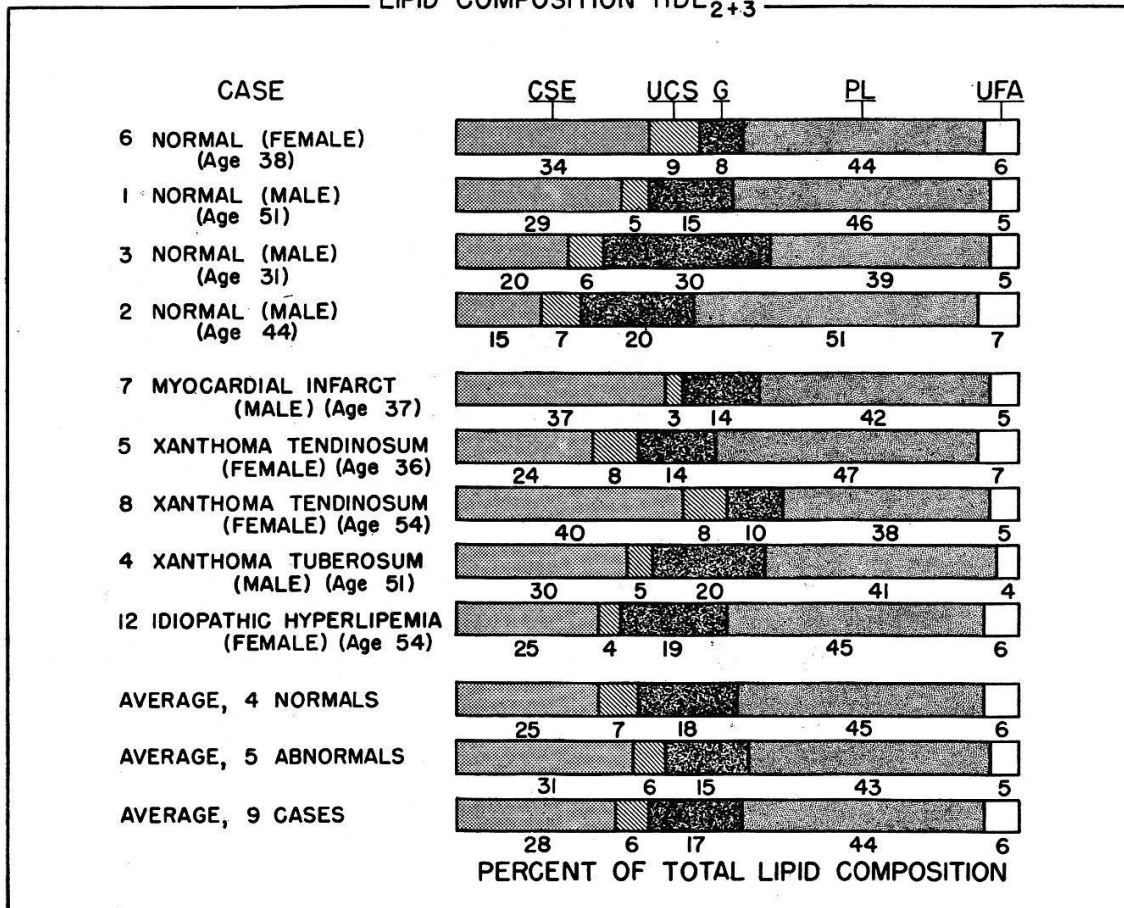


Fig. 4. Lipid chemical composition for the major high density lipoproteins (HDL<sub>2</sub> and HDL<sub>3</sub>).

amount of cerebral arteriosclerosis and the amount of coronary arteriosclerosis present at autopsy in the same individual. The assumption that persons who have experienced a myocardial infarction have on the *average* more *coronary* arteriosclerosis than members of a "non coronary" population is well established by autopsy studies (7). Thus, if a biochemical variable measures in some way the presence of arteriosclerosis it will segregate to *some* extent a coronary population from a normal population of the same age and sex. We expect this partial separation since it must be assumed that the normal population has a wide distribution of coronary arteriosclerosis and that many clinically normal individuals will have more arteriosclerosis than the average member of a living coronary population. If this were not so, we would have to assume that an individual's level of a biochemical variable was elevated as the result of experiencing a myocardial infarction. Our follow up studies clearly indicate that if there is a lipoprotein elevation such elevation *precedes* the myocardial infarction.

The two major low density lipoprotein classes consisting of the S<sub>f</sub><sup>0</sup> 0-12 and S<sub>f</sub><sup>0</sup> 12-400 lipoproteins have been studied in a large group of normals and coronaries in the

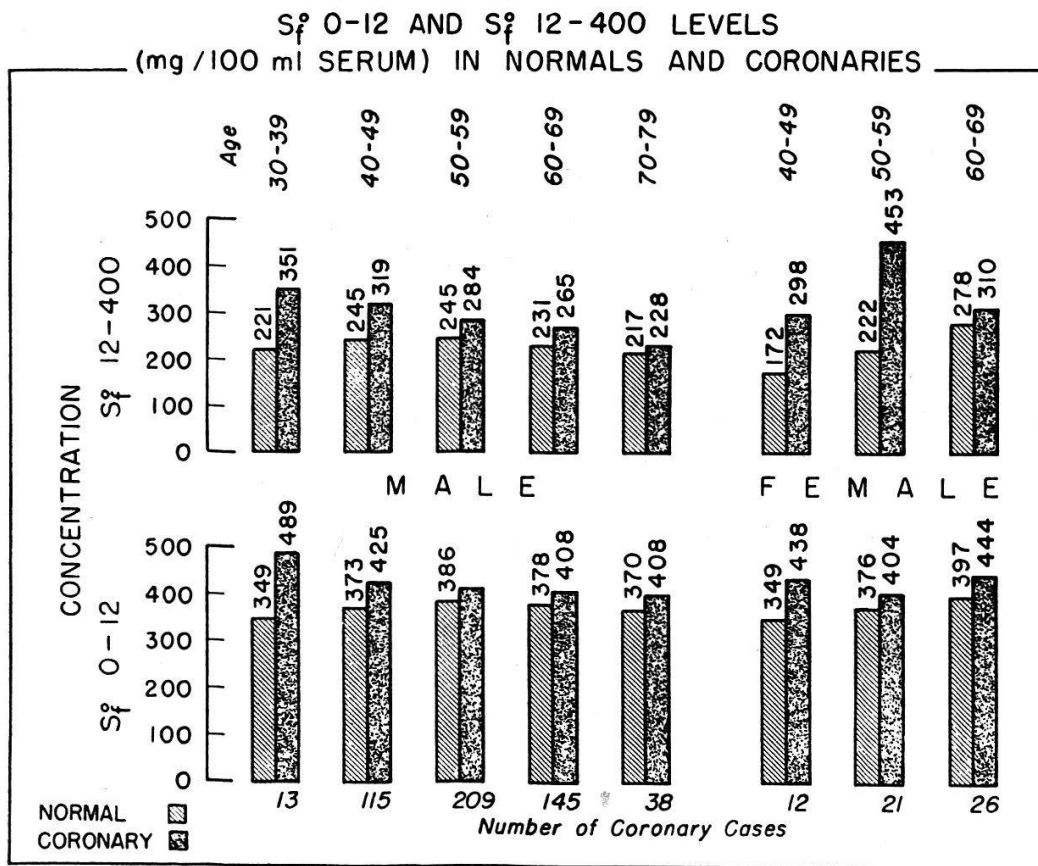


Fig. 5. Comparison of  $S_f^0$  0-12 and  $S_f^0$  12-400 lipoprotein levels for clinically normal individuals and individuals who have experienced a proven myocardial infarction (no coronary was studied until at least 6 weeks following the acute episode).

age group 30-70 years. The lipoprotein data for both coronaries and normals are presented in Fig. 5. It is seen that in all age groups of both sexes there exist elevated average lipoprotein levels in the coronary population as compared to a matched "non coronary" normal group.

From the above data it is clear that on the basis of elevated lipoprotein levels both the  $S_f^0$  0-12 and  $S_f^0$  12-400 lipoproteins contribute toward separation of the coronary groups from the corresponding normal groups. Intercorrelation studies between the  $S_f^0$  0-12 and  $S_f^0$  12-400 show that there exists *independent* contribution toward segregation from both lipoprotein classes. We could, of course, simply combine the two levels without regard to their relative weights in discriminating coronaries from normals. However, the method best suited to combine two variables, each of which contributes *independently* toward discrimination between two categories, is the Fisher linear discriminant analysis. When such an analysis is applied to the above data a new derived function is obtained which is hereafter referred to as "alpha". The  $\alpha$  function is as follows:

$$" \alpha " = 0.1 (S_f^0 \text{ 0-12}) + 0.16 (S_f^0 \text{ 12-400})$$

Here lipoprotein concentration is expressed in mg/100 ml serum and the absolute values of the factors (0.1 and 0.16) are chosen to give a convenient scale of " $\alpha$ " values (it is the relative value of the two factors that is important since the absolute values are arbitrary). The application of this function (with the constants 0.1 and 0.16) represents the best way in which we are able to discriminate members of a coronary population from a normal control population utilizing the present data of  $S_f^0$  0-12 and  $S_f^0$  12-400 lipoprotein concentrations. Where the constant 0.175 (derived from earlier analysis) has been used instead of 0.16 the  $\alpha$  value has been called the Atherogenic Index (or A.I.). The relation between  $\alpha$  and A.I. for the average individual is simply:  $\alpha = (\text{A.I.} - 4)$  units. It should be clear that we may expect small further refinements in the relative weighting factors for the  $S_f^0$  12-400 class as more data are accumulated.

If the  $\alpha$  is some measure of the presence of arteriosclerosis in an individual, we would expect to find a consistent elevation in certain common disease categories in which excessive arteriosclerosis is ordinarily found. Table 1 presents the  $\alpha$  levels for several clinical abnormalities,

Table 1  
Mean  $\alpha$  values for several disease categories

Disease category	Number of cases	Mean $\alpha$ value in disease	Mean $\alpha$ value for matched controls
Nephrotic state . . . . .	13	266.5	51.1
Essential hyperlipemia . .	9	260.2	78.0
Xanthoma tuberosum . .	23	243.6	76.0
Xanthoma tendinosum .	18	129.5	67.7
Spontaneous myxedema .	2	123.5	74.9
Xanthelasma . . . . .	43	87.8	71.4

together with  $\alpha$  levels for matched controls. In each case there is a significant  $\alpha$  elevation in all the clinical groups (in addition to clinical coronary disease itself) where we might expect excessive arteriosclerosis compared to that found in normal controls of the same age and sex. Of particular interest are very high  $\alpha$  levels found in the nephrotic state, idiopathic hyperlipemia, and xanthoma tuberosum. All these observations strongly indicate that elevated  $\alpha$  levels are associated with the presence of greater than average arteriosclerosis.

Our present hypothesis is that the  $\alpha$  value represents indirectly, at the present time, and perhaps with more knowledge, directly a measure of the *rate* of development of arteriosclerosis.

The operation of a rate of development of arteriosclerosis involves an accumulative phenomenon. Further, an accumulative concept of arterio-

sclerosis is consistent with autopsy data over the entire human life span (18). Thus, an estimate of the amount of arteriosclerosis accumulated by an individual over a 10 year period during which time he maintained an  $\alpha$  level of 90 would be proportional to  $90 \times 10$  or  $(K) (10) (\alpha)$ . In order to estimate the total amount of accumulated arteriosclerosis present in a particular individual we ideally should know the  $\alpha$  value for that individual over his entire life span up to the present moment. Thus, we would obtain a summation of all  $\alpha$  values times the time intervals over which each  $\alpha$  value was maintained. In the notation of the calculus this would be  $K \int_0^T \alpha dT$  or accumulated coronary disease (A.C.D.). In order to obtain a convenient scale of A.C.D. values  $K$  is set equal to  $1/10$  in which case the relationship between  $\int \alpha dT$  and A.C.D. is simply:  $\text{A.C.D.} = 1/10 \int \alpha dT$ . Obviously, such  $\alpha$  values for a given individual are unavailable. However, knowing the present  $\alpha$  value, an estimate of  $K \int \alpha dT$  can be made if we assume that an individual maintains the same position on an  $\alpha$  scale relative to his fellows over his previous life span. This assumption is a reasonable one if made during the active life span of an individual at a time which is clearly typical of the environmental, dietary and other factors known and unknown that may bear upon an individual's overall metabolism. Our own observations for individuals in the 20–50 age group support this assumption. However, recent data (19, 20) demonstrate clearly that this assumption cannot be made for institutionalized individuals in the very high age brackets. For this reason estimates of A.C.D., and from it an estimate of degree of arteriosclerosis may be applicable only to those individuals from which a determination of  $\alpha$  during their typically active period can be obtained.

In order to test the hypothesis that A.C.D. represents a measure of accumulated coronary disease we can compare A.C.D. values for all age and sex groups with available quantitative autopsy data. *Lober* (7) has presented such autopsy data from which it is possible to calculate the volume of intimal sclerosis per unit length of coronary artery for both normal males and females over the entire life span. If we compare his recalculated data with plots of A.C.D. for the normal male and female we observe a striking similarity (see Fig. 6). These observations are consistent with the hypothesis that A.C.D. measures in some way the total amount of accumulated arteriosclerosis.

The question now arises as to what is the value of an “ $\alpha$ ” determination and a subsequent A.C.D. calculation. One consequence is that it is possible to extend the concept of A.C.D. to provide an estimate of coro-

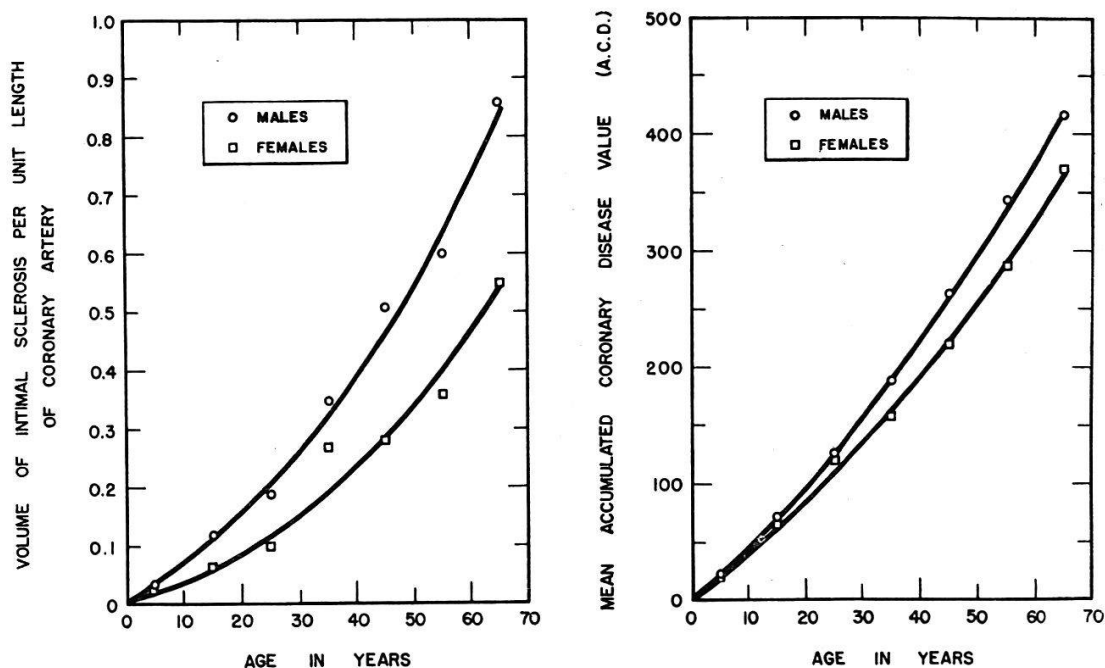


Fig. 6. Plotted on the left are the mean values of volume of sclerotic intimal tissue (calculated from Lober's data) per unit length of coronary artery for both sexes. Plotted on the right for comparison are the mean values of Accumulated Coronary Disease (A.C.D.) for a representative United States Population.

nary disease risk in the form of an estimate of coronary mortality. We know, for instance, that for each age and sex group the coronary population must grow out of a population whose A.C.D. distributions are the *same* as that found in a normal population. It remains to calculate for each age interval and sex group the total number of normals in the total population with a particular A.C.D. interval and to compare this with the number of coronary mortalities that occur in the same age and sex group with that same A.C.D. interval. For this consideration all coronary cases were combined irrespective of age and sex to give an average A.C.D. distribution for all coronaries. Thus, from the coronary mortality of each group of the general population, the distribution of A.C.D. within the coronary population and the number of persons from the general population with a given A.C.D. value we can calculate a relative probability of a coronary for an individual knowing *only his* A.C.D. The actual number of individuals in the population at large were obtained from the 1949 U.S. vital statistics data. Knowing the A.C.D. distribution for samples of subpopulations by age and sex gives immediately the A.C.D. distribution in the entire normal population for each age group and sex category.

Since the relative probability of a person dying of a coronary (over a time interval) at a particular A.C.D. is just the ratio of the number of coronaries occurring (over the same time interval) having that A.C.D.

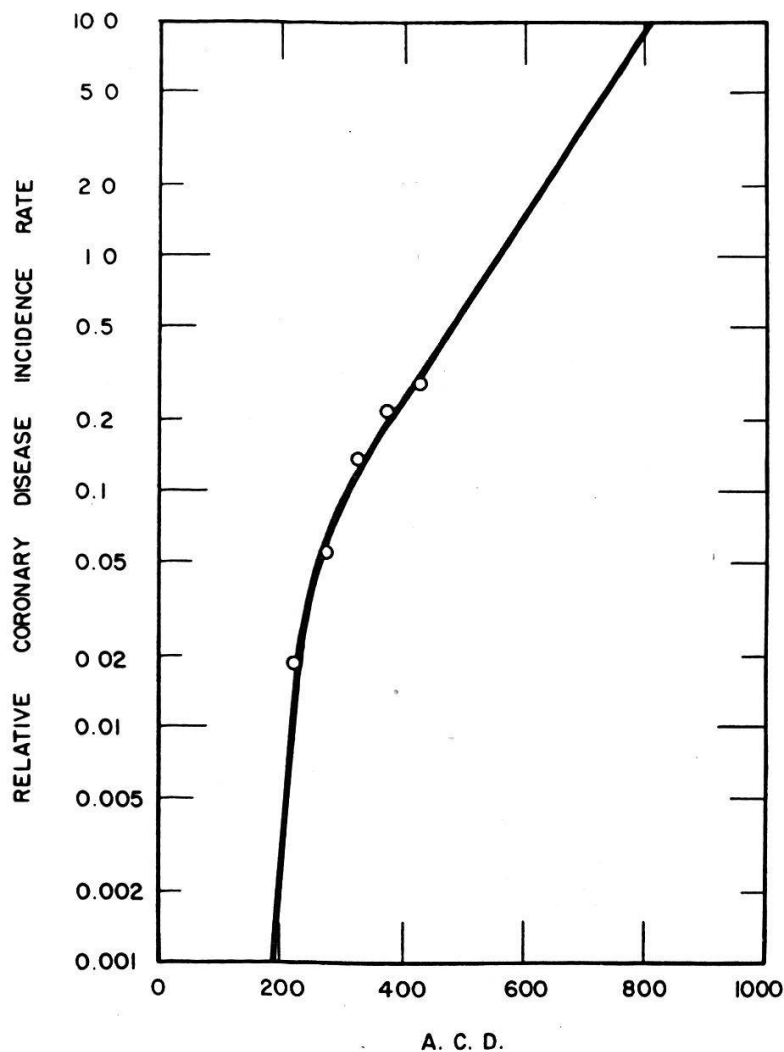


Fig. 7. Relative coronary disease incidence (or mortality) plotted as a function of accumulated coronary disease values. This curve is made on the basis of the distribution of A.C.D. values in the normal and coronary population studied.

value to the *total* number of normals having that value, a scale of relative probability can be set up for either mortality or incidence (since it is a reasonable assumption that incidence and mortality are proportional). Such a relative coronary mortality (or incidence) plot is shown in Fig. 7.

It is possible using this relative mortality (or incidence) plot together with the A.C.D. distribution within each age and sex group to calculate for each such group the mean relative mortality. In order to compare these mean relative mortality rates derived from A.C.D. values with actuarial data<sup>2</sup> it is convenient to normalize the mortality rates of both at, say, 1.00 for the 40-49 year old male population. If this is done we obtain two comparable scales of relative mortality; one from actuarial

<sup>2</sup> The following categories were selected to represent coronary arteriosclerosis and its complications: 420 (arteriosclerotic heart disease, including coronary heart disease); 422 (other myocardial degeneration); 440-443 (hypertension with heart disease) (1949 U.S. Vital Statistics).

data, the other from A.C.D. values alone. Comparison of the relative mortality from actuarial data with the relative mortality predicted from A.C.D. values is given in Table 2. With the exception of the somewhat higher mortality rates predicted in the younger females there is very good quantitative agreement between the actuarial data and A.C.D.

Table 2  
Comparison of predicted coronary disease mortality rates with those from U.S. Vital Statistics\*

Population group	Mortality rates from U.S. Vital Statistics	Predicted mortality rates from A.C.D. values
<b>Males:</b>		
35.1 years	0.27	0.32
44.3 years	1.00	1.00
54.2 years	3.39	2.94
62.1 years	6.73	7.99
<b>Females:</b>		
35.2 years	0.06	0.12
44.0 years	0.26	0.46
53.9 years	1.06	1.41
62.1 years	2.97	2.94

\* U.S. Vital Statistic Tables, 1949 data.

predictions—including the age and sex trends. Further, the rate of increase in mortality predicted from A.C.D. values is also in close agreement with the actuarial data.

#### *Prognosis of a high "α" or A.I. level*

In order to be of use, a biochemical variable that measures to some extent the amount of arteriosclerosis must demonstrate prognostic value. Therefore, in normal individuals in which an  $\alpha$  or A.I. determination has been made it is of the greatest importance to know which individuals go on to develop clinical coronary disease. To date we have observed 5 individuals develop such clinical evidence de novo in the form of a myocardial infarction (21). The A.I. levels for these individuals together with the A.I. levels of matched controls are shown in Table 3. The elevated A.I. values of these normal individuals determined before the occurrence of the myocardial infarction strongly support the hypothesis that  $\alpha$  (or A.I.) levels are a good estimate of the rate of development of arteriosclerosis and that when combined with the age factor may provide a good estimate of total accumulated arteriosclerosis.

Further evidence of the prognostic value of the  $\alpha$  (or A.I.) determination is available from a clinical study in which the A.I. values were

determined in a study (22) of 119 coronary insufficiencies (as manifested by angina pectoris) who had no previous evidence of myocardial infarction. Table 4 shows the data for all 119 angina patients. Over a five year period 31 individuals went on to develop a myocardial infarction. The

Table 3  
Atherogenic index values for 5 normal male individuals who later developed a myocardial infarction

Case	Age at time of study	A.I. value
1	35 years	167 units
2	42 years	136 units
3	36 years	104 units
4	40 years	132 units
5	42 years	99 units

Mean for 5 cases developing infarction = 127.6 units ( $P < .001$ )  
Mean for matched controls = 77 units

Table 4  
Atherogenic index values in angina patients developing myocardial infarction and in angina patients remaining uncomplicated

Category	Number of cases	A.I. value
Anginas becoming infarctions	31	104.3
Anginas remaining uncomplicated	88	86.4

Difference = 17.9 units ( $P = 0.004$ )

average A.I. for the myocardial infarctions was 104.3 whereas the average A.I. for the remaining 88 uncomplicated coronary insufficiencies was 86.4. The highly significant ( $P = 0.004$ ) elevation of A.I. values (average of 17.9 A.I. units) in those individuals developing the clinical complications of myocardial infarction demonstrate the relative prognostic value of the  $\alpha$  (or A.I.) determination.

### Therapy

If the  $\alpha$  (or A.I.) value is a good estimate of the present rate of development of arteriosclerosis as the data strongly suggest, it would be of great potential importance to be able to lower a persistently high  $\alpha$  (or A.I.) level. There is substantial evidence that reducing the A.I. level is of benefit, at least within a group of coronary patients (22). In this study 155 out of a group of 280 patients with manifest coronary disease went

on a low fat-low cholesterol diet. Although the recommended diet was restricted to 25 g of fat and approximately 0.2 g of cholesterol per day the actual diet for each "dieter" must have varied considerably. It is safe to assume, however, that the group voluntarily going on the diet and who stated they remained on this diet consumed on the average less fat and cholesterol than did members of the non-dieting group. Table 5 shows the A.I. data for the dieters and non-dieters. Before dieting began the mean A.I. levels for the dieters was 1.9 units less than the non-dieting group. Over the 4 year period the dieters maintained a mean A.I. of 11 units lower ( $P < 0.01$ ) than the mean A.I. for the non-dieters (after correction for the initial differences in means). In the non-dieting group over the 4 year period the recurrence and mortality was 41% and 10.4% respectively. On the other hand, within the dieting group the recurrence and mortality was only 10% and 2.6% respectively.

Table 5  
Four year dietary follow-up on 280 coronary patients

Group	No. cases	Average follow-up	Average maintained A.I.	Mean difference
Non-dieters	125	4.2 years	95.3	11.1 A.I. units ( $P < .01$ )
Dieters	155	3.8 years	84.2 (corrected)	

Mean difference = 11.1 A.I. units ( $P < .01$ ).

This limited study would indicate the great desirability of reducing the A.I. level, if only by the order of 10 units. Thus, from this study the prognosis over a 4 year period is approximately 4 times as favourable for a coronary who lowers his A.I. level by the order of 10 units (in this case apparently via dietary fat and cholesterol restriction) than for a coronary who maintains his A.I. level.

### *Lipoprotein reduction*

#### *a) Pharmaceutical agents*

In our experience only two drugs, thyroid and heparin, have clearly proven effective in lipoprotein reduction. Intravenous administration of 100 mg of sodium heparin acutely reduces  $S_f^0$  20-400 lipoprotein levels (23) over a period lasting up to 6-12 hours following administration. Since lipoprotein reduction is only accomplished over a relatively short period, the long term prophylactic use of heparin, unless administered daily, is doubtful. However, *Engelberg* (24) has recently reported favourably on the intermittent use of heparin in the long term management of

coronary patients. The usefulness of heparin, however, is definitely limited both by the price of the drug and the considerable inconvenience of parenteral administration.

Thyroid substance taken orally (25) in the form of 4–5 grains of desiccated thyroid per day significantly lowers not only  $S_f^0$  0–20 lipoprotein levels, but also  $S_f^0$  20–400 lipoprotein levels. Fortunately and usefully the higher the initial lipoprotein level the greater is the absolute lipoprotein reduction. However, at lower dosage levels such as 3 grains or less per day the effect of thyroid on  $S_f^0$  0–20 lipoproteins is only temporary and by 24 weeks the  $S_f^0$  0–20 level returns to the pre-thyroid value (26). The application of thyroid therapy in  $S_f^0$  0–20 lipoprotein reduction has proven very useful (27) in such disease categories as xanthoma tendinosum where as much as a two fold reduction in  $S_f^0$  0–20 lipoprotein levels are commonly observed.

#### b) Dietary studies

Dietary studies of any type are difficult to conduct for at least two reasons. First there is the difficulty in conducting long term studies which would be of much greater reliability in evaluating dietary therapy and secondly, there is the difficulty in completely controlling the quantity and exact composition of the food not only prepared but actually eaten.

Several studies (28) provide information on the effect of diet on the low density lipoproteins and hence on the  $\alpha$  (or A.I.) value. In the first study, 28 subjects were followed on a weight reducing program before and after 2 months on a 1000 calory low fat-low carbohydrate diet. The results of this study showed a highly significant reduction in lipoprotein levels throughout the  $S_f$  0–400 spectrum. As the result of the low density lipoprotein reduction the mean A.I. value was substantially reduced 20 units from 81 to 61.

In a second dietary study, a long term controlled iso-caloric study of 5 patients involving three principal periods was made. The principal periods included a low fat-high carbohydrate period, a high vegetable fat period and a high animal fat-high cholesterol period. In each of the dietary periods there was observed different responses within *different* parts of the  $S_f^0$  0–400 lipoprotein spectrum. The results of this study indicated that with respect to the  $S_f^0$  0–20 lipoprotein class the high vegetable fat and the low fat diet were essentially equivalent whereas the high animal fat diet significantly *raised* the  $S_f^0$  0–20 class. On the other hand, the  $S_f^0$  20–400 lipoprotein levels were relatively equivalent on both the high vegetable fat and high animal fat periods but were

significantly elevated in the low fat-high carbohydrate period. Considering the effects of all three diets upon the  $\alpha$  function (or A.I.) it would appear that *without* caloric restriction it would be more beneficial (4/5 cases) to be on a high vegetable fat diet than on either a low fat or high animal fat diet (one case out of the five did best on a low fat diet). Although in itself, vegetable fat may not be exerting any positive action in lowering lipoprotein levels the data indicate that *substitution* of vegetable fat for animal fat is indicated. Also caloric substitution of vegetable fat for some carbohydrate *may* also be of benefit. The present status of the therapeutic value of *adding* unsaturated vegetable fat to the diet needs more careful study, especially with regard to what the vegetable fat may be calorically replacing.

The significant reduction of *both*  $S_f^0$  0-12 and  $S_f^0$  20-400 levels observed in the weight reduction study in which a low fat-low carbohydrate diet was employed suggest that a diet high in carbohydrate or in total calories may tend to elevate  $S_f^0$  20-400 levels. Thus, on a carbohydrate restriction  $S_f^0$  20-400 lipoprotein levels were lowered whereas on carbohydrate supplementation the  $S_f^0$  20-400 levels rose. Indeed, on a nearly fat free, high carbohydrate dietary study (29) there was observed an appreciable elevation in serum neutral fat which was reflected in elevations in lipoproteins of  $S_f^0$  20 and above.

#### *Estimation of lipoprotein $\alpha$ (or A.I.), $S_f^0$ 0-12 and $S_f^0$ 12-400 lipoprotein concentrations*

The evidence presented strongly suggests the potential usefulness of an  $\alpha$  (or A.I.) determination in the evaluation of coronary risk, coronary prognosis and evaluation of therapy whether for normal individuals or for coronary patients. The major obstacle in the use of such a determination is, of course, the very limited availability of preparative and analytic ultracentrifugal facilities. To date we have developed two methods that can be used to approximate the A.I. values as well as to approximate the levels of  $S_f^0$  0-12 and  $S_f^0$  12-400 lipoproteins.

The first method (30) depends upon two chemical determinations on the whole serum; the total gravimetric lipid concentration and the total serum cholesterol concentration.

There is a theoretical basis for predicting that the total serum lipid concentration (G.T.L.C.) above can be used to approximate the total  $S_f^0$  0-400 lipoprotein classes and hence lipoprotein A.I. This is possible because a large part of the total lipid (80%) is to be found on the *average* within the  $S_f^0$  0-400 class (see Fig. 8). Also, since the *total*  $S_f^0$  0-400 class

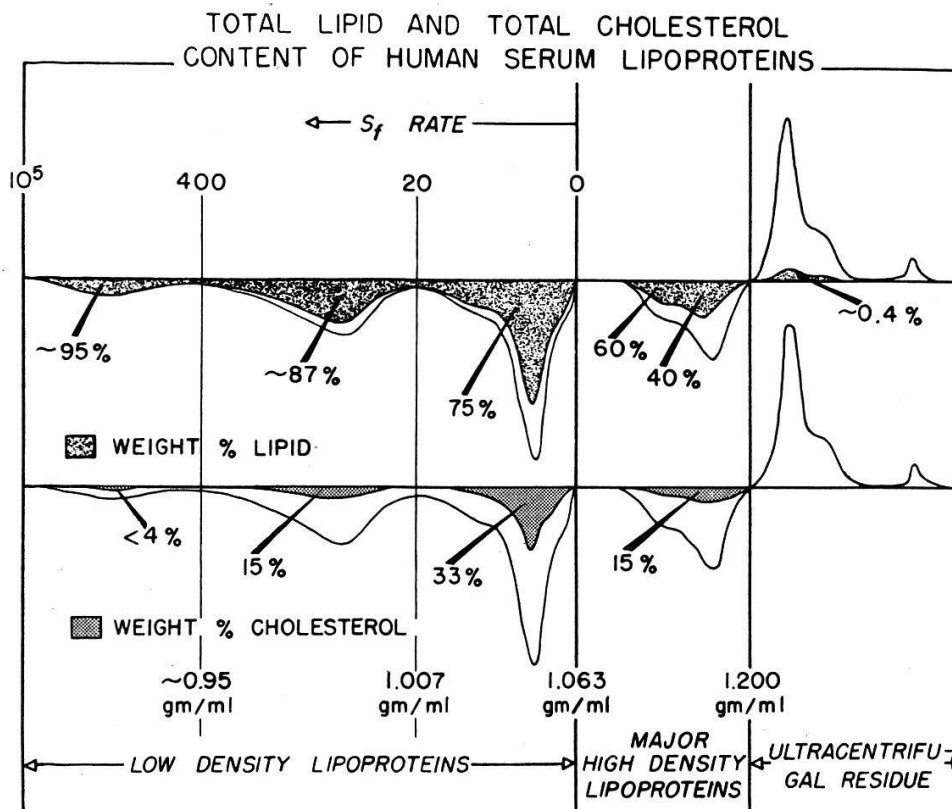


Fig. 8. The average composition of total lipid and total cholesterol (expressed as per cent of total lipoprotein component) present in the major ultracentrifugal lipoprotein classes.

is approximately 80% lipid we get a good estimation of *total*  $S_f^0$  0-400 lipoprotein concentration. Further the amount of lipid variability outside this low density lipoprotein class is relatively low. On the other hand, the total cholesterol is present on the average to the extent of about 33% of total component in the  $S_f^0$  0-12 class but only about 15% within the  $S_f^0$  12-400 class. Thus a given elevation in the concentration of  $S_f^0$  12-400 will be reflected chemically by less than half the increment of serum cholesterol as the same elevation of lipoproteins of the  $S_f^0$  0-12 class. In other words, serum cholesterol is sensitive to lipoprotein elevation within the  $S_f^0$  0-12 class but is not sensitive to lipoprotein elevation in the  $S_f^0$  12-400 class. This is perhaps the most important limitation of a serum cholesterol measurement, especially since with respect to coronary arteriosclerosis there exists strong evidence that the lipoproteins of the  $S_f^0$  12-400 class are more important milligram for milligram than the  $S_f^0$  0-12 class.

We might expect, therefore, that total serum gravimetric lipid (G.T.L.C.) would be a good estimate of lipoproteins of the  $S_f^0$  0-400 class which in turn is a good estimate of lipoprotein A.I. (lipoprotein A.I. =  $1(S_f^0$  0-400) +  $0.075(S_f^0$  12-400). Also G.T.L.C. measures to some extent

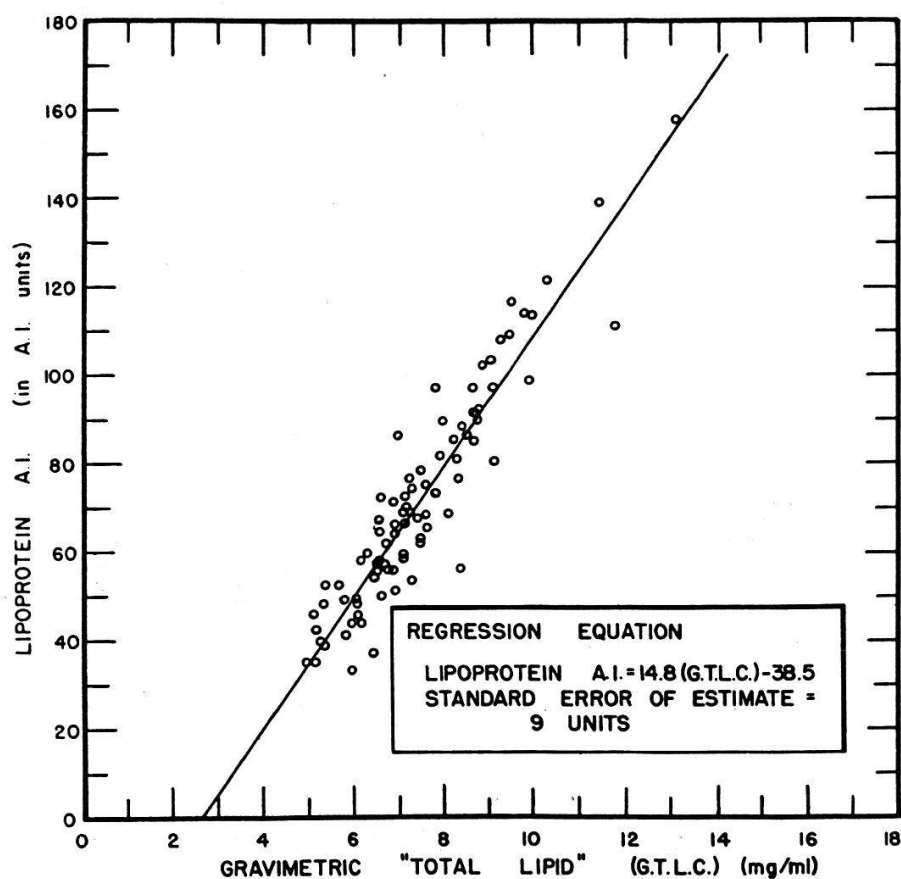


Fig. 9. The relationship between Gravimetric Total Lipid Concentration (G.T.L.C.) and lipoprotein Atherogenic Index (A.I.).

all additional lipoproteins above  $S_f^{0} 400$  and so compensates somewhat for the failure of G.T.L.C. to weight the  $S_f^{0} 12-400$  class by the factor 1.75. Fig. 9 shows the observed relationship between G.T.L.C. and lipoprotein A.I. The regression equation for lipoprotein A.I. is as follows: Lipoprotein A.I. = 14.8 (G.T.L.C. - 38.5). For the 88 cases of normal males of ages 19-70 years the close approximation of lipoprotein (or A.I.) by G.T.L.C. is striking. The correlation coefficient between lipoprotein A.I. and G.T.L.C. is 0.93 indicating a very strong association. The low standard error of estimate of 9 A.I. units further suggests the reliability of lipoprotein A.I. estimation by G.T.L.C. Also, it is apparent that any other reproducible method of measuring total serum lipid could be used in lipoprotein A.I. estimation. The exact relationship including the regression equation would of course have to be established since methods for evaluation of total serum lipid are not strictly equivalent.

In comparison with G.T.L.C. Fig. 10 shows there is a much poorer correlation of total serum cholesterol with lipoprotein A.I. The actual correlation coefficient is 0.76 for 86 cases. Further, a standard error of estimate of 16 units is too large to make estimation of A.I. by serum

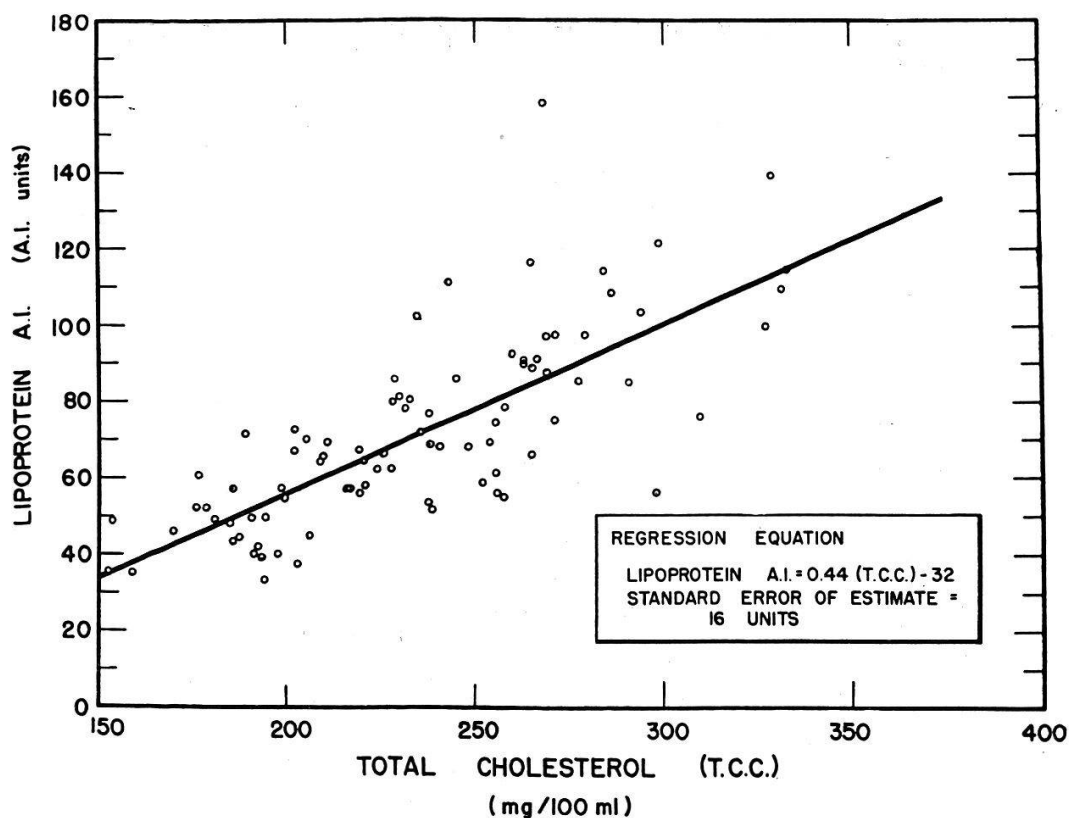


Fig. 10. The relationship between Total serum Cholesterol Concentration (T.C.C.) and Lipoprotein Atherogenic Index (A.I.).

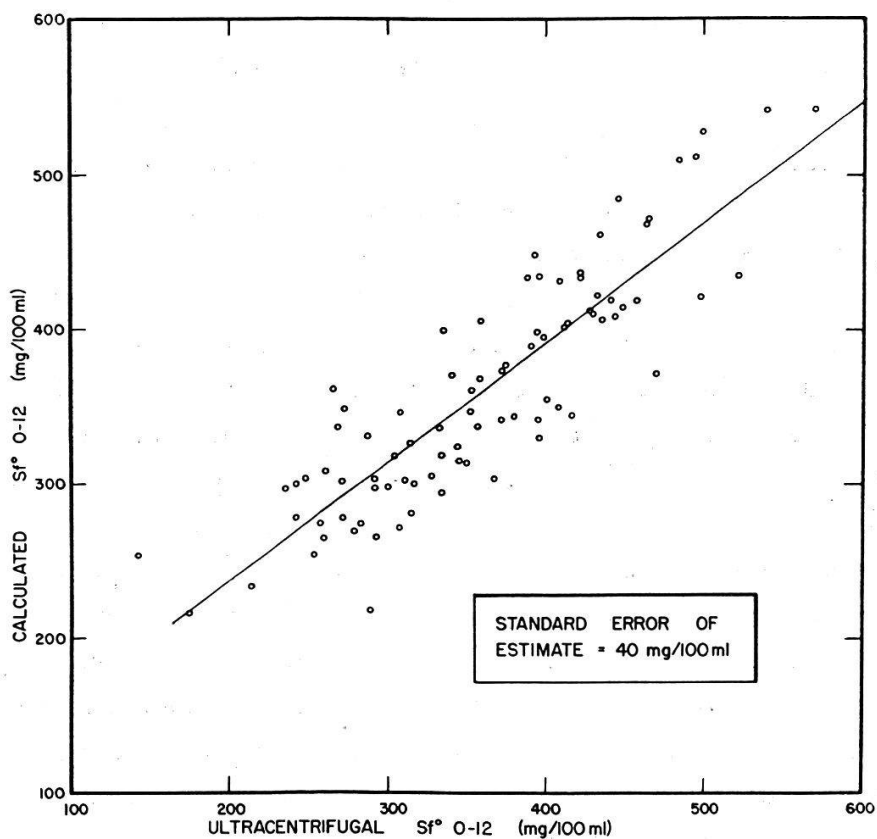


Fig. 11. The relationship between estimated  $S_f^0$  0-12 lipoproteins (as determined by total serum gravimetric lipid and total serum cholesterol) and ultracentrifugally determined  $S_f^0$  0-12 lipoproteins.

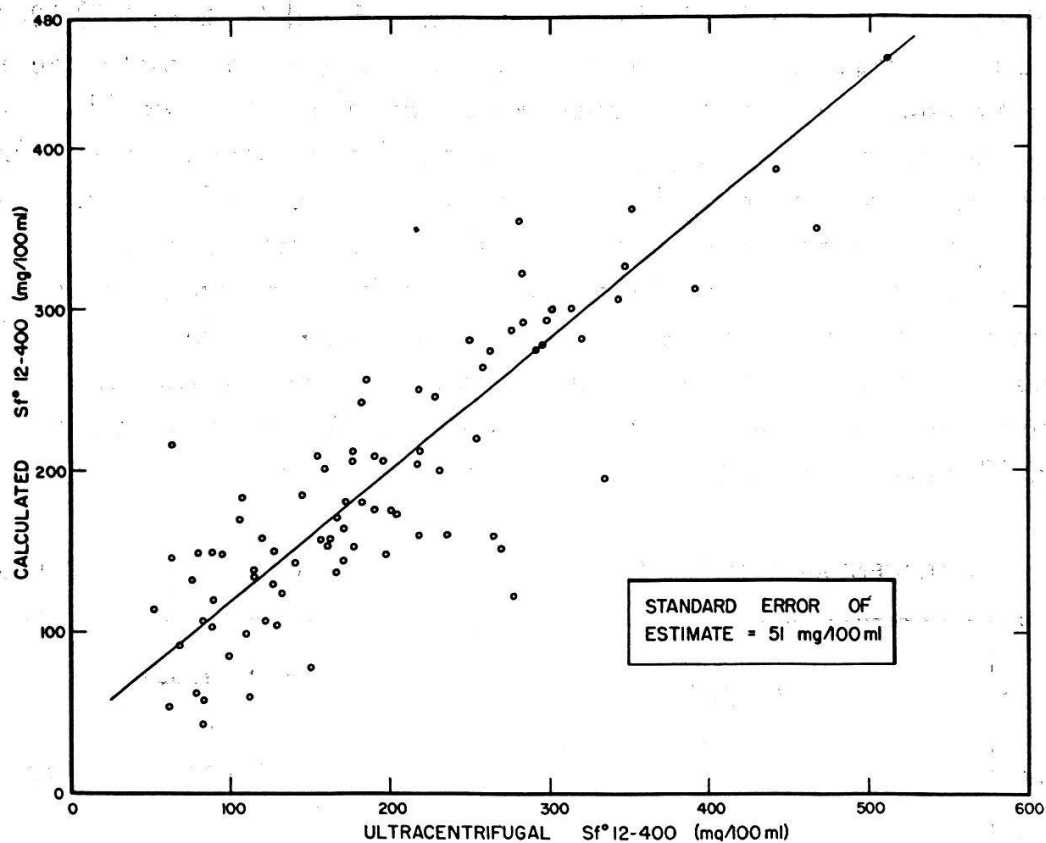


Fig. 12. The relationship between estimated  $S_f^0$  12-400 (as determined by total serum gravimetric lipid and total serum cholesterol) and ultracentrifugally determined  $S_f^0$  12-400 lipoproteins.

cholesterol sufficiently reliable for any practical use. However, the serum cholesterol and G.T.L.C. can be combined to give valuable additional data concerning the distribution of lipoproteins within the  $S_f^0$  0-12 and  $S_f^0$  12-400 class (see Figs. 11 and 12). Thus, we can estimate both these lipoprotein classes by using the following appropriate multiple regression equations:

$$S_f^0 \text{ 0-12} = -39.8 + 2.1 (\text{T.C.C.}) - 12.6 (\text{G.T.L.C.})$$

$$S_f^0 \text{ 12-400} = -209.5 - 1.1 (\text{T.C.C.}) + 88.5 (\text{G.T.L.C.})$$

The reliability of lipoprotein class estimation from the above regression equations is indicated by a standard error of estimate of 40 mg per 100 ml for the  $S_f^0$  0-12 and a standard error of estimate of 51 mg/100 ml for the  $S_f^0$  12-400. These determinations on the amount and distribution of lipoproteins within the low density class may be of considerable value in deciding what therapy may be most effective in overall  $\alpha$  (or A.I.) level reduction. As was pointed out in the dietary section, the dietary procedures for effective A.I. reduction may be dependent upon knowing the distribution of the lipoproteins among the flotation classes.

The second method (31) for lipoprotein A.I.,  $S_f^0$  0-12 and  $S_f^0$  12-400 estimation requires a preparative ultracentrifuge to isolate the low density lipoprotein fraction from serum. Such isolation would involve the same methodology (15) as that required for analytic ultracentrifugal analysis. However, instead of an analytic ultracentrifugal analysis a total refractive increment and a total cholesterol determination is made on the low density lipoprotein fraction. The refractive increment measurement provides the estimate of  $S_f^0$  0-400 and, hence, lipoprotein A.I. The combination of refractive increment and total top fraction cholesterol allows the estimation of both  $S_f^0$  0-12 and  $S_f^0$  12-400 class lipoproteins. Fig. 13 shows the relationship between lipoprotein A.I. and refractive

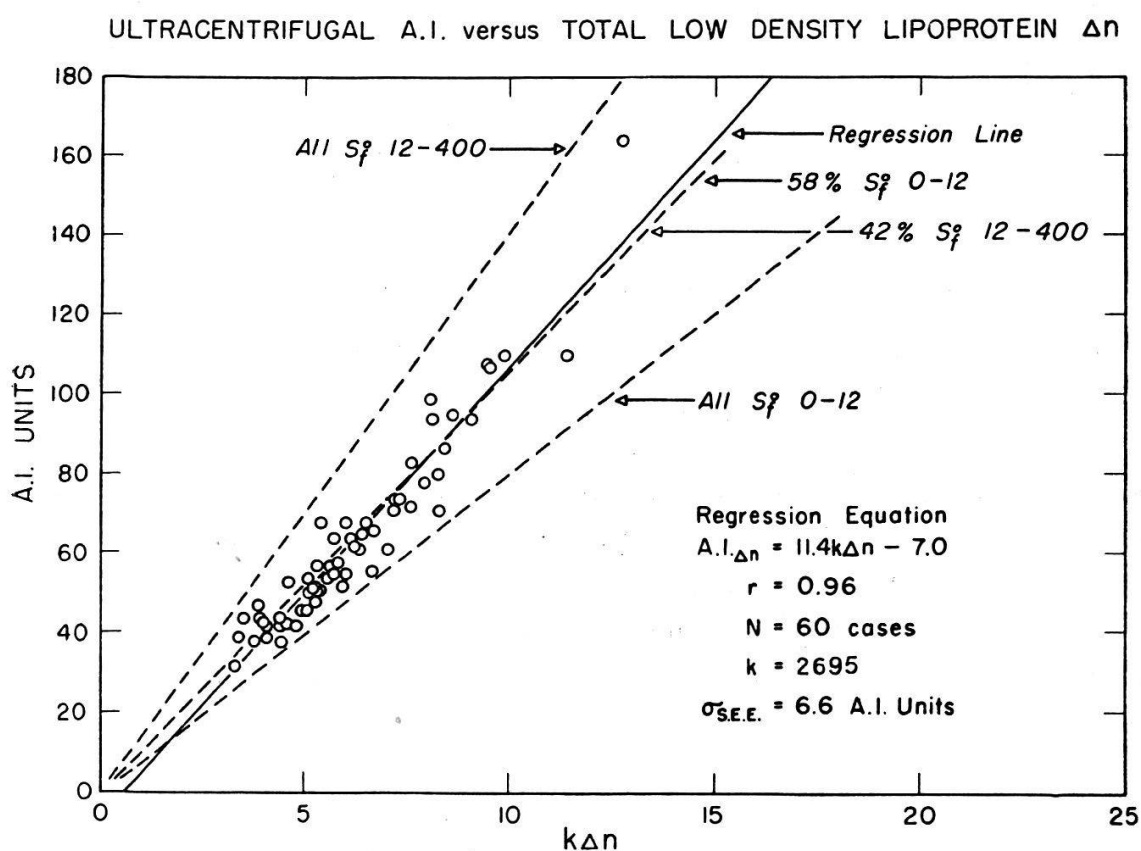


Fig. 13. The relationship between the concentration of total low density lipoproteins (as determined by  $k\Delta n$ ) and ultracentrifugal lipoprotein Atherogenic Index. The two extreme dotted lines illustrate the expected relationship if all the lipoproteins were either in the form of  $S_f^0$  12-400 or  $S_f^0$  0-12 lipoproteins. The intermediate dotted line is the expected relationship for the mean distribution between  $S_f^0$  12-400 and  $S_f^0$  0-12 lipoproteins for the normal 40-49 year old male population.

increment obtained for 60 normal individuals of mixed sex over the age range 20-60 years. The regression equation for lipoprotein A.I. is as follows:

$$\text{Lipoprotein A.I.} = 11.4 K\Delta n - 7.0$$

The very high degree of correlation ( $r = 0.96$ ) between lipoprotein A.I. and  $k\Delta n$  indicates a very high degree of association between A.I. and  $k\Delta n$ . The high reliability of lipoprotein A.I. estimation from  $k\Delta n$  is indicated by a standard error of estimate of only 6.6 units. Again, a total cholesterol determination on the low density top fraction failed to give a satisfactory estimate of lipoprotein A.I. as is indicated by the low correlation coefficient of 0.76 and the high standard error of estimate of 16 units (see Fig. 14).

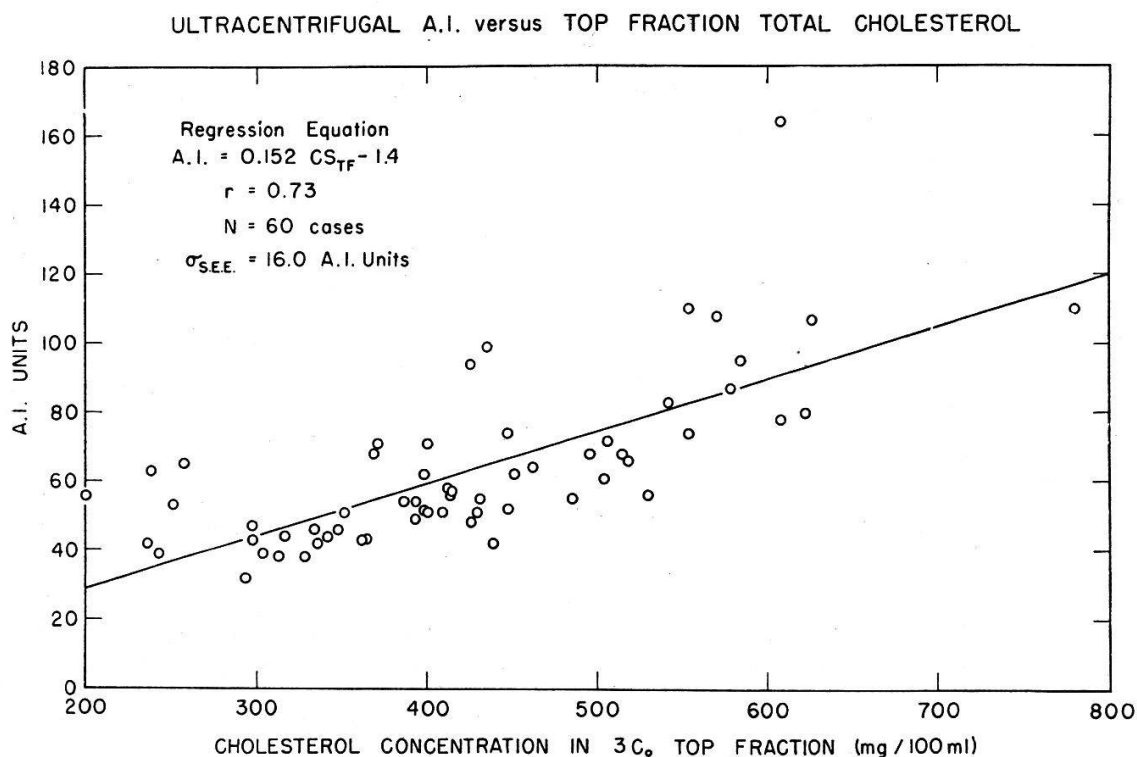


Fig. 14. The relationship between low density top fraction total cholesterol and ultracentrifugal lipoprotein A.I.

Both  $k\Delta n$  and Cholesterol values in the top fraction can be combined to give estimations of  $S_f^0$  0-12 and  $S_f^0$  12-400 lipoprotein concentrations (see Fig. 15 and 16). The appropriate multiple regression equations for each lipoprotein class are as follows:

$$S_f^0 \text{ 0-12} = 120.9 - 8.39 K\Delta n + 0.619 (CS_{TF})$$

$$S_f^0 \text{ 12-400} = -90.8 + 75.6 K\Delta n - 0.484 (CS_{TF})$$

The reliability of such estimates is indicated by a standard error of estimate of 49 mg/100 ml for  $S_f^0$  0-12 and a standard error of estimate of 54 mg/100 ml for  $S_f^0$  12-400.

#### *The value of lipoprotein A.I. estimation*

The above two methods provide rapid and relatively inexpensive means whereby low density lipoproteins and particularly lipoprotein

ESTIMATION OF  $S_f^0$  0-12 FROM  $\Delta n$  AND  
TOP FRACTION TOTAL CHOLESTEROL

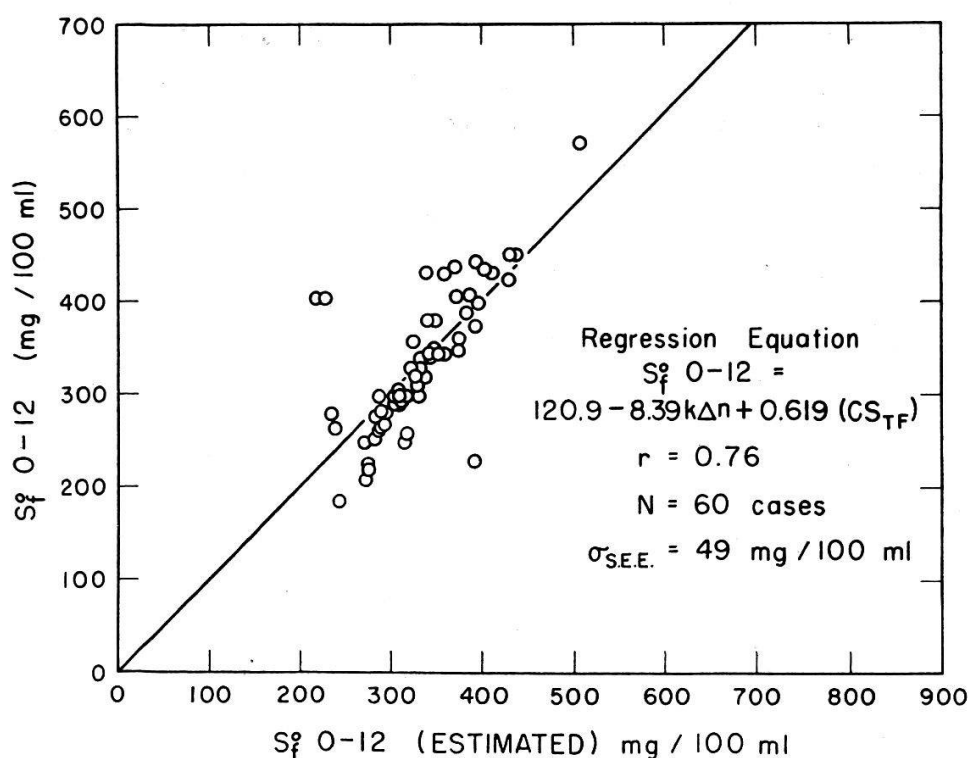


Fig. 15. The relation between estimated  $S_f^0$  0-12 lipoproteins (by total top fraction refractive index and cholesterol determinations) and ultracentrifugally determined  $S_f^0$  0-12 lipoproteins.

A.I. may be estimated. Such estimations, whether in the present form or in modifications thereof, are of considerable potential value as an inexpensive screening procedure to pick out from the general population high coronary risk individuals. Further, such methods may also be of potential value in evaluation of the effectiveness of therapy, whether in the form of preventive therapy on high risk "non coronary" normals or in the form of routine coronary therapy evaluation. Moreover, as the result of the economy of these methods, the health authorities of any country, large or small, could establish the validity, on their own population, of the relationship between serum lipoproteins and arteriosclerosis. Further, these methods could be used as a screening procedure in a program of prevention of premature arteriosclerosis.

*Summary*

1. The transport phase of lipid metabolism is accomplished by the blood lipids which are almost wholly in the form of serum lipoproteins.
2. Elevated levels of low density serum lipoproteins, characterized ultracentrifugally as the "standard  $S_f^0$  0-12 and standard  $S_f^0$  12-400

ESTIMATE OF  $S_f^0$  12-400 FROM  $\Delta n$  AND TOP FRACTION CHOLESTEROL

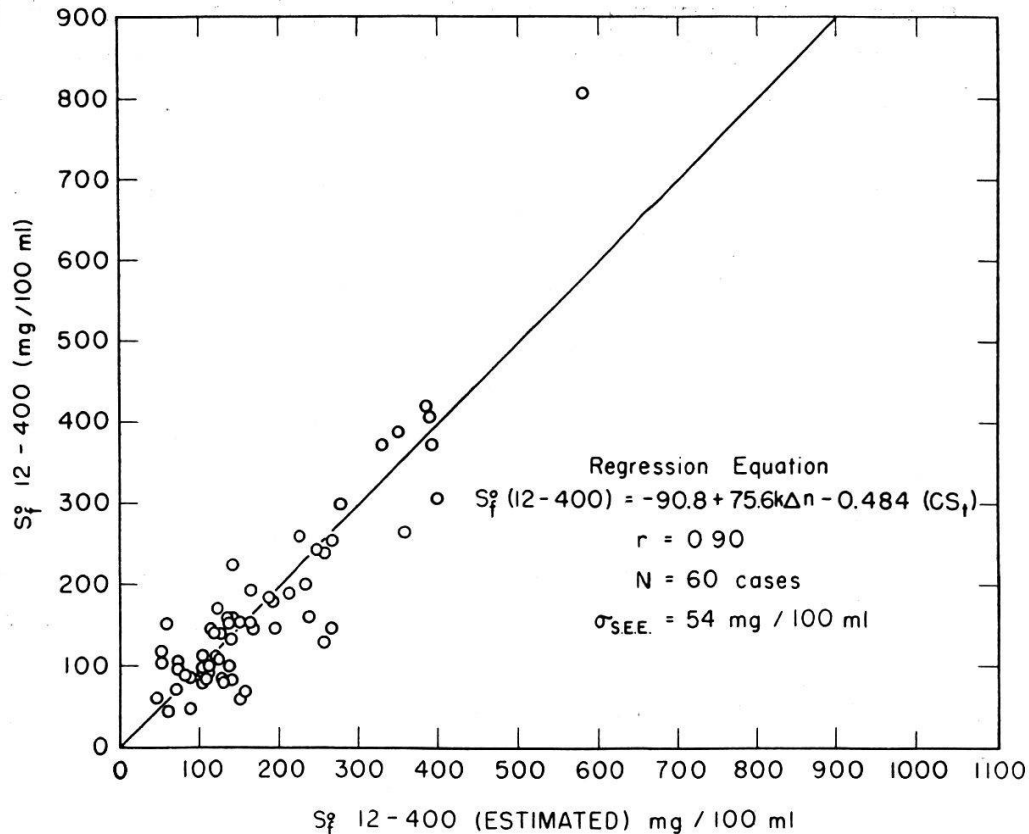


Fig. 16. The relationship between estimated  $S_f^0$  12-400 lipoproteins (by total top fraction refractive index and cholesterol determinations) and ultracentrifugally determined  $S_f^0$  12-400 lipoproteins.

lipoproteins”, are strongly associated with the presence as well as the development of human arteriosclerosis.

3. Our current concept of arteriosclerosis is that it represents an accumulative process over time. The rate of development of arteriosclerosis may be estimated by an “ $\alpha$ ” function (also called atherogenic index) which is related to the serum concentrations of the  $S_f^0$  0-12 and  $S_f^0$  12-400 lipoproteins.

4. Follow-up studies over a 6 year period show that lipoprotein levels may be used to predict the risk of development of the clinical consequences of arteriosclerosis.

5. Follow-up studies show that prognosis of clinical coronary artery disease is related to these serum lipoprotein levels.

6. Dietary measures are definitely effective in the control of elevated serum lipoprotein levels.

7. Thyroid substances and heparin administration to humans can produce profound alterations in serum lipoprotein levels.

8. Two methods have been presented whereby the ultracentrifugally defined  $S_f^0$  0-12 lipoprotein, the  $S_f^0$  12-400 lipoproteins and the lipo-

protein  $\alpha$  (or A.I.) value may be approximated. The first method utilizes a combination of total serum gravimetric lipid and total serum cholesterol. The second method, requiring the use of a preparative ultracentrifuge, is based upon a total refractive increment and a total cholesterol determination on the ultracentrifugally separated low density lipoprotein fraction. These approximations can furnish information, almost equivalent to the analytic ultracentrifugal lipoprotein values useful for the prediction of coronary risk as well as the therapy most likely to be effective in overall lipoprotein reduction.

### *Zusammenfassung*

1. In der Transportphase des Lipoidstoffwechsels treten Blutlipoide und vor allem Serumlipoproteide auf.

2. Die Erhöhung des Gehaltes an Serumlipoproteiden von niedrigem spezifischem Gewicht, die mit Hilfe der Ultrazentrifuge als Standard- $S_f^0$  0–12 und Standard- $S_f^0$  12–400 Lipoproteide gekennzeichnet werden können, steht mit der Arteriosklerose des Menschen und der Entwicklung dieser Krankheit in engem Zusammenhang.

3. Unserer heutigen Ansicht nach ist die Arteriosklerose ein Akkumulierungsvorgang. Die Schnelligkeit in der Entwicklung dieser Krankheit kann mittels einer  $\alpha$ -Zahl, auch «atherogenic index» genannt, ermessen werden. Diese Zahl steht mit der Serumkonzentration der  $S_f^0$  0–12 und  $S_f^0$  12–400 Lipoproteine in Beziehung.

4. Spätere, sich über 6 Jahre erstreckende Studien haben gezeigt, daß der Lipoproteidspiegel zur Voraussage des Risikos in der klinischen Entwicklung der Arteriosklerose benützt werden kann.

5. Aus weiteren Untersuchungen geht hervor, daß der Serumlipoproteidgehalt auch für die Prognose der klinischen Coronarsklerose maßgebend ist.

6. Diätetische Maßnahmen vermögen einen erhöhten Serumlipoproteidspiegel entscheidend zu senken.

7. Auch Schilddrüsenpräparate und Heparininjektionen können den Serumlipoproteidgehalt günstig beeinflussen.

8. Es werden Methoden gezeigt, wie auch ohne Ultrazentrifuge  $S_f^0$  0–12 und  $S_f^0$  12–400 Lipoproteide und die Lipoproteid- $\alpha$ -Zahl annähernd bestimmt werden können. Auch diese approximativen Werte, die den analytischen, mit der Ultrazentrifuge ermittelten Lipoproteidwerten beinahe entsprechen, können zur Voraussage des Risikos einer Coronar-erkrankung verwendet werden oder Hinweise auf die für die Senkung des Lipoproteidspiegels wirksame Therapie geben.

## Résumé

1. La phase de transport dans le métabolisme des lipides est objectivée par les lipides sanguins, qui sont presque tous sous la forme de lipoprotéines sériques.

2. Une teneur élevée en lipoprotéines sériques de basse densité, caractérisées à l'ultracentrifugation par des lipoprotéines standard  $S_f^0$  0-12 et standard  $S_f^0$  12-400, est associée chez l'homme à l'existence ainsi qu'à la progression de l'artériosclérose.

3. Notre conception générale de l'artériosclérose est qu'elle consiste en un processus d'accumulation dans le temps. La rapidité de l'évolution de l'artériosclérose peut être estimée au moyen d'un facteur  $\alpha$  (appelé aussi index athérogénique), qui est en relation avec les concentrations de lipoprotéines  $S_f^0$  0-12 et  $S_f^0$  12-400.

4. Une étude suivie pendant une période de 6 ans montre que le niveau des lipoprotéines peut être utilisé pour évaluer les risques de complications cliniques de l'artériosclérose.

5. Des études suivies montrent que le pronostic des maladies cliniques des artères coronaires est en relation avec les taux des lipoprotéines sériques.

6. Des mesures diététiques sont nettement efficaces pour normaliser les lipoprotéines sériques.

7. L'administration à l'homme de substances thyroïdiennes et d'héparine provoque des altérations profondes dans le taux des lipoprotéines sériques.

8. Des méthodes sont présentées, au moyen desquelles, on peut déterminer approximativement, sans l'emploi d'ultracentrifuge les lipoprotéines  $S_f^0$  0-12 et  $S_f^0$  12-400 ainsi que le facteur lipoprotidique  $\alpha$ .

9. Ces renseignements approximatifs peuvent fournir des données presque équivalentes aux valeurs obtenues par ultracentrifugation analytique des lipoprotéines pour prédire les risques coronariens ainsi que la portée vraisemblable de la thérapeutique destinée à réduire massivement les lipoprotéines.

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