

THE CANADIAN MEDICAL ASSOCIATION
LE JOURNAL DE
L'ASSOCIATION MÉDICALE CANADIENNE

JUNE 9, 1962 • VOL. 86, NO. 23

Serum Lipids and Lipoproteins in Healthy Adult Canadian Males

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IN THE course of studies on atherosclerotic coronary heart disease,¹ the need for an examination of the serum lipids and lipoproteins in healthy Canadians arose. In recent years, the concentrations of the serum lipids, especially cholesterol, have been intensively studied in many parts of the world and have been found to differ widely.² It appears that each survey of serum lipids is valid only for the specific population studied;³ there have been no previous detailed reports of the serum lipid levels in Canadians. Some of the data to be reported here have already appeared in several interim abstracts.⁴⁻⁶

The subjects of this investigation were 121 Canadian men, ranging in age from 30 to 80 years. Careful clinical and laboratory studies were carried out to ensure that only healthy persons were included. The serum lipids studied were: total, free and ester cholesterol; phospholipids; C/P (cholesterol/phospholipid) ratio and standard S₁ 0-400 lipoprotein fractions. The relationships of the various lipid fractions to one another and to age, body measurements, physical activity and family history of coronary heart disease were also evaluated.

CLINICAL MATERIAL

Selection

The subjects were chosen at random from hospital files classified under such minor diseases as "hemorrhoids" and "respiratory infection". A review of their past military and medical records had shown no evidence of cardiovascular or any other important disease. All were free of hypertension, arbitrarily defined as the persistent elevation of blood pressure levels above 150/90 mm. Hg for those under 70 years of age and above 175/100 mm. Hg for those over 70. All were ambulatory,

residing at home, and none was receiving any special dietary or drug treatment.

The rate of refusal to participate in the study was less than 10%. About 20% of the subjects called in on the basis of their documents had to be disqualified after their initial physical and laboratory examinations. The serum lipid concentrations were not a factor in rejection.

Procedure

After an overnight fast, the investigation of each participant included basal metabolism test; fasting blood specimen for determination of lipids and lipoproteins; detailed family, medical and dietary history; complete physical examination; fluoroscopic and orthodiagraphic examinations; 12-lead electrocardiogram; and chest radiographs. A urinalysis, a hemogram, determination of blood sugar and non-protein nitrogen levels and a Wassermann reaction were also carried out on each subject. Where indicated, determinations of serum protein-bound iodine, radioactive iodine uptake, glucose tolerance test, liver function tests and fasting electrocardiographs were performed.

Composition and Characteristics

All of the participants were males. Their numbers and ages are shown in Table I.

The characteristics of the 100 men in the fourth to seventh decades have previously been described.¹ More than half were born in Canada and most of the remainder had come from the British Isles in their youth. There was one Jew but no Negroes. All occupational groups were represented. About two-thirds of the men were moderately, and one-quarter very, physically active. The men in the eighth decade had similar characteristics but were less active, 16 being retired.

A dietary survey⁷ involving 70 of these men showed that the mean daily caloric intake decreased gradually by decade from 2565 calories in the

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Aided by a grant from the Ontario Heart Foundation.

TABLE I.—COMPOSITION OF THE GROUP: NUMBER AND AGE

Decade	n	Mean age	s
4th.....	25	34.4	2.9
5th.....	25	43.6	2.7
6th.....	27	56.0	2.4
7th.....	23	64.1	2.8
8th.....	21	73.0	2.9
	121		

n = number; s = standard deviation.

fourth to 2148 in the seventh decade. In 14 men in the eighth decade the average caloric intake was 1784. However, in each decade the average fat consumption was 38% of the total calories.

Laboratory Methods

Serum lipid determinations for each man were performed on a single fasting blood specimen. The fasting state is important because although serum cholesterol does not change significantly after a meal containing fat, the serum phospholipid⁸ (and consequently the C/P ratio) and the serum lipoproteins do change.

Total and free serum cholesterol were determined in duplicate by a modified Schoenheimer and Sperry method;⁹ the phospholipid in duplicate by the method of Zilversmit and Davis.¹⁰ All of the cholesterol and phospholipid determinations were performed by one biochemist. The duplicates were carried out simultaneously but not blindly. Serum lipoproteins were determined by the technique of De Lalla and Gofman¹¹ in the Ultracentrifuge Laboratory, McGill University, Montreal.

The standard technical errors* for the determination of each serum lipid fraction are as follows:

	Number of pairs	Technical error
Cholesterol		
Total.....	121	1.47
Free.....	120	0.68
Phospholipid.....	134	4.75
Standard Sf lipoproteins		
0 - 12.....	57	17.13
12 - 20.....	57	5.71
20 - 100.....	57	9.82
100 - 400.....	57	8.05

In the case of the lipoproteins, the 57 pairs of observations were consecutive. The technical errors for the other lipids are derived from all determinations including those which were repeated because of a greater than 5% disagreement in duplicates. These technical errors compare favourably with those reported in the literature.^{12,13}

RESULTS

Individual

The results of the serum lipid fractionations on each individual are recorded in the Appendix to this paper.

*Technical Error = $\sqrt{\sum d^2/2k}$, where k = number of pairs and d = difference between duplicates.

Age

The mean lipid values for each decade are shown in Table II. Analysis of variance demonstrated no significant difference in the concentration of any of the fractions over the age period of this study, i.e. from 30 to 80 years of age. Therefore, the data for each lipid fraction in all decades could be pooled, as shown in the final column of Table II.

Frequency Distributions

The distribution of total serum cholesterol values for the entire group is shown in Fig. 1. In comparison to a normal distribution, it is slightly too peaked in the middle and drawn-out at each end

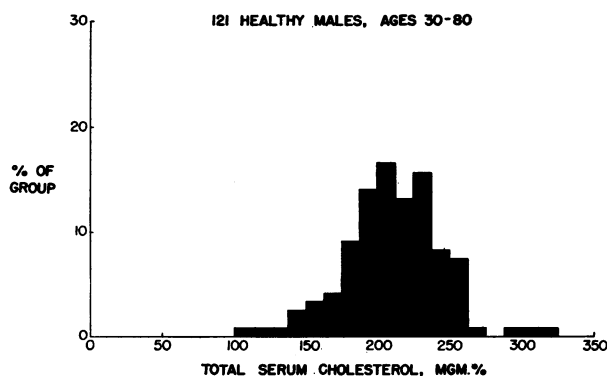


Fig. 1.—Distribution of total serum cholesterol.

(positive kurtosis). This can be demonstrated more conveniently by plotting the cumulative distribution on normal probit paper. A normal distribution produces a straight line. Fig. 2 shows that for total, ester and free cholesterol and for phospholipid, the central portions of the curve are fairly straight while both ends bend slightly, indicating positive kurtosis. However, for practical purposes, these lipids may be considered to have a normal distribution, and any distortions were of a trivial character.

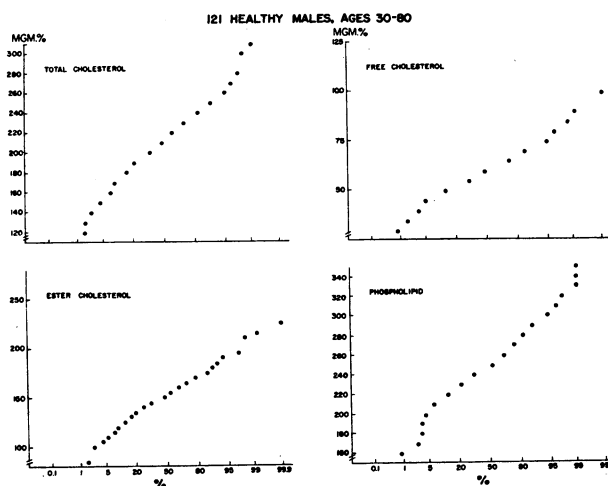


Fig. 2.—Cumulative frequency distributions (on normal probit paper) for serum lipids.

TABLE II.—SERUM LIPIDS AND LIPOPROTEINS, MG.%, MEAN AND STANDARD DEVIATION
*(Geometric Mean and 95% Limits)

Decade	4th	5th	6th	7th	8th	4th - 8th
Number:	25	25	27	23	21	121
Cholesterol, total.....	204.9	214.9	206.5	215.8	218.5	210.7
Ester.....	28.7	38.0	30.5	36.6	38.0	34.4
Free.....	147.5	154.8	147.8	154.1	150.8	150.9
Free.....	20.0	27.3	22.9	25.2	27.4	24.6
Free.....	57.4	60.1	58.7	61.7	61.7	59.8
Free.....	9.1	11.4	8.2	12.0	11.8	10.5
% free.....	28.0	27.8	28.4	28.5	29.0	28.4
Free.....	1.1	1.4	1.4	1.4	1.8	1.5
Phospholipid.....	242.4	255.0	244.5	260.0	254.2	250.9
Free.....	29.0	32.5	30.3	36.5	33.0	32.5
C/P ratio.....	0.845	0.842	0.848	0.829	0.835	0.840
Free.....	0.062	0.101	0.076	0.068	0.105	0.084
Lipoproteins, Std. S _f 0-12	286.0	299.6	289.1	281.1	294.9	290.1
12-20	40.1	51.1	45.5	52.7	71.3	52.4
20-100*	51.4	54.8	57.3	49.2	51.1	53.0
100-400*	21.3	23.1	28.5	21.3	23.5	23.8
12-400*	75.9	75.8	70.5	63.5	63.5	70.0
0-400	27.9-206.6	30.0-191.4	27.8-179.2	14.7-274.8	20.1-201.1	23.4-209.2
12-20	22.8	23.6	17.2	15.5	20.3	19.6
20-100*	3.4-150.6	5.2-107.6	4.5-65.2	1.4-168.1	2.5-161.4	3.1-124.9
100-400*	155.6	155.3	145.8	131.4	138.6	145.6
12-400*	62.5-386.4	65.1-370.5	68.0-312.7	35.9-480.5	46.1-416.6	54.3-389.5
0-400	456.9	469.7	445.0	444.6	452.3	453.7
12-20	93.4	104.8	83.9	153.1	117.4	111.7

*Log normal distribution.

Among the several lipoprotein fractions, as shown in Fig. 3, there is an interesting variation in distribution. Std. S_f 0-12 (in Fig. 3A) has a normal distribution, while Std. S_f 12-20, 20-100 and 100-400 (in Fig. 3B, C and D) show an increasing amount of upward concavity indicating positive skewing. The entire Std. S_f 0-400 fraction (in Fig. 3F) has a normal distribution. This may be explained by the fact that 0-12 constitutes approximately two-thirds

by weight of the total 0-400 fraction (see Table II) and masks the skewed distribution of the 12-400 fractions.

Fig. 3E also illustrates that Std. S_f 100-400 lipoprotein has a nearly perfect logarithmic normal distribution. In fact, Fisher's K₃ value for the skewness in the normal Std. S_f 100-400 distribution is 185,308 ($t = 28.21, p < < 10^{-8}$). The skewness present for the logarithms gives $K = -0.0012$ ($t = 0.0819, p > 0.9$), which is negligible and is due merely to sampling error. As an alternative means of explaining the skewness in the distribution of Std. S_f 100-400 lipoprotein, an attempt was made to sort the subjects into two populations, but this failed.

Each of the Std. S_f 20-100, 100-400 and 12-400 fractions conforms better to a logarithmic than to a normal distribution. For this reason their geometric means and 95% limits, rather than their arithmetic means and standard deviations, are given in Table II.

RELATION BETWEEN THE VARIOUS SERUM LIPID AND LIPOPROTEIN FRACTIONS

Free and Total Serum Cholesterol

The range of per cent of free to total cholesterol is small, being 25.8% to 33.6% (see Appendix), and the mean is 28.4%, with a S.E. of 0.13. The correlation coefficient between the free and total cholesterol is very high ($r = 0.96, p < < 0.001$). This constancy of relationship between free and total (and therefore between ester and total) cholesterol in the serum holds true at all ages and throughout the range of cholesterol readings described.

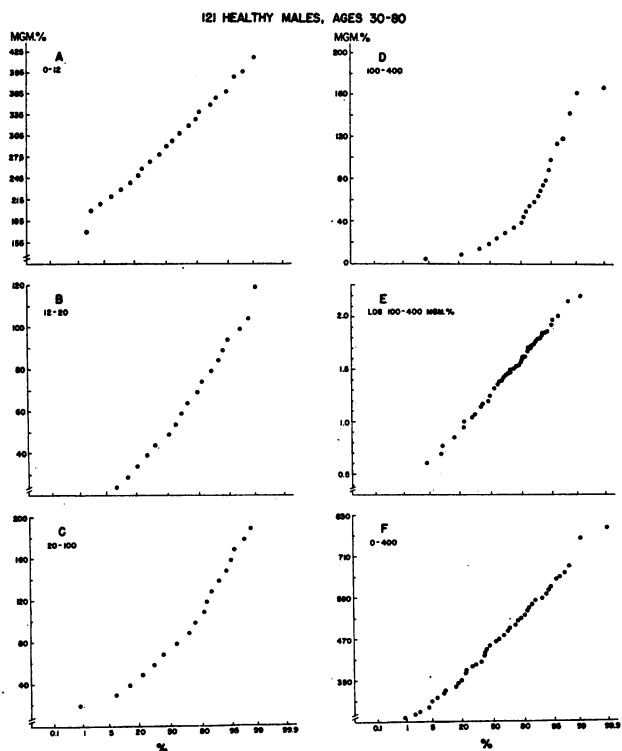


Fig. 3.—Cumulative frequency distributions (on normal probit paper) for Std. S_f lipoproteins.

Serum Cholesterol and Phospholipid (C/P Ratio)

It has been known for some time that there is a close correlation between the concentrations of serum cholesterol and serum phospholipid. Since, as described above, the correlation between free and total cholesterol is very high, either the total or the free cholesterol may be used as the numerator in the so-called C/P ratio. Wilkinson¹⁴ recommended the use of free cholesterol because it maintains its close relationship to phospholipids even in the presence of liver disease. However, in these subjects there is no liver disease, and total cholesterol was preferred as it has been so much more widely studied.

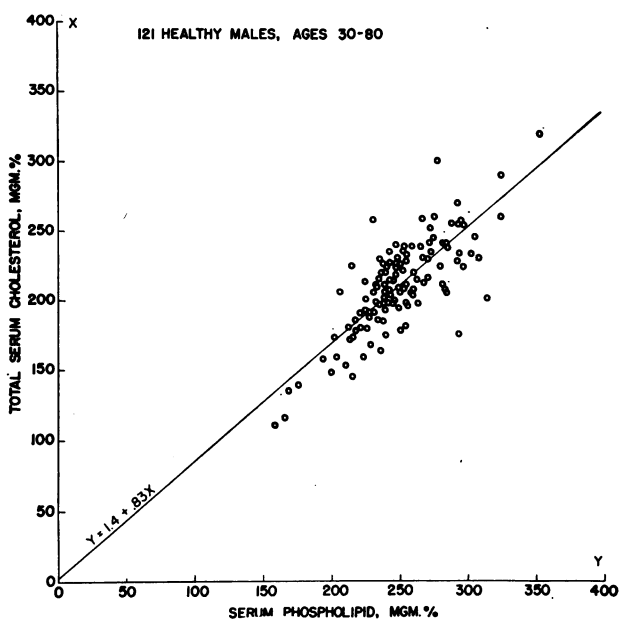


Fig. 4.—Relation between total serum cholesterol and phospholipid.

Table II shows that the C/P ratio, like serum cholesterol and serum phospholipid, does not vary significantly by decade. Since there is no variation with age, the total data may be pooled. Fig. 4 is a scatterdiagram of total serum cholesterol plotted against phospholipid, showing the regression line of cholesterol on phospholipid. Table III shows that in each decade the correlation coefficient between total cholesterol and phospholipid is similar, that for the entire group being +0.79. The intercepts of the regression lines are not significantly different from zero; that is, cholesterol and phospholipid may be taken as proportional. The regression coefficients are similar in each decade, that for the entire group being 0.83 ± 0.06 (S.E.).

Adlersberg³ has stated that, in healthy males, for each increase in the serum cholesterol level of 1 mg. %, there was an increase in phospholipid of only 0.68 mg. % and the C/P ratio was a function of the change in serum cholesterol level, so that it increased as the serum cholesterol increased. The

TABLE III.—RELATION BETWEEN TOTAL SERUM CHOLESTEROL AND PHOSPHOLIPID AS SHOWN BY THE CORRELATION COEFFICIENT (r), THE REGRESSION COEFFICIENT OF CHOLESTEROL ON PHOSPHOLIPID (b) AND THE INTERCEPT (a) OF THE REGRESSION EQUATION

Decade	r	b ± s.e.	*a ± s.e.
485	.83 ± .10	3.7 ± 15.5
573	.86 ± .17	3.3 ± 16.2
674	.74 ± .14	3.6 ± 15.3
788	.88 ± .10	-0.1 ± 16.6
873	.83 ± .18	1.5 ± 16.3
Pooled 4 - 8.....	.79	.83 ± .06	1.4

*"a" is based on average "b".

mathematical basis for this conclusion was not given in detail. It seemed probable that the association between C/P ratio and total serum cholesterol was of a kind that is spurious in nature;¹⁵ that is, the C/P ratio simply reflects the imperfect correlation between serum cholesterol and phospholipid so that high C/P ratios tend to be associated with high serum cholesterol values.

This was shown to be the case in the present study. In Fig. 4 the imperfect correlation between total cholesterol and phospholipid results in a scattering of points above and below the regression line. The points above the line are associated with higher total serum cholesterol values and higher C/P ratios than points below the line. The correlation coefficient between C/P ratio and total serum cholesterol is 0.63, which is similar to that expected (0.60) from this imperfect correlation between total serum cholesterol and phospholipid.¹⁵ This indicates that the correlation between total serum cholesterol and C/P ratio has no fundamental significance in healthy males.

RELATION BETWEEN CHOLESTEROL AND LIPOPROTEIN FRACTIONS

Previous workers have shown that cholesterol and phospholipid are present in varying concentrations in different segments of the lipoprotein spectrum.^{16 17} Consequently, the data were analyzed in order to examine this relation further.

TABLE IV.—CORRELATION COEFFICIENTS OF SERUM LIPOPROTEINS WITH CHOLESTEROL AND WITH PHOSPHOLIPID

Lipoprotein Std. S _f	r cholesterol	r phospholipid
0 - 12.....	.625	.312
12 - 20.....	.544	.325
20 - 100.....	.473	.419
100 - 400.....	.366	.411
0 - 400.....	.692	.548

In Table IV the simple correlation coefficient between cholesterol and the lipoproteins is greatest for the 0-12 fraction. There is a progressively decreasing correlation with the 12-20, 20-100 and 100-400 fractions. This trend is not due to chance because the difference between $r_{\text{cholesterol } 0-12}$ and $r_{\text{cholesterol } 100-400}$ is significant ($.001 < p < .005$),

although there is no significant difference between any other pair of correlation coefficients. Logarithmic transformation of the lipoprotein values did not significantly influence these correlations

The correlation between cholesterol and the lipoprotein fractions could be improved by using multiple regression analysis. The equation was found to be: $y = 88.1 + 0.33 x_1 - 0.19 x_2 + 0.17 x_3 + .13 x_4$, where $y =$ mg. % cholesterol and $x_{1-4} =$ mg. % Std. S_r lipoproteins 0-12, 12-20, 20-100 and 100-400, respectively. The multiple correlation coefficient, $R = .73$, is greater than the simple correlation coefficient, $r_{\text{cholesterol } 0-400} = .692$.

RELATION BETWEEN PHOSPHOLIPID AND LIPOPROTEIN FRACTIONS

In Table IV the simple correlation coefficients between phospholipid and the lipoprotein fractions are all significantly different from zero, but there is no significant difference between any pair of them. Logarithmic transformations of the lipoprotein values did not significantly influence these correlations, nor did multiple regression analysis improve them.

CORRELATION OF TOTAL SERUM CHOLESTEROL TO BODY MEASUREMENTS

An attempt was made to determine whether, in health, the body measurements taken in "routine" clinical practice are related to the level of serum cholesterol. The following measurements were recorded on all subjects: (1) height (without shoes, to the nearest half inch), (2) chest circumference (midsternal level, midway between inspiration and expiration, to the nearest half inch), and (3) weight (in underclothes, to the nearest half pound).

A. To Height, Weight and Chest Circumference

The data for the body measurements of subjects in the 4th-7th decades inclusive have been presented previously.¹ The 21 men in the 8th decade were slightly shorter (mean 65.2 inches, S.E. $\pm .37$) and lighter (139.0 lb. ± 4.9), and had a similar chest circumference (36.7 inches $\pm .62$).

TABLE V.—BODY MEASUREMENTS AND CORRELATION WITH CHOLESTEROL

Measurement	Range	Mean	S.E.	Correlation coefficient
Height (inches)	62.0 - 76.0	68.2	.26	-.09
Weight (pounds)	104 - 240	156.9	2.3	.14
Chest circumference (inches)	30.0 - 44.0	37.0	.29	.16

Table V gives the range of values and the means for the height, the weight and the chest circumference of the entire group. The relation of serum total cholesterol to each of these was computed by regression analysis, and the correlation coefficients

are given in the last column of Table V. None is significant. Further analysis by decade also failed to produce significant correlation.

Within the range of these data, there is, then, no linear relation between total serum cholesterol and height, weight or chest circumference.

B. To a Height-Weight Index

In the present study, height and weight were both found to be normally distributed. However, the simple weight/height ratio could not be employed as an index of body weight as these two variates are not proportional, since the regression lines did not pass through the origin. Consequently, the regression of height against weight was set up and the intercept calculated. Then a height-weight index was obtained for each subject, using the formula: (weight-intercept)/height. The correlation coefficient between total serum cholesterol and this index for the entire group was 0.18, which was not significant. The correlation coefficients in the various decades were similar and also not significant.

Within the range of these values, there is, then, no relation between total serum cholesterol and the height-weight index.

C. To a Body Build Index

For the same reasons and in the same manner as outlined in the previous paragraph, a body build index was calculated, using the formula: (chest circumference-intercept)/height. Total cholesterol was not related to this body build index, the correlation coefficient for the entire group being only 0.18.

D. To Height, Weight and Chest Circumference Combined

A final attempt was made to correlate serum total cholesterol with a combination of these three measurements by multiple regression analysis. The following equation was obtained:

$$y = 28.8 + .16a + 1.32b - 1.26c$$

where $y =$ mg. % cholesterol, $a =$ weight in pounds, $b =$ height in inches and $c =$ chest circumference in inches. The multiple regression coefficient, $R = .033$, is not significant; that is, there is no demonstrable correlation between serum total cholesterol and the combination of these three body measurements. It is concluded, therefore, that it is impossible to predict a healthy man's serum total cholesterol from any combination of height, weight and chest circumference.

CORRELATION OF SERUM CHOLESTEROL TO PHYSICAL ACTIVITY

A. Present Physical Activity

The physical activity of the subjects at the time of investigation was assessed by a consideration of

recreational activity as well as occupation. The results are summarized in an arbitrary manner in Table VI. There are no real differences in serum cholesterol between the subgroups. It appears that in this group these degrees of physical activity at time of investigation did not cause differences in the serum cholesterol concentration.

TABLE VI.—PHYSICAL ACTIVITY: PRESENT

Activity	n	Mean serum total cholesterol
Slight.....	9	200.0
Moderate.....	85	215.9
Most.....	27	198.1

B. Life-Long Physical Activity

As emphasized in one editorial comment,¹⁸ the usual practice of noting the last occupation of the subject, rather than the occupation in which he has spent most of his working life, may lead to fallacious conclusions. Accordingly, "life-long physical activity" of the subject was assessed by inquiring into his physical activity in his childhood, youth and adult life.

Table VII demonstrates that approximately half of the group were classified as being physically "moderately" active and half "most" active. The mean cholesterol of the two groups is identical.

TABLE VII.—PHYSICAL ACTIVITY: LIFE-LONG

Activity	n	Mean serum total cholesterol
Moderate.....	64	211.1
Most.....	57	210.2

In this group then, these degrees of life-long physical activity did not cause differences in the serum cholesterol concentration.

RELATION OF SERUM CHOLESTEROL TO FAMILY HISTORY OF CORONARY HEART DISEASE

A considerable body of evidence testifies to the existence of a familial tendency to coronary heart disease.^{1, 19} It was therefore thought to be of interest to determine whether those healthy subjects with a positive family history have higher serum cholesterol levels than those without.

A detailed family history with particular reference to the presence of coronary heart disease was obtained by direct questioning of each subject. In instances in which information was doubtful, no record was made. The data proved to be unreliable in regard to the incidence of coronary heart disease in aunts and uncles, and too few in number in regard to siblings and grandparents. The prevalence of coronary heart disease in the fathers of the subjects is shown in Table VIII. The average serum

TABLE VIII.—PREVALENCE OF CORONARY HEART DISEASE IN FATHERS

Decade	Number
4th	7/24
5th	5/20
6th	3/23
7th	5/14
8th	0/18
Total	20/99

The lower figure is the number of fathers about whom reliable information was obtained.

total cholesterol of the 20 subjects whose fathers had had coronary heart disease was 210.5 mg. %, almost exactly the same as for the group as a whole.

It is concluded that within this group of healthy men, there is no tendency for a higher serum cholesterol level among those subjects whose fathers had clinical coronary heart disease.

DISCUSSION

Material

All of the participants in the study were, by careful selection and by personal clinical and laboratory examination, found to be free of any cardiovascular or major disease, and none was receiving any treatment, drug or dietary, which might readily alter the serum lipids. Unlike some other workers,^{20, 21} we have made no attempt to exclude individuals with a family history of such conditions as coronary heart disease or diabetes often associated with changes in the serum lipids. Since only truly healthy subjects were accepted, the results may not be representative of the general population.

Although the subjects are called "Canadians", these 121 men do not represent a true sampling of this broad country with its heterogeneous population groups. The subjects were urban or suburban dwellers, and it may be that men who live in the country have different lipid levels. The fact that nearly all of the subjects originated from the British Isles could also affect the results, although it is probable that so-called "ethnic" differences in lipid levels only reflect economic, cultural and dietary differences.²²

Total Serum Cholesterol Level

The subjects in this study are "clinically healthy". However, it may be safely assumed that they all have subclinical disease, since pathological evidence indicates that atherosclerosis is universal in adult males in Western civilization.²³ It is evident, therefore, that the serum lipid values in these men, despite their freedom from clinical disease, can hardly be employed as an index of the "normal" range in the ideal sense. If the magnitude of lipid levels noted in those almost immune to clinical atherosclerosis, i.e. most animals, children and primitive populations, is accepted as the "normal"

or desirable level, it would be in the order of 150 mg. % or less for total cholesterol for the adult human. The so-called "normal" average cholesterol level in Western society may then represent a moderate form of hypercholesterolemia in regard to the association with a high incidence of clinical atherosclerosis. This is illustrated by the fact that four of the 121 "healthy" men studied died of coronary heart disease within five years.

As previously emphasized,³ each survey is only valid for the specific population studied, because of the influence of environment on serum lipids. It is interesting that the mean total serum cholesterol of adult Canadians, 210 mg. %, falls neatly in the centre of the range of mean values (180-230) reported for various population groups who share a similar Western type of socio-economic and dietary environment.²

Variation in Serum Lipid Levels

It is all too common for the clinician to seize upon the mean value as representing the "normal level" and to ignore the wide variations that normally exist for most biological measurements. The normal ranges for the serum lipids in this study are indicated by the standard deviations or the 95% limits shown in Table II. For example, the "normal" range for total serum cholesterol may be considered to be the mean \pm 2 standard deviations, i.e. 140-280 mg. %. The relative variations about the mean are even greater for the lipoprotein fractions.

Frequency Distributions of the Serum Lipids

As in a large American study,¹³ total serum cholesterol was found to have a fairly normal distribution. Other workers report the distribution to be more lognormal.^{24, 25}

Frequency distributions of the lipoproteins are rarely described in the literature. However, there is a previous report¹³ of positive skewing for the S_r 12-20 and 20-100 fraction similar to the findings in this study. Logarithmic transformations resulted in more normal distributions in the Std. S_r 20-400 lipoprotein fractions. Because of this, geometric means and 95% limits are preferable to arithmetic means and standard deviations for these fractions. These transformations were used in statistical tests, such as correlations, in order to improve their efficiency. This precaution was not evidently observed in some studies of lipoproteins.²⁶

Serum Cholesterol and Age

In the male, the level of serum cholesterol gradually increases to the age of 30.^{3, 27} Further increases up to age 60 have been reported by some^{28, 29} but not by others.^{3, 30} Mann found that the serum cholesterol rose with age in prosperous, sedentary and well-fed city dwellers whether American or Guatemalan, but not in rural, lean Guatemalans.³¹

Not unlike these rural Central Americans, this group of Canadians maintained their physical activity,¹ and their caloric consumption was not excessive and decreased with age.⁷ Consequently, the average weight tended to decrease with increasing age,¹ and this may explain, in part at least, why their serum lipids remained stable with age. The rigid exclusion of individuals with diabetes, clinical atherosclerosis, etc., may also have influenced the age trend for serum lipid values. The fact that within this relatively small homogeneous group there was no apparent influence of physical activity, weight or body type on serum cholesterol does not mean that these variables exert no influence on the average serum cholesterol level for the entire group.

Relation Between Free and Total Serum Cholesterol

The results of this study show that the percentage of free to total cholesterol in these normal subjects is confined within the narrow limits of about 25 to 32%, averaging 28%. This agrees with the findings of previous workers.³² Indeed, in the absence of liver disease, a gross variation of the percentage of free to total cholesterol indicates poor laboratory technique. At least one prominent study reported grossly abnormal per cent free fractions,³³ and most other studies did not determine free cholesterol.

Since in health the relation between the free and total cholesterol is a physiologic constant, further ratios such as that between the ester and the free cholesterol can only be tautologous. Nor is there anything to be gained by the calculations of the correlations of the ester or of the free cholesterol to any variate when the latter's relationship to total cholesterol has already been established.

Serum Cholesterol-Phospholipid Relationship and C/P Ratio

The concentrations of cholesterol and of phospholipid in the serum maintain a fairly close relationship.³⁴ Since cholesterol is hydrophobic and phospholipids are hydrophilic, this may be of physiological importance in stabilizing the lipids and lipoproteins in solution. It has been claimed that excess of serum cholesterol in relation to phospholipid, i.e. a high C/P ratio, is more closely related to the incidence of atherosclerotic coronary heart disease than is cholesterol or phospholipid concentration alone.³³ The C/P ratio has also been considered to be a function of serum cholesterol concentration.³

In the present study, there was no correlation between cholesterol and C/P ratio, only a spurious one. Thus in health, there is no fundamental significance to the C/P ratio. It may be added that, in coronary patients studied by the authors, the relationship between serum cholesterol and phospholipid is the same as that in the healthy subjects, and

here too the calculation of the C/P ratio is a worthless exercise.⁸

Serum Lipoproteins

The Std. S_r 0-400 lipoproteins represent only a portion of the total lipoprotein spectrum and comprise the low-density β-lipoproteins as distinct from the high-density α-lipoproteins.^{35, 36}

It is difficult to compare the Std. S_r lipoprotein values in this study with the earlier reports of non-standard S_r lipoproteins. The ultracentrifugal methods used in most other studies did not make the corrections suggested by De Lalla and Gofman¹¹ for the self-slowning of lipoproteins with increasing concentration. These corrections were employed in the present study as denoted by the term "standard". A further complication is the fact that all other reports in the literature give arithmetic mean values for all the lipoprotein fractions, although it would appear that geometric means are better suited for certain of the fractions. Although not so informative, arithmetic means for the entire group are listed here to facilitate comparison with other results reported in the literature.

Std. S _r	Arithmetic mean	S.E.
0 - 12.....	290	4.7
12 - 20.....	53	2.2
20 - 100.....	81	4.3
100 - 400.....	30	2.7
12 - 400.....	164	7.6
0 - 400.....	454	10.1

The Std. S_r 0-12 and 12-400 fractions are lower than those of Gofman's "non-coronary" subjects of the same age,³⁷ and the Std. S_r 12-20 and 20-100 fractions are again lower than those reported by Gofman *et al.* in Table 19 of the Co-operative Study.¹³ The Std. S_r 12-20 and 20-100 fractions reported here are slightly higher than the non-standard S_r 12-20 and 20-100 counterparts in the literature.^{13, 38, 39}

Relation of Cholesterol and of Phospholipid to the Lipoproteins

Various workers have isolated lipoprotein fractions and determined chemically the amount of cholesterol and phospholipid in the separate fractions.^{16, 17, 40} There is general agreement that the denser beta lipoproteins, namely Std. S_r 0-12, contain a much larger percentage of cholesterol and an equal or slightly greater percentage of phospholipid than the less dense 12-400 fractions. The data of this study confirm these relations. Of the partial regression coefficients in the multiple regression equation of cholesterol on lipoprotein fractions, *q.v.*, the largest is for Std. S_r 0-12, and the remainder become progressively smaller for the higher S_r fractions. The correlations in Table IV between total serum cholesterol and the lipoproteins are higher for Std. S_r 0-12 than for the 12-400 fractions.

In general, these correlations are slightly higher than those reported by Lawry *et al.*³⁹ The correlations between phospholipid and the lipoproteins show no differences for the various lipoprotein fractions.

The reported chemical studies have been performed on only two fractions which divide the beta lipoproteins somewhere between S_r 12 and S_r 20. In the present study, there are two findings which indicate that it might be informative to fractionate further the Std. S_r 0-400 lipoproteins in preparation for chemical analysis. The progressively changing distribution of lipoproteins from normal at Std. S_r 0-12 to lognormal at 100-400 and the progressively increasing correlation between cholesterol and lipoprotein concentration from Std. S_r 0 to Std. S_r 400 both suggest a gradually changing chemical composition.

Serum Cholesterol and Body Weight

Because of the erroneous assumption that obesity (excessive fat) is synonymous with overweight (excessive fat, muscle or fluid), and because of the confusion of both with body build, most of the data on the relationship of cholesterol to "obesity" and "obesity indices" are unreliable.⁴¹ In the Co-operative Study of normal Americans, the correlations of lipid levels with weight was positive but very low.⁴² Keys⁴³ has summarized his experience in this field: "Among clinically healthy men of given age in restricted socio-economic classes and cultures, there is at most only a small correlation between relative obesity or relative body weight, and serum cholesterol concentration."

The data presented here show that, in healthy Canadian men with a relatively high average serum cholesterol, there is no relation between the level of cholesterol and absolute weight. Nor is there any relation of cholesterol to a body weight index compounded of weight and height. However, it should be added that in other populations characterized by a lower average serum cholesterol and a lower average body weight, a better relation of weight to cholesterol can be demonstrated.^{44, 45}

Serum Cholesterol and Body Build

The reliability of earlier investigations of the correlation of lipid levels with body build was limited by the difficulty of physique classification.⁴⁶ In 1950, Kornerup²⁰ found that the serum lipid values tended to be higher in the pyknic than in the leptosomatic subjects, but the differences were not significant. Gertler and White⁴⁶ employed the Sheldon system of somatotyping,⁴⁷ but even this technique with its use of anthropometry still requires a subjective "impression" of the patient and his photograph. Average cholesterol was somewhat higher in the endomorph (the rounded, soft type) than in the mesomorph (the squared, muscular type), and was lowest in the ectomorph (the linear,

fragile type). Tanner²⁷ found a significant correlation of cholesterol with endomorphy and no correlation with ectomorphy or mesomorphy.

In this study, a simple body build index was constructed employing the objective measurements of height and chest circumference. No relation to serum cholesterol could be demonstrated. Possibly a more refined index of body build is necessary, but it can be concluded that, popular impression to the contrary, an unequivocal relation between serum cholesterol level and body build has not been established.

Serum Cholesterol and Physical Activity

As reported in our previous paper, a group of coronary subjects were physically more active throughout their lives as compared to the controls.¹ Others have reported the opposite, that coronary heart disease is more prevalent in the sedentary.¹⁸ If exercise really is preventive for atherosclerosis, one could speculate that it might lower serum lipids. In this study of healthy subjects the amounts of current physical activity encountered did not affect fasting serum cholesterol levels. The possibility remains that exercise lowers post-prandial serum lipid peaks. Also greater amounts of exercise than are customary in our society may lower fasting serum lipids. Therefore the effect of exercise on serum lipid levels is still an open question requiring additional investigation.

Serum Cholesterol and Familial Coronary Heart Disease

Coronary heart disease is often familial and is often associated with hypercholesterolemic states. It appeared probable, then, that healthy subjects with a positive family history of coronary disease would exhibit elevated levels of serum cholesterol. This was found to be true in young medical students⁴⁸ and military cadets,⁴⁹ although the elevations in cholesterol were quite small. This study did not confirm those findings, but there were fewer and older subjects involved.

SUMMARY AND CONCLUSIONS

Serum cholesterol (total, free and ester), phospholipid, C/P ratio and standard S_r 0-12, 12-20, 20-100 and 100-400 lipoprotein fractions were determined in 121 healthy Canadian males, aged 30-80. The relations of the lipid fractions to one another and to body measurements, physical activity and family history were examined.

Cholesterol and its fractions, and phospholipid were found to have a normal distribution. Of the lipoprotein fractions, standard S_r 0-12 had a normal distribution, while standard S_r 12-20, 20-100 and 100-400 showed progressively increasing positive skewing and the last two conform better to a lognormal distribution. These last two fractions therefore must be characterized by their geometric means and limits.

The variation within the group for all serum lipid measurements was considerable. This was especially true of the lipoprotein fractions.

The means of the lipid concentrations were in the mid-range of values of other population groups in Western countries. These averages are considerably higher than those of population groups which enjoy a relative immunity to clinical coronary atherosclerosis.

The levels of the serum lipid and lipoprotein fractions did not change between the ages of 30 and 80.

The proportion of free to total serum cholesterol is a physiologic variable with a very small dispersion from the mean of 28%.

The imperfect correlation between total serum cholesterol and phospholipid produces a spurious correlation between the C/P ratio and total serum cholesterol. Because of this, the C/P ratio is worthless in characterizing the serum lipids.

The correlation of serum cholesterol with lipoprotein was greatest for the Std. S_r 0-12 fraction and progressively decreased for the other fractions from 12 to 400.

The correlation of phospholipid and lipoprotein was positive and similar for each fraction.

Total serum cholesterol showed no relation to height, weight or chest circumference or to any combination of these body measurements; nor was there any demonstrable correlation of cholesterol with a body weight index or with a body build index. There was no relation between total serum cholesterol and physical activity, past or present, in this group. There was also no relation to a family history of coronary heart disease. It should be cautioned that, within this group, there were relatively small internal variations in some of the factors analyzed, so that the negative results do not necessarily apply to other populations.

The support of Professor R. Ian Macdonald is gratefully acknowledged. The biochemical determinations were performed by Mrs. C. Radecka, M.A., and the lipoproteins, by the University of McGill Ultracentrifuge Laboratory under the direction of Dr. R. F. Robertson. Dr. E. A. Murphy reviewed the manuscript and contributed to the statistical analysis along with Professor D. B. W. Reid and Mrs. A. Csima. Miss Ruth Yano has been a most cheerful and capable secretary.

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APPENDIX

SERUM LIPID FRACTIONATION (mg.%) IN HEALTHY CANADIAN MALES

No.	Study No.	Age	Cholesterol				Phospho-lipid	C/P ratio	Std. S _f lipoproteins				
			Total	Ester	Free	% free			0-12	12-20	20-100	100-400	0-400
Fourth decade													
1	C2	30	188	136	52	27.6	228	0.82	267	47	50	9	373
2	C3	39	198	145	54	27.3	257	0.77	262	55	89	55	461
3	C6	34	168	122	46	27.4	229	0.73	258	34	31	5	328
4	C7	34	207	152	55	26.4	222	0.93	271	60	90	23	444
5	C10	37	180	130	50	28.0	213	0.85	227	19	44	16	306
6	C15	35	197	137	60	30.3	236	0.85	292	36	93	50	471
7	C16	34	242	172	70	29.0	283	0.85	308	45	53	16	422
8	C23	31	204	148	56	27.6	261	0.78	263	36	77	95	471
9	C25	38	201	146	55	27.2	226	0.88	310	30	41	11	392
10	C29	37	228	162	66	29.0	293	0.78	283	21	146	119	569
11	C32	39	185	134	51	27.7	238	0.78	232	36	78	63	409
12	C33	37	220	158	62	28.4	241	0.91	271	62	63	12	408
13	C40	31	246	182	64	26.0	307	0.80	318	80	141	21	560
14	C44	32	236	168	68	28.7	259	0.91	269	46	136	24	475
15	C50	31	231	167	64	27.9	268	0.86	314	46	151	70	581
16	C55	32	172	127	45	26.2	214	0.80	365	60	61	15	501
17	C58	33	174	125	49	28.4	203	0.86	237	32	25	7	301
18	C59	31	242	172	70	28.8	274	0.88	287	94	188	86	655
19	C61	31	225	160	65	29.0	240	0.94	301	80	80	18	479
20	C65	35	135	98	37	27.6	169	0.80	221	30	56	14	321
21	C66	35	179	129	50	28.1	227	0.79	317	65	62	23	467
22	C80	33	238	170	68	28.6	254	0.94	391	96	85	9	581
23	C85	33	186	135	51	27.8	235	0.79	273	42	77	4	396
24	C88	39	210	153	57	27.0	234	0.90	315	73	108	32	528
25	C123	38	231	160	71	30.6	249	0.93	299	60	104	60	523
Fifth decade													
1	C5	40	232	165	67	28.8	251	0.92	294	51	58	16	419
2	C11	43	290	209	81	28.0	326	0.89	328	67	170	67	632
3	C24	46	199	147	52	26.0	233	0.85	306	45	40	11	402
4	C26	45	228	165	63	27.4	256	0.89	368	37	60	14	479
5	C31	41	258	184	74	28.5	232	1.11	351	65	131	51	598
6	C34	40	260	187	73	28.0	277	0.94	324	67	136	143	670
7	C37	41	209	154	55	26.2	250	0.84	245	48	78	27	398
8	C38	41	186	138	48	26.0	218	0.85	265	74	79	34	452
9	C39	44	270	194	76	28.1	294	0.92	349	98	158	29	634
10	C45	42	198	146	52	26.2	238	0.83	257	32	51	21	361
11	C49	43	234	170	64	27.2	256	0.91	348	39	38	14	439
12	C53	43	164	115	49	30.0	237	0.69	227	21	82	32	362
13	C57	48	206	149	57	27.8	232	0.89	306	47	73	32	458
14	C64	45	178	128	50	27.8	252	0.71	273	103	106	35	517
15	C71	40	252	181	71	28.2	274	0.92	366	70	61	39	536
16	C73	41	193	139	54	28.2	240	0.81	295	42	49	4	390
17	C77	43	207	143	64	30.8	279	0.74	337	53	166	35	591
18	C78	48	206	143	63	30.6	252	0.82	306	67	81	16	470
19	C79	41	255	181	74	28.8	298	0.86	361	96	80	48	585
20	C84	43	111	82	29	26.0	158	0.70	178	18	44	14	254
21	C116	47	176	124	52	29.2	294	0.60	196	36	72	39	343
22	C117	49	224	166	58	25.9	281	0.80	276	42	125	25	468
23	C118	44	208	154	54	25.8	243	0.87	288	47	38	11	384
24	C124	45	211	150	61	28.7	256	0.82	317	28	52	7	404
25	C129	47	218	157	61	28.0	248	0.88	331	76	77	14	498

SERUM LIPID FRACTIONATION (mg.%) IN HEALTHY CANADIAN MALES

No.	Study No.	Age	Cholesterol				Phospho-lipid	C/P ratio	Std. S ₁ lipoproteins				
			Total	Ester	Free	% free			0 - 12	12 - 20	20 - 100	100 - 400	0 - 400
<i>Sixth decade</i>													
1	C4	57	148	102	46	30.8	200	0.74	311	47	36	9	403
2	C14	55	261	187	74	28.4	326	0.80	272	45	76	21	414
3	C17	56	198	135	63	31.8	244	0.81	231	78	132	41	482
4	C18	54	259	187	72	27.9	268	0.97	325	67	124	30	546
5	C20	57	236	172	64	27.0	249	0.95	305	55	125	35	520
6	C22	59	226	159	67	29.6	241	0.94	340	43	84	21	488
7	C27	58	202	146	56	27.9	316	0.64	221	23	48	29	321
8	C35	51	199	146	53	26.6	247	0.80	241	47	69	11	368
9	C36	51	198	144	54	27.4	243	0.81	284	28	73	23	408
10	C46	56	173	123	50	28.8	216	0.80	213	30	51	6	300
11	C56	56	224	163	61	27.1	242	0.92	316	82	80	14	492
12	C62	56	213	156	57	26.8	233	0.91	345	65	95	30	535
13	C63	54	239	170	68	28.6	267	0.90	347	66	75	16	504
14	C67	59	158	112	46	28.8	194	0.81	243	40	34	9	326
15	C74	52	214	152	62	29.0	246	0.87	273	62	171	42	548
16	C75	59	178	124	54	30.2	218	0.82	264	47	103	34	448
17	C83	53	239	175	64	26.8	260	0.92	321	86	72	25	504
18	C101	59	220	156	64	29.3	238	0.92	381	78	106	11	576
19	C104	57	238	168	70	29.2	286	0.83	302	171	56	7	536
20	C106	57	208	148	60	28.6	261	0.80	290	67	78	30	465
21	C110	58	193	134	59	30.6	226	0.86	287	56	91	25	459
22	C111	57	159	110	48	30.6	224	0.79	245	34	40	5	324
23	C113	53	214	157	57	26.7	244	0.88	313	53	100	31	497
24	C115	55	159	116	43	27.0	204	0.78	217	25	48	7	297
25	C126	59	229	166	63	27.4	237	0.97	358	57	48	10	473
26	C127	57	192	137	55	28.6	232	0.83	268	51	23	7	349
27	C130	57	199	145	54	27.1	240	0.83	292	45	63	32	432
<i>Seventh decade</i>													
1	C8	65	234	172	62	26.7	294	0.80	246	40	45	11	342
2	C19	66	181	132	49	27.2	222	0.82	208	25	25	5	263
3	C21	63	206	147	59	28.4	240	0.86	260	26	29	5	320
4	C28	60	235	168	67	28.6	274	0.86	383	33	32	5	453
5	C41	62	231	167	64	27.5	309	0.75	264	38	22	9	333
6	C42	64	202	148	54	26.6	240	0.84	238	35	93	14	380
7	C60	69	236	172	64	27.4	244	0.97	246	51	82	27	406
8	C70	67	213	148	65	30.3	268	0.79	295	56	82	18	451
9	C72	60	205	151	54	26.5	260	0.79	235	28	27	9	299
10	C81	60	245	177	68	27.8	276	0.89	362	74	92	15	543
11	C82	66	198	144	54	27.5	264	0.75	242	18	85	38	383
12	C97	62	139	102	37	27.0	176	0.79	212	23	25	2	262
13	C98	62	319	222	97	30.4	355	0.90	345	80	301	160	886
14	C99	64	153	108	45	29.2	211	0.72	201	40	65	11	317
15	C103	67	220	157	63	28.8	267	0.82	283	91	117	113	604
16	C108	69	197	140	57	28.9	257	0.77	317	76	54	11	458
17	C112	62	208	147	61	29.0	253	0.82	324	52	32	2	410
18	C114	61	217	153	64	29.3	272	0.80	308	54	103	48	513
19	C120	63	256	181	75	29.3	289	0.89	320	54	124	69	567
20	C135	65	257	176	81	31.3	296	0.87	283	86	308	75	752
21	C136	68	182	125	57	31.3	256	0.71	223	32	64	5	324
22	C139	65	216	154	62	28.7	236	0.92	327	56	54	18	455
23	C140	65	214	154	60	28.1	226	0.95	343	63	71	27	504
<i>Eighth decade</i>													
1	C9	73	256	188	68	26.4	294	0.87	288	51	43	7	389
2	C52	73	116	82	34	29.6	166	0.70	123	21	83	7	234
3	C86	75	225	160	65	28.8	216	1.04	344	76	74	23	517
4	C87	76	222	158	64	28.6	253	0.88	350	67	65	62	544
5	C91	70	191	141	50	26.0	222	0.86	257	36	62	14	369
6	C95	72	204	145	59	29.0	244	0.84	278	41	46	21	386
7	C96	71	225	161	64	28.4	249	0.90	396	79	112	55	642
8	C107	70	192	137	55	28.6	228	0.84	293	36	84	28	441
9	C109	72	206	144	62	29.8	285	0.72	288	45	112	166	611
10	C119	74	195	143	52	26.6	250	0.78	418	46	70	21	555
11	C121	70	145	105	40	27.8	216	0.67	234	25	53	9	321
12	C125	71	242	170	72	29.6	284	0.85	272	47	141	113	573
13	C128	70	224	162	62	27.9	298	0.75	231	23	24	11	862
14	C131	71	234	160	74	31.8	304	0.77	278	115	92	16	501
15	C132	71	216	143	73	33.6	267	0.81	259	55	135	53	502
16	C133	77	175	120	55	31.4	241	0.73	278	43	72	38	431
17	C134	77	212	145	67	31.6	240	0.89	336	66	68	15	485
18	C138	71	301	212	88	29.4	279	1.08	441	78	54	12	585
19	C141	79	240	173	67	27.9	249	0.96	310	60	87	28	485
20	C142	73	212	154	58	27.4	282	0.75	217	17	13	2	249
21	C143	78	230	162	68	29.4	272	0.84	302	47	30	7	386