Title

ESTIMATION OF ATHEROGENIC INDEX AND ACCUMULATED CORONARY DISEASE IN HUMAN MALES: EVALUATION FROM SERUM GRAVIMETRIC ""TOTAL LIPID"" OR TOTAL CHOLESTEROL CONCENTRATION

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ABSTRACT

1. A fairly rapid and simple gravimetric method is presented for estimation of the atherogenic index (A.I.) ordinarily obtained from analytical ultracentrifugation of serum lipoproteins. This method entails the weighing of a total lipid extract from a 1-ml aliquot of serum. The approximate A.I. is subsequently calculated from a regression equation relating lipoprotein A.I. values to total lipid concentration values. Serum lipoprotein distibutions that account for differences between the lipoprotein A.I. and the calculated Index are evaluated and discussed.

2. For the 88 normal subjects studied, the total lipid concentration was found to be more strongly correlated with the lipoprotein A.I. than the serum total cholesterol concentration.

3. Equations are presented for estimation of an individual's S_f^o 0-12 and S_f^o 12-400 serum lipoprotein concentration values from the serum total lipid concentration value together with the total cholesterol concentration value.

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INTRODUCTION

Data have been presented[1, 2, 3] that indicate that an ultracentrifugal serum lipoprotein measurement provides predictive and prognostic information on coronary atherosclerosis and clinical coronary heart disease risk. Application of these findings in clinical medicine is already widespread, but has been limited by unavilability of the necessary ultracentrifugal equipment in many clinical centers, especially in countries other than the U. S. A. This report considers possible methods for providing the predictive and prognostic information inherent in lipoprotein determination through the use of biochemical or other techniques that are readily available and inexpensive for centers not having ultracentrifugal facilities.

In the utilization of serum lipoprotein data with respect to atherosclerosis, the concentration values of the two lipoprotein classes implicated (Standard S_f 0-12 and Standard S_f 12-400) are mathematically combined to give what has been designated as the "effective concentration" of lipoproteins, or the lipoprotein Atherogenic Index (A. I.).* The term "effective concentration" is a consequence of a statistical evaluation of the ability of the various serum lipoprotein measures to separate a population sample of overt coronary disease from a matched sample of individuals without clinically manifest disease. The Standard S_f 12-400 lipoproteins were found to be approximately 1.75 times as effective, milligram for milligram, in the statistical segregation of these two population samples. Mathematically the A. I. value of effective concentration value is defined as follows:

$$\text{A. I. (from lipoprotein measurement)} = 0.1\ (S_f^\circ\ 0\text{-}12) + 0.175\ (S_f^\circ\ 12\text{-}400)^{**}$$

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* A. I. values have also been designated as "alpha" values, primarily to indicate that the findings in coronary disease are general and do not depend upon any preconceived notion as to the relationship of atherogenesis with clinical coronary disease.

** The factors 0.1 and 0.175 are used (rather than 1.0 and 1.75) so as to yield a convenient scale of values for A. I.; this is an arbitrary choice.

An additional function, the A. C. D. value (accumulated coronary disease), is based on the hypothesis that the rate at which coronary disease accumulates is directly proportional to the lipoprotein A. I. In essence, then, the A. C. D. function is evaluated by the summation of the products of all previous lipoprotein values times the length of the time periods in which each of these A. I. values existed from birth to the present age. The factor relating the rate of accumulation of coronary disease to the lipoprotein A. I. is arbitrarily set at unity. Mathematically, the relation may be stated

$$\text{A. C. D.} = \int \text{A. I.} \times dt,$$

where t = time (essentially a measure of age).

Since it is not feasible to have continuous determinations of the serum lipoprotein concentrations, the following reasonable assumption is made: that an individual's position on the scale of lipoprotein A. I. values remains the same relative to his fellows throughout life. With this assumption it is possible to calculate an A. C. D. value for an individual at any age after a single serum lipoprotein determination. Tables of coronary disease risk in relation to age and A. C. D. values are available,[2] and it is thus possible to translate an A. C. D. value into relative risk of coronary disease for any individual.

The striking agreement between the mortality rates from Vital Statistics data and the mortality rates as predicted by the A. C. D. function, calculable from serum lipoprotein concentrations, supplies a valid and much needed relationship for preselection of candidates for future coronary heart disease. Likewise the age and sex trends with coronary disease mortality are well predicted from the A. C. D. function.

The question now arises whether there is some other biochemical variable that could be substituted for the lipoprotein A. I. in the A. C. D. function. It is certainly obvious that in order for this new variable to yield satisfactory predictive data from the A. C. D. function it must have a very high correlation with the lipoprotein A. I. value. Two biochemical variables were chosen for the initial study. These were the gravimetric "total lipid" concentration (abbreviated G. T. L. C.)* and the total cholesterol concentration (abbreviated T. C. C.) of human male serum. This report deals with the extent of correlation of these two biochemical variables with the lipoprotein A. I.

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* It is conceivable that in the gravimetric determination there may be present small amounts of extractable substances that are not specifically lipids, but which make up together with the identifiable lipids the gravimetric "total lipid" concentration or the G. T. L. C. From subsequent considerations itis shown that more detailed information concerning such substances is not immediately essential for application of the existing G. T. L. C. values to the problem at hand.

METHODS

Blood samples (30 ml) were drawn from 88 human male subjects. These samples were obtained during routine physical examinations of job applicants and employees.* A wide variety of occupational categories is included. The samples used in the study were from 88 consecutive examinations; there was no rejection or selection of samples. The serum was analyzed for the concentrations of the Standard S_f^o 0-12, the Standard S_f^o 12-400, and the H.D.L. (high-density lipoproteins) classes by ultracentrifugal techniques described elsewhere.[4]

The total cholesterol concentrations (T.C.C.) of the sera were determined according to the method of Colman and McPhee.[5]

The gravimetric "total lipid" concentrations (G.T.L.C.) of the sera were determined gravimetrically following extraction of the lipids. The procedure consisted of the following operations:

(a) Extraction: 1 ml of serum was pipetted into a screw cap capsule vial (1 in. in diameter and 4.25 in. in length); 4 ml of absolute ethanol was then added. The vial was carefully capped and swirled. (An inert Teflon gasket was used in the screw cap.) The vial was then placed into an aluminum block heated to a temperature of 80° C for 1 hour. A periodic swirl of the vial during the heating dispersed whatever gelatinous masses tended to form.

After the hour of heating, the vial was allowed to cool to room temperature. Then 9 ml of distilled water was added to the mixture and again the vial was swirled. Hydrochloric acid (6N) was added dropwise into the vial to obtain a pH value in the mixture (tested by means of indicator paper) of approximately 2.

Twenty to twenty-five ml of ethyl ether was added to the vial. The aqueous and ether phases were then shaken together mechanically for 5 minutes (at a speed of approximately 240 strokes per minute). After shaking, the vial was centrifuged in a clinical centrifuge (at approximately 2,000 rpm) to completely separate the two phases. The ether layer was drawn off under suction into a 125-ml Erlenmeyer flask. This ethyl ether extraction procedure was repeated 3 more times.** After the fourth extraction the ether extract

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* University of California Radiation Laboratory

** Thoroughness of the extraction by the above procedure was ascertained by prolonged saponification of the remaining aqueous serum residue; extraction of all the saponified residue lipids with petroleum ether; and, finally, quantitative analysis by means of infrared spectrophotematic techniques described elsewhere.[6] It was found that the residue lipids accounted for approximately 3 to 5% of the "total lipid" extract weight. Hence, in all subsequent considerations of the "gravimetric total lipid" as extracted by the above procedure, it is understood that the recovery is in the neighborhood of 95% of the "total extractable lipid" weight.

was placed in a drying box for evaporation of the solvents. With the first signs of dryness the extracts were placed in a desiccator under vacuum, and were kept there for at least 6 hours.

(b) Transfer of extracts for weighing: The extracted lipids were carefully transferred in chloroform from the Erlenmeyer flask into a previously weighed weighing bottle. A total of 8 ml of chloroform was used for dissolving the lipid extract and for rinsing the flask. The weighing bottle was placed in the drying box until the chloroform evaporated, then in a desiccator for 6 or more hours.

(c) Weighing operations: All weighing bottles used in this study were weighed only after they had been in a vacuum desiccator for at least 6 hours. Thus, the weighing bottle receiving the above lipid extract dissolved in chloroform had been in a desiccator for 6 hours and had been weighed empty, and then, with the lipid extract in it, was kept in the vacuum desiccator for at least 6 hours prior to the final weighing. Weighings were made at approximately constant temperature (25-26° C) on a Mettler microbalance type M-5.

Prior to extraction of the serum samples the gross visible physical appearance of the serum was noted and recorded. The prime objective was to keep a record of serum samples that were turbid because of lipemia.

RESULTS

Table I shows the distribution of the male group according to age and the lipoprotein A.I. Males in the age range of 30-39 years account for half the total distribution. The mean value of the lipoprotein A.I. for the total male group was 71 units.

The correlation coefficients between the lipoprotein A.I. and (a) G.T.L.C., and (b) T.C.C., together with the conditions of the calculation of each coefficient, are tabulated in Table II. It is seen that for all conditions of calculation, the coefficients between the lipoprotein A.I. and the G.T.L.C. value are significantly higher than those between the lipoprotein A.I. and the T.C.C. This prompted calculation of the regression line for the lipoprotein A.I. and the G.T.L.C., and indicated the possible application of the G.T.L.C. as a close approximation to the lipoprotein A.I. in the calculation of the predictive A.C.D. values. The regression line equation was

$$\text{Gravimetric A.I.} = 14.8 \times (\text{G.T.L.C.}) - 38.5.$$

Plots of lipoprotein A.I. versus (a) G.T.L.C. and (b) T.C.C., together with the calculated regression lines, are presented in Figs. 1 and 2.

Table III presents the correlation coefficients between the major lipoprotein groups and (a) the lipoprotein A.I., (b) the G.T.L.C., and (c) the T.C.C. These data indicate the basis for the strong correlation of the G.T.L.C. with the lipoprotein A.I.

Table IV tabulates the lipoprotein and lipid values for subjects whose gravimetric A.I. values differ from lipoprotein A.I. values by 11 or more units (this value was considered an appreciable difference from the lipoprotein A.I. value). The table is divided into two sections (A and B) according to whether the subject's lipoprotein A.I. value was above or below the mean value of 71 units. This table points out serum lipoprotein distributions that can give rise to such discrepancies. (See discussion).

Table I

Characterization of male group from which blood samples were drawn

Frequency distribution with age

Age Range (years)	N (number of subjects)
10-19	2
20-29	21
30-39	44
40-49	13
50-59	7
60-69	1
Total	88

Frequency distribution with lipoprotein A.I.

Lipoprotein A.I. range (units)	N (number of subjects)
30-39	5
40-49	11
50-59	17
60-69	16
70-79	11
80-89	9
90-99	8
100-109	4
110-119	4
120-129	1
130-139	1
140-149	-
150-159	1
Total	88

Table II

Correlation coefficients[a] between lipoprotein A. I. and (a) G. T. L. C. and (b) T. C. C.

Conditions of calculation	Between lipoprotein A. I. and G. T. L. C.		Between lipoprotein A. I. and T. C. C.	
	n	r	n	r
For all values of lipoprotein A. I. and (a) all G. T. L. C.	88	0.93	--	--
(b) all T. C. C.	--	--	87	0.76
For all values of lipoprotein A. I. greater than the mean lipoprotein A. I. units (71)	38	0.91	37	0.53
For all values of lipoprotein A. I. less than the mean lipoprotein A. I.	50	0.75	50	0.54
For all values of S_f^o 12-400 greater than the mean S_f^o 12-400 (201 mg%)	34	0.92	34	0.68
For all values of S_f^o 12-400 less than the mean S_f^c 12-400	53	0.80	53	0.67
For all values of S_f^o 0-12 greater than the mean S_f^o 0-12 (360 mg%)	43	0.91	42	0.63
For all values of S_f^o 0-12 less than the mean S_f^o 0-12	45	0.93	45	0.72
For all values of H. D. L. greater than the mean H. D. L. (278 mg%)	41	0.92	39	0.69
For all values of H. D. L. less than the mean H. D. L.	45	0.95	44	0.85
For all values of cholesterol greater than the mean cholesterol (234 mg%)	42	0.91	45	0.49
For all values of cholesterol less than the mean cholesterol	45	0.86	42	0.70

S_f^o 12-400 = concentration of S_f^o 12-400 lipoproteins
S_f^o 0-12 = concentration of S_f^o 0-12 lipoproteins
H. D. L. = concentration of high-density lipoproteins (H. D. L. $_1$, H. D. L. $_2$, and H. D. L. $_3$)

[a] Pearson product-moment correlation coefficient

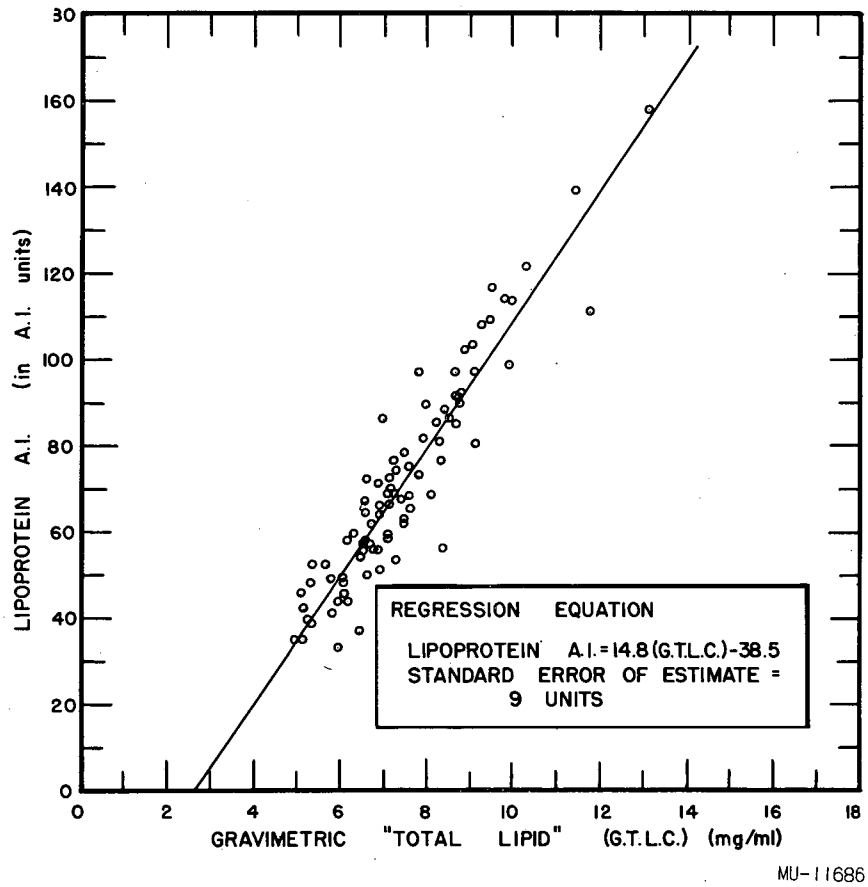

Fig. 1. The relationship between the Lipoprotein A.I. and Gravimetric "Total Lipid" Concentration. (Based on data collected for 88 normal human males.)

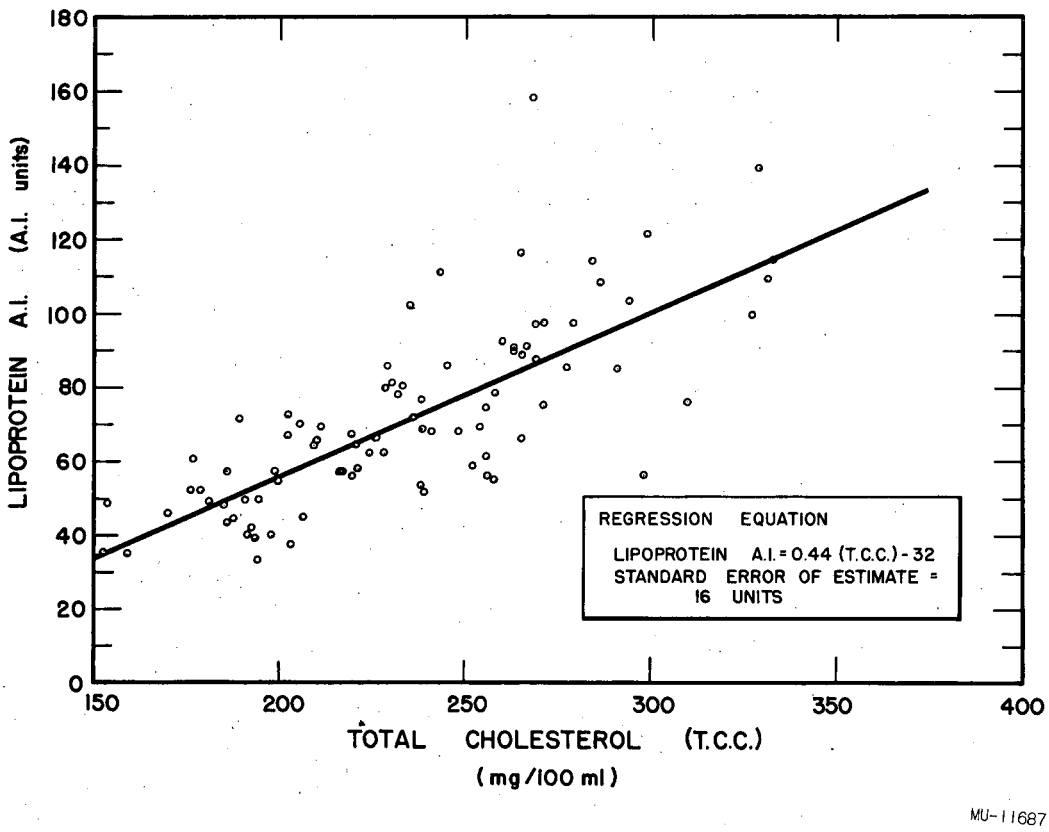

Fig. 2. The relationship between the Lipoprotein A.I. and the Total Cholesterol Concentration. (Based on data collected for 87 normal human males.)

Table III

Correlation coefficients[a] between the major lipoprotein classes and (a) the Lipoprotein A.I., (b) the G.T.L.C., and (c) the T.C.C.

Lipoprotein Classes	Between lipoprotein A.I. and serum concentrations of lipoprotein classes at left.		Between G.T.L.C. and serum concentrations of lipoprotein classes at left.		Between T.C.C. and serum concentrations of lipoprotein classes at left.	
	n	r	n	r	n	r
S_f^o 12-400	87	0.95	87	0.87	87	0.55
S_f^o	87	0.61	87	0.60	87	0.87
Total H.D.L.	84	-0.23	84	0.01	83	0.22

[a] Pearson product-moment correlation coefficient

Table IV

Mean lipoprotein and lipid values

No. of subjects	Mean total H.D.L. (mg%)	Mean s_f^o 0-12 (mg%)	Mean s_f^o 12-400 (mg%)	Mean cholesterol (mg%)	Mean G.T.L.C. (mg/ml)
A. Subjects having lipoprotein A.I. values less than the mean (71)					
1. Value differing by 11 or more units from gravimetric A.I. values calculated from G.T.L.C. values					
(a) Lipoprotein A.I. less than gravimetric A.I.					
7	409	338	99	238	7.21
(b) Lipoprotein A.I. greater than gravimetric A.I.					
1	239	260	150	176	5.32
2. Value not differing by 11 or more units from gravimetric A.I. values calculated from G.T.L.C. values					
40	276	311	136	206	6.33
B. Subjects having lipoprotein A.I. values greater than the mean (71)					
1. Value differing by 11 or more units from gravimetric A.I. values calculated from G.T.L.C. values					
(a) Lipoprotein A.I. less than gravimetric A.I.					
2*	218	329	360	236	10.42
(b) Lipoprotein A.I. greater than gravimetric A.I.					
4	230	356	327	246	7.70
2. Values not differing by 11 or more units from gravimetric A.I. values calculated from G.T.L.C. values					
30	262	433	291	270	8.72

* both very turbid sera

DISCUSSION

In this group of 88 normal male subjects the correlation coefficient between the G. T. L. G. value and the lipoprotein A. I. value was significantly higher than that observed between the T. C. C. and the lipoprotein A. I. value (Table II). By virtue of this relationship between the G. T. L. C. and the lipoprotein A. I. a fairly rapid and simple approximation of the lipoprotein A. I. value, which measures coronary disease risk at a particular age, becomes feasible with the gravimetric determination. Likewise, it would appear that any other accurate method for the determination of total serum lipid concentration would yield data with the same order of significant correlation with the lipoprotein A. I. Of course the exact relationship, including the regression equation, would have to be established by a study similar to this one.

The reason for the lower correlation coefficient between the T. C. C. and the lipoprotein A. I. becomes clear after perusal of Table III. As seen in Table III, the correlation coefficient between the T. C. C. and the S_f^o 12-400 lipoprotein concentration is only 0.55. Since, by definition, the S_f^o 12-400 lipoprotein concentration is weighted more heavily (by a factor of 1.75) than the S_f^o 0-12 lipoprotein concentration in the calculation of the lipoprotein A. I., the observation of the lower correlation coefficient is not at all surprising.

An evaluation of lipoprotein and lipid values for subjects in which there were differences of 11 or more units between the lipoprotein A. I. and the gravimetric A. I. is outlined in Table IV. For subjects having values of lipoprotein A. I. less than the mean lipoprotein A. I. there appear two categories: (a) Cases ($\overline{n}$ = 7) in which the lipoprotein A. I. values are less than the gravimetric A. I. values (mean difference of 18 units), and (b) one case in which the lipoprotein A. I. value is greater than the gravimetric A. I. value (difference of 12 units).

In category (a) there is observed a higher total H. D. L. concentration than in the comparison group. This is believed to be the main cause of the higher G. T. L. C. values which lead, in turn, to higher gravimetric A. I. values (Table IV, A). Although its total H. D. L. and S_f^o 0-12 lipoprotein concentrations tend to be lower than the comparison group, the one case in category (b) presents no distinct extreme of lipoprotein distribution to account for the difference in A. I. values.

For subjects having values of lipoprotein A. I. values greater than the mean lipoprotein A. I. there appear two categories: (a) Cases ($\overline{n}$ = 2) in which the lipoprotein A. I. values are less than the gravimetric A. I. values (mean difference of 20 units), and (b) cases (n = 4) in which the lipoprotein A. I. values are greater than the gravimetric A. I. values (mean difference of 17 units). Cases in category (a) were very lipemic, as evidenced by a marked turbidity of the serum. Since the lipoprotein A. I. values are calculated from concentrations of serum lipoproteins in the range S_f^o 0 to S_f^o 400, it is clear that lipoproteins of S_f^o values greater than S_f^o 400--including chylomicrons, which are primarily responsible for the visible lipemia--could increase the gravimetric A. I. value above the lipoprotein A. I. value. Cases in category

(b) do not present any consistent explanation for their discrepancy and are believed at present to have arisen from possible errors in the analyses at the higher levels of lipoprotein and lipid concentrations in these samples.

From the above evaluation it is apparent that the gravimetric A.I. determination can give values higher than the lipoprotein A.I. (mean difference of 18 to 20 units) in the following instances: (a) whenever the serum total H.D.L. concentration is appreciably higher than normally observed and (b) whenever there is an elevated concentration of lipoproteins with S_f^o values greater than S_f^o 400. Case (a) above occurred in individuals with lipoprotein A.I. values appreciably below the mean, and thus their gravimetric A.I. values were not so inordinately increased as to place the individual into a much higher risk category than is correct for him. In case (b) the observation of a lipemic serum immediately indicates a probable discrepancy in agreement between the lipoprotein A.I. and the gravimetric A.I. In these cases it would be advisable to ascertain the origin of the lipemia, i.e., whether it is due to a postprandial state or whether the individual has some abnormality in his serum lipoprotein distribution. Depending on the diagnosis, a reasonable interpretation can be made of the significance of the gravimetric A.I. in question.

In the five cases wherein the gravimetric A.I. determination was lower than the lipoprotein A.I. value (mean difference of 16 units) there are no obvious explanations other than possible methodological difficulties in these samples.

Thus, 84% of all the gravimetric A.I. determinations in the present study are within 11 units of the ultracentrifugally determined A.I. values. Only 10% of the gravimetric A.I. values fall 11 units or more above their corresponding lipoprotein A.I. values, and only 6% of the gravimetric A.I. values fall 11 units or more below their corresponding lipoprotein A.I. values. These findings support the conclusion that the gravimetric A.I. value can be used to estimate the ultracentrifugal lipoprotein A.I., and hence to provide a very good approximation of the lipoprotein information with respect to coronary disease if ultracentrifugal facilities are unavailable.

For many purposes such as metabolic studies and in the clinical management of lipoprotein abnormalities, it is highly desirable to know the contribution of each segment of the lipoprotein spectrum to the A.I. value. These data are, of course, immediately available in the ultracentrifugal analysis. We have considered the question of estimation of the S_f^o 0-12 and S_f^o 12-400 lipoproteins from the G.T.L.C. and T.C.C. values.

Multiple regression equations were calculated for the ultracentrifugal S_f^o 0-12 and S_f^o 12-400 serum lipoprotein concentrations, with the T.C.C. and the G.T.L.C. values as the independent variables. The final equations are:

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

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

The correlation coefficients between the ultracentrifugal lipoprotein concentration values and those calculated from the above regression equations were: 0.88 between the ultracentrifugal S_f^o 0-12 and the calculated S_f^o 0-12 lipoprotein concentrations; 0.90 between the ultracentrifugal S_f^o 12-400 and the calculated S_f^o 12-400 lipoprotein concentrations. A plot of S_f^o lipoprotein concentrations obtained ultracentrifugally versus S_f^o 0-12 lipoprotein concentrations obtained by calculation from the G. T. L. C. and T. C. C. values is presented in Fig. 3. A plot of S_f^o 12-400 lipoprotein concentrations obtained ultracentrifugally versus S_f^o 12-400 lipoprotein concentrations obtained by calculation from the G. T. L. C. and T. C. C. values is presented in Fig. 4. Again it must be emphasized that the occurrence of specific lipoprotein, distributions which was responsible for discrepancies in the gravimetric A. I. values, is likewise responsible for the major disagreements between the ultracentrifugal and the calculated lipoprotein concentrations.

The regression equation for an estimate of the lipoprotein A. I. from both the T. C. C. and the G. T. L. C. values was

$$\text{Estimated A. I.} = -40.7 + 0.03\,(\text{T. C. C.}) + 14.2\,(\text{G. T. L. C.}).$$

Application of this equation for calculation of the estimated A. I. value results in a standard error of the estimate of 9 units. Comparison of this equation with the equation previously stated for the calculation of the gravimetric A. I. shows that, because the coefficient of the T. C. C. term is relatively small, the T. C. C. value does not supplement the G. T. L. C. value in more precisely estimating the A. I. value.

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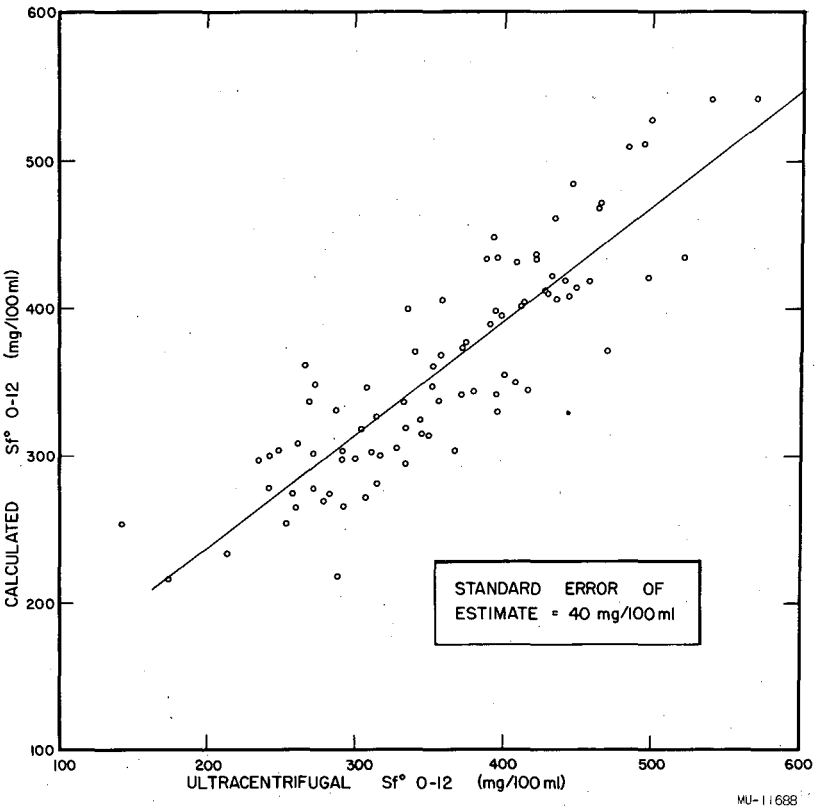

Fig. 3. The relationship between the S_f^o 0-12 lipoprotein concentration calculated from G. T. L. C. and T. C. C. values and S_f^o 0-12 lipoprotein concentration obtained from ultracentrifugal analysis. (Based on data collected for 87 normal human males.)

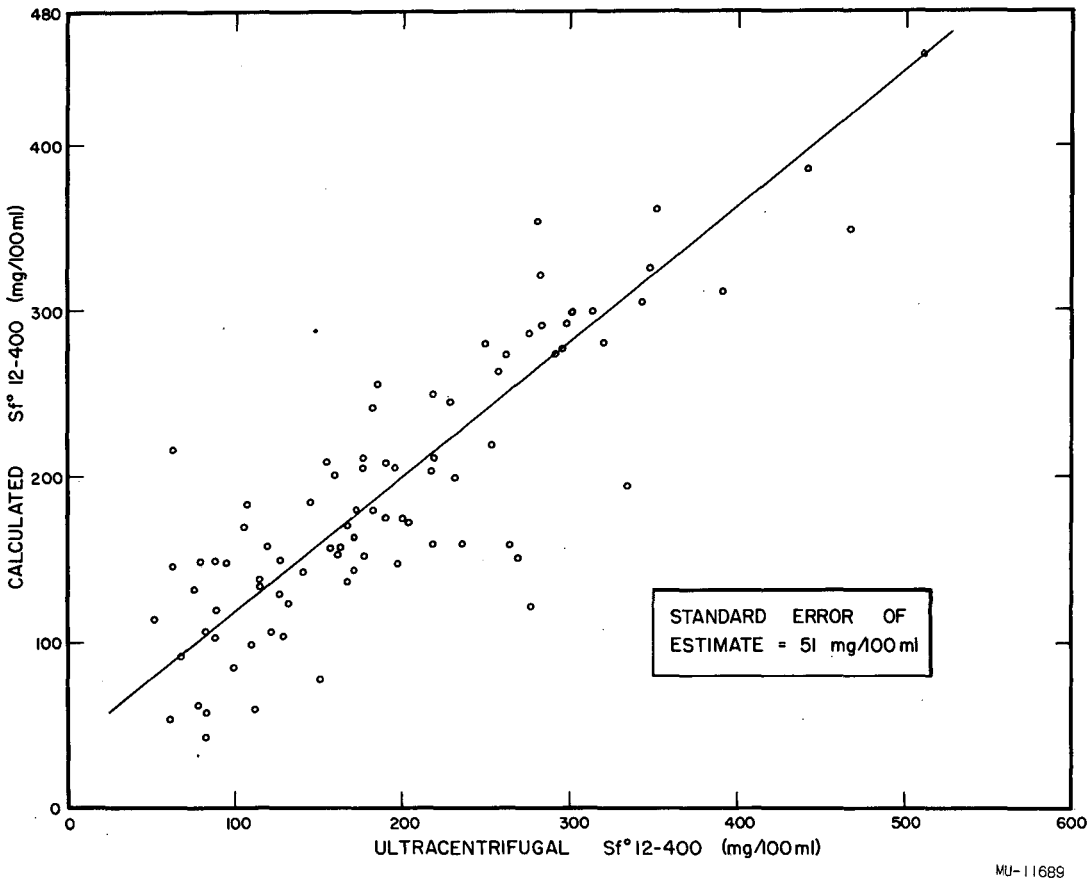

Fig. 4. The relationship between the S_f^o 12-400 lipoprotein concentration calculated from G. T. L. C. and T. C. C. values and the S_f^o 12-400 lipoprotein concentration obtained from ultracentrifugal analysis. (Based on data collected for 87 normal human males.)

SUMMARY

1. The serum gravimetric "total lipid" concentrations and the total cholesterol concentrations of 88 normal male human subjects were tested for correlation with ultracentrifugal serum lipoprotein A.I. values.

2. The correlation coefficient between the lipoprotein A.I. value and the gravimetric "total lipid" concentration was found to be 0.93. The correlation coefficient between the lipoprotein A.I. value and the total cholesterol concentration was found to be 0.76.

3. Serum lipoprotein distributions that account for differences between the lipoprotein A.I. and the calculated gravimetric A.I. are evaluated and discussed.

4. A fairly rapid and simple method is presented for approximation of the A.I. value--and hence of the Accumulated Coronary Disease function--in estimating coronary disease risk in human males.

5. Regression equations are presented for estimation of S_f^o 0-12 and S_f^o 12-400 lipoprotein concentrations in male serum from gravimetric "total lipid" and total cholesterol concentration values.

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