

# THE SERUM PHOSPHOLIPID LEVELS AND TOTAL CHOLESTEROL: PHOSPHOLIPID RATIOS OF APPARENTLY NORMAL ADULT FILIPINO STUDENTS\*

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The interrelationships of serum cholesterol and phospholipids in health and in coronary heart disease have drawn considerable interest among many investigators and the work done on the subject from 1950 to July, 1957 has been summarized in an excellent review by Mattill (1). Aspects of the problem that have attracted our interest are the reported findings that the serum total cholesterol: phospholipid ratio, usually designated as the C:P ratio, has been shown to be more constant in a given individual than the actual cholesterol level and this C:P ratio tends to be elevated in patients with coronary heart disease. Some investigators feel that the C:P ratio may be more important than serum total cholesterol levels in the study of atherosclerosis because it has been contended that "normal C:P ratio is a prerequisite for a normal vascular system even if the absolute concentrations are higher than normal" (1).

It was therefore felt that a study of the serum phospholipid levels and the serum cholesterol: phospholipid ratios in Filipino subjects would provide valuable information in addition to those secured in studies we have conducted (2) and are still conducting on serum cholesterol levels in this country.

Correlation studies were also done on C:P ratio and relative body weight, C:P ratio and serum total cholesterol level, serum phospholipid level and amount of total fat habitually taken at breakfast, and serum phospholipid level and amount of coconut oil contained in the same meal.

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## MATERIAL AND METHODS

The subjects of this study were 66 male and 30 female first year medical students aged from 18 to 24, averaging 19.9 years. They had no overt disease revealed by a physical and medical examination and their blood pressure readings were below 140 mm. Hg. systolic and 90 mm. Hg. diastolic.

Detailed dietary diaries were kept for seven days by the subjects.

Blood samples were obtained by venipuncture between 2 to 3 hours after breakfast. These were allowed to clot and the serum obtained. Serum total cholesterol was determined on duplicates by the method of Abell *et al.* (3). The serum phospholipids were determined on duplicates, on the same day as the blood withdrawal, by the method of Youngburg (4) which employs the phosphate procedure of Fiske and Subbarow.

## RESULTS AND DISCUSSION

*Serum phospholipids.* The serum phospholipids of the 66 male subjects ranged from 120 to 310 mg. per 100 ml. with a mean of  $192.4 \pm 36.6$  mg. per 100 ml. (S.D.) Those of 30 female subjects ranged from 110 to 332.5 mg. per 100 ml. with a mean of  $207 \pm 54.8$  mg. per 100 ml. (S.D.) Statistical analysis showed that the difference of 14.6 mg. per 100 ml. between the mean values of the 2 sexes is not significant ( $t = 1.454$ ). Likewise, Peters and Man (5) did not find any significant difference between the serum phospholipids of normal male and female adults.

Comparing our results with findings abroad, we find that our values are comparable to those of Kornerup in Denmark (6), but are lower than those of Russ *et al.* (7) and of Peters and Man (5), and Gertler *et al.* (8) on American subjects. These are summarized in the following table.

COMPARISON OF SERUM PHOSPHOLIPID VALUES AND CHOLESTEROL: PHOSPHOLIPID RATIOS FOR "NORMALS" OF THIS SERIES AND THOSE OF WORKERS ABROAD.

	No. of subjects	Serum phospholipid in mg. per 100 ml.		Serum total cholesterol: phospholipid ratio	
		Range	Mean $\pm$ S.D.	Range	Mean
Filipinos This series	96	110-332.5	197 $\pm$ 45.8	0.564-1.791	1.043
Gertler et al. (Boston) Unselected normals	146	213-415	299.3 $\pm$ 39.9	0.520-1.056	0.7408
Normals "matched" to myocardial infarction points	90	221-397	305.7 $\pm$ 39.8	0.5184-1.146	0.776
Peters and Man (New York)	103	152.5-362.5	230.2 $\pm$ 35.25	0.775-0.968	0.8556
Russ et al. (New York)	38	187-327	253	0.76-1.14	0.95
Kornerup (Denmark)	104	80-260*	180 $\pm$ 40.0	0.736-2.318**	1.227**

\* Range = mean + 2 S.D.

\*\* Computed from data given in paper.

The frequency distribution of the serum phospholipid values of the 96 subjects of this study is shown in Figure 1.

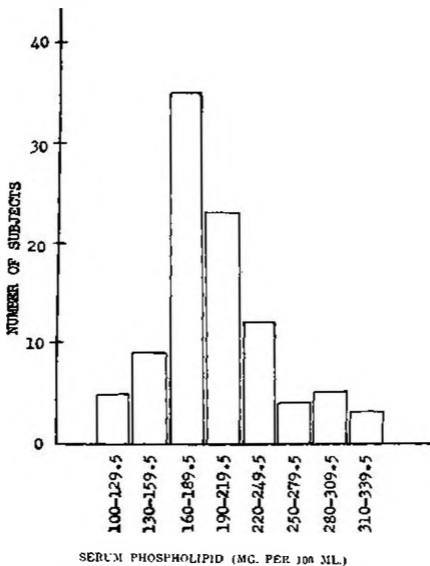


Figure 1. Frequency distribution of serum phospholipid values in 96 male and female Filipino students.

*Cholesterol: Phospholipid ratios.* The C:P ratios in our 66 male subjects varied from 0.564 to 1.469, with a mean of  $1.041 \pm 0.19$  (S.D.). In the 30 female subjects, the C:P ratios ranged from 0.660 to 1.791, averaging  $1.049 \pm 0.274$  (S.D.). Although the female subjects gave a higher range and mean, the difference between means of the two sexes, 0.008, is not statistically significant ( $t = 0.157$ ). The frequency distribution of the C:P ratios is shown in Figure 2. The mean for the subjects of both sexes is  $1.043 \pm 0.218$  (S.D.).

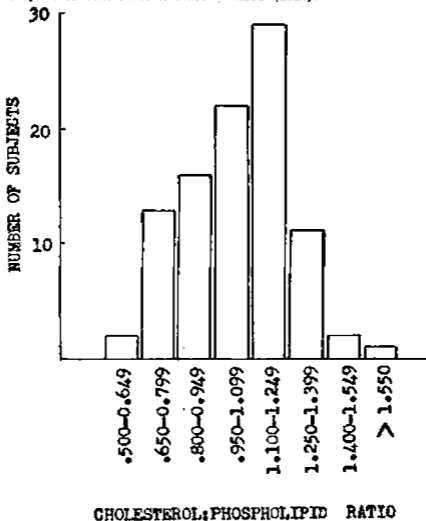


Figure 2. Frequency distribution of cholesterol: phospholipid ratios of 96 male and female Filipino students.

Comparing with findings of other workers shown in the table, the C:P values of our Filipino subjects are strikingly higher than those obtained by American investigators. We computed the C:P ratios from data given by Kornerup in his paper and found that they were also higher in his Danish subjects than those of Americans. However, the factor of differences in analytical methods used for cholesterol and phospholipid determinations must be remembered in making this comparison. Serum cholesterol was determined by means of the Lieberman-Burchard reaction after extraction by varying methods in all the investigations included in the table except in that of Peters and Man where the gravimetric method was employed. Serum phospholipid was extracted and digested in different ways and the P analyzed by the colorimetric method of Fiske and Subbarow, in this series and those of Gertler *et al.* and Russ *et al.* Kornerup employed a modification of the method of Fiske and Subbarow using amidol instead of aminonaphthol sulfonic acid in the color reaction. In all the reports being compared here, the lipid P value was multiplied by 25 to get the phospholipid level.

It is nevertheless of interest to mention that our mean for this series of normal adults is 1.043 which is the value (1.04) quoted from Oliver and Boyd (1) as the mean of their 60 patients with myocardial infarction. It is also higher than those of 60 patients with coronary disease obtained by Gertler *et al.* (8), to wit: 0.578 to 1.32, with a mean of 0.894.

Gertler *et al.* (8) attribute the importance of the phospholipid in maintaining the other lipids in solution to the fact that it is a colloid stabilizer. Since phospholipids are hydrophilic, they tend to keep the hydrophobic cholesterol in solution, much like the action of lecithin and bile salts in holding the cholesterol of the bile in suspension. They observed that in normal individuals, a rise of serum cholesterol is usually accompanied by a concomitant rise in the serum phospholipids, while in coronary artery disease, the interrelationships between the lipids are disturbed and the phospholipid does not rise *pari passu* with the cholesterol. This was corroborated by Steiner *et al.* (1) in his study comparing 82 coronary infarction patients with 112

healthy controls. They found twenty-five of the coronary groups with cholesterol values within the normal range but with low phospholipid levels resulting in high C:P ratios.

*Correlation studies.* A contributory factor to the high C:P ratios is the fact that the serum phospholipid levels in this series are lower on the average than those reported in the literature for normal groups. The lower serum phospholipid values were obtained in spite of the fact that the blood samples were withdrawn not in the fasting state but 2 to 3 hours after breakfast. It has been claimed in the literature (6) that unlike cholesterol, phospholipid levels are affected by the precedent meal and rise in the blood to a maximum within four or five hours after a meal rich in fat.

This finding made us look into the quantities of fat habitually taken by the subjects at breakfast from data gathered through their individual seven-day dietary diaries. We found that total fat of the daily breakfast ranged from 11.8 to 75.2 Gm., with a mean of  $29 \pm 13.4$  Gm. (S.D.) This shows that as a group, our subjects took breakfast with moderately high fat content. There was no correlation between the serum phospholipid level and the total fat intake at breakfast in the subjects of either sex. The statistical data are as follows:\*

Male	Female
$n = 66$	$n = 30$
$b = 0.58$ mg. serum phospholipid per 100 ml. per gram total breakfast fat	$b = -0.303$ mg. serum phospholipid per 100 ml. per gram total breakfast fat
$S_b = 0.344$	$S_b = 1.189$
$t = 1.682$	$t = 0.255$

It was thought of interest to determine the amount of coconut fat taken habitually at breakfast for several reasons. Blood phospholipids normally contain essential fatty acids (9). Because of the low content of such fatty acids in coconut oil,

\* For this and subsequent correlation studies the symbols used are:

$n$  = No. of cases

$S_b$  = Standard error of regression coefficient

$b$  = Regression coefficient

$t = b/S_b$

could the common use of coconut oil in food preparation in this country have been a factor in the production of low levels of serum phospholipids in this series? Coconut oil has consistently been reported as a highly saturated fat with only 1 to 2 per cent linoleic acid content (10,11).

The coconut oil content of the daily breakfast varied from 1.0 to 16.9 Gm., with a mean of  $7.04 \pm 3.64$  Gm. (S.D.) No correlation was found between the serum phospholipid levels and the amount of coconut oil expressed in grams, taken at breakfast among the male subjects. However, the female subjects exhibited a significant decrease of 9.03 mg. serum phospholipids per 100 ml. per gram increase in the coconut oil intake at breakfast. The statistical data are as follows:

Male	Female
n = 66	n = 30
b = -0.106 mg. serum phospholipid per 100 ml. per gram coconut oil taken at breakfast	b = -9.03 mg. serum phospholipid per 100 ml. per gram coconut oil taken at breakfast
$S_b = 1.315$	$S_b = 3.559$
t = 0.081	t = 2.537

Expressing the coconut oil content as per cent of the total fat at breakfast, the range was from 3.7 to 62.6 per cent with a mean of 24.7 per cent among the female subjects. A decrease of 2.08 mg. per 100 ml. serum phospholipid accompanied a rise of 1 per cent in the coconut oil content of the total breakfast fat, significant at the 2 per cent level ( $t = 2.66$ ). Among the male subjects a corresponding decrease of 0.26 mg. per 100 ml. serum phospholipid was not found to be significant ( $t = 0.59$ ). The coconut oil constituted from 5.7 to 53.8 per cent with a mean of 26.3 per cent for the male subjects.

The influence of body build on the serum lipid level has been brought out in the literature (3, 8). In this series only relative body weights were studied. No correlation was found



between C:P ratios and relative body weight expressed as per cent of the standard weight for Filipinos of the corresponding height and age (12). The statistical data are as follows:

Male	Female
$n = 66$	$n = 30$
$b = -0.0013$ units C:P per 1 per cent relative body weight	$b = -0.0042$ units C:P per 1 per cent relative body weight
$S_b = 0.0022$	$S_b = 0.0053$
$t = 0.575$	$t = 7.802$

In this series, the C:P ratios rose as the serum cholesterol level increased. This means that the serum phospholipid level did not increase at the same rate as the serum cholesterol. A similar trend has been reported by Peters and Man (5), although their different means of C:P ratios for increasing levels of serum cholesterol are below the values obtained in this study. Among our 66 male subjects, there was found to be a rise of C:P value of 0.0017 per mg. per 100 ml. rise in serum cholesterol, significant at the 1 per cent level ( $t = 2.916$ ). In the 30 female subjects, the rise of C:P ratio was 0.0035 per mg. per 100 ml. rise in serum cholesterol, significant at the 5.5 per cent level ( $t = 2.034$ ). The scattergrams and regression line calculated by the least squares method are shown in Figures 3 and 4.

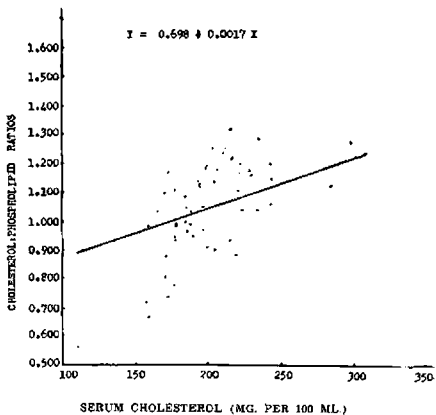


Figure 3. Relation between serum cholesterol: phospholipid ratio and serum total cholesterol level in 66 male Filipino students.

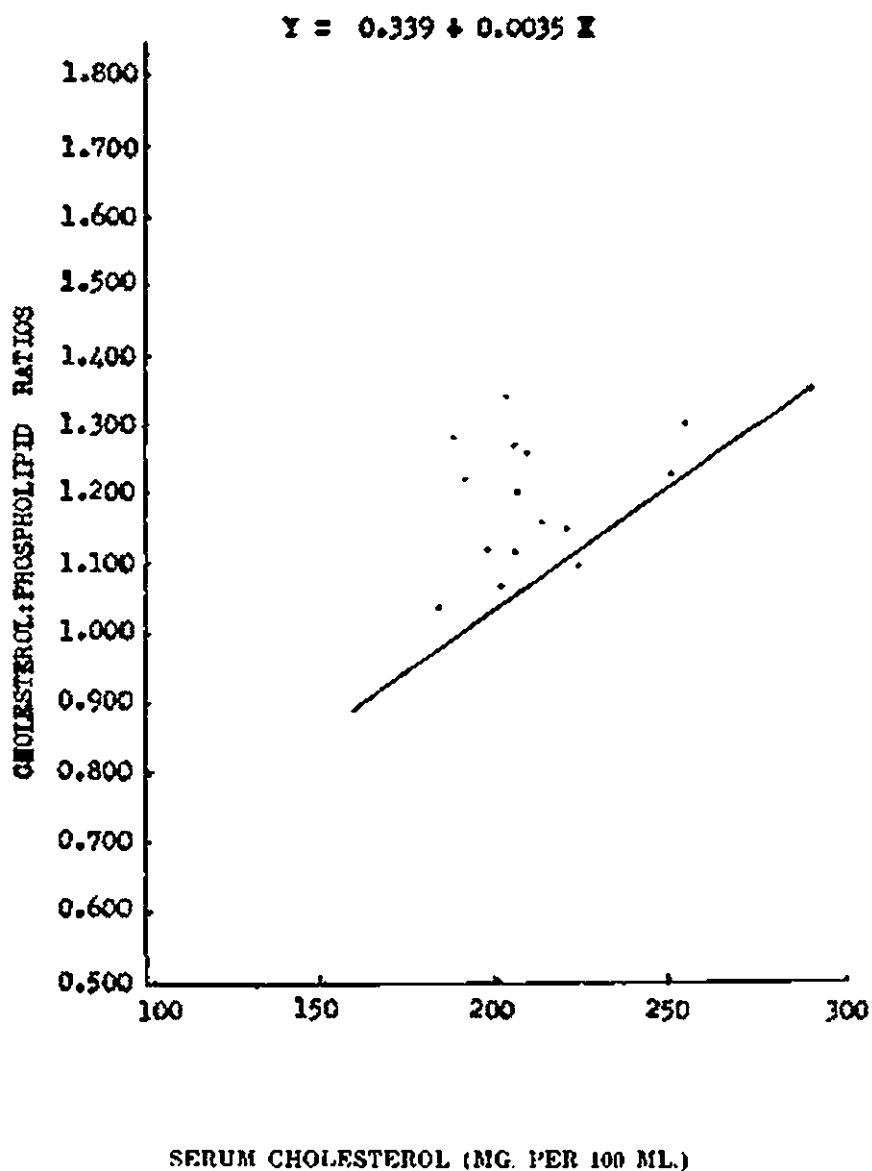


Figure 4. Relation between serum cholesterol: phospholipid ratio and serum total cholesterol level in 30 female Filipino students.

## SUMMARY

1. The non-fasting serum phospholipid values of 96 apparently healthy adult male and female subjects 18 to 24, averaging 19.9 years old and habitually taking breakfast with moderately high fat content (mean 29 Gm., 25 per cent of which was coconut oil) varied from 110 to 332.5 mg. per 100 ml. serum, with a mean of  $197 \pm 45.8$  mg. per 100 ml. (S.D.) No significant sex difference was found.
2. The serum cholesterol: phospholipid ratios ranged from 0.564 to 1.791, with a mean of  $1.043 \pm 0.218$  (S.D.). These are high compared to reports in the literature, so much so that our mean is the same as the 1.04 given by Oliver and Boyd (1) for their subjects with myocardial infarction. No significant sex difference in C:P ratios was obtained in our study.
3. No correlation was found between the serum phospholipid level and the total grams of fat taken at breakfast.

However, expressed as grams or as per cent of the total fat, there was observed a significant decrease of serum phospholipid levels with an increase in the coconut oil content of breakfast among the 30 female subjects. No correlation was obtained among the 66 male subjects.

4. No correlation was found between C:P ratio and relative body weight in both sexes.
5. The C:P ratio rose significantly with the level of serum cholesterol in the subjects of both sexes: 0.0017 per mg. per 100 ml. rise of serum cholesterol in the males, and 0.0085 per mg. per 100 ml. in the females.

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