



## THE SERUM LIPIDS II REFERENCE VALUES IN CHIANG MAI

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The values of the serum lipids, especially the lipoproteins, have shown a rather wide acceptable variation according to the different methods being used. Ultracentrifugation, electrophoresis, low temperature fractionation, direct precipitation and immunochemical methods are used for the determinations of the serum lipoproteins. These methods involve a wide variety of physical and chemical technics and, as a consequence, the values obtained may not agree amongst these different methods.

The purpose of this study is an attempt to establish "REFERENCE VALUES" of the serum lipids, especially the lipoproteins, rather than "NORMAL" or "STANDARD VALUES" in some apparently healthy persons in Chiang Mai.

### METHODS AND MATERIAL :

As many different procedures for the determination of Lipoproteins are used, none of them is ideal. The ones that are of greatest value at the present time have one common feature, that is the ability to provide a qualitative separation of lipoproteins and particles carrying mainly endogenous glycerides from those contain-

ing exogenous glycerides from those containing exogenous glycerides. Fredrickson (I) and colleagues emphasized the value of lipoprotein electrophoresis in rapid separation of plasma lipoprotein in to five different bands. They also pointed out that this classification should not be considered rigid and should be changed if

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new facts revealed additional disorders.

Modification of Beking and Ellefson's (2) method is used for the determination of lipoproteins and albumin-bound free fatty acids. The cholesterol was estimated by Abell's (3) method, triglycerides by micro-method of Van Handel-Zilversmit (4) and total lipids by the method of Merkotest (5) et al. After extraction of serum with  $\text{CH}_3\text{Cl}_3\text{-CH}_3\text{OH}$  mixture, Phosphorous was then measured (13), and determined as lipid P.

A total number of 32 (29 males and 3 females) blood specimens were drawn early in the mornings for study and almost all of them were fasting specimens. The ages ranged from 20 to 59 years. All of the subjects were apparently healthy, no one had the history of secondary hyperlipoproteinemia or familial history of primary hyperlipoproteinemia. After clotting for approximately 1 hour, the sera were centrifuged at 2,000 r.p.m. for 15 minutes and as soon as possible, the analyses were performed in duplicate. Another group of 18 subjects (13 males, 5 females), ages ranged from 20-24 years was studied separately for serum phospholipid.

RESULTS: All data were summarized in table I, II and III.

#### A. Lipoproteins. (Table I)

The "REFERENCE VALUES" for lipoproteins in young adults (20-29 years

of age) were from 12-23% with the mean of 17% in the alpha fraction; 13-48% with the mean of 29% in the pre-beta fraction and 19-43% with the mean of 28% in the beta fraction.

Between the ages of 30-39, the values were 14-23% with the mean of 17% in the alpha fraction; 13-33% with the mean of 22% in the pre beta fraction and 26-39% with the mean of 32% in the beta fraction.

At the ages of 40-49, they were 13-23% with the mean of 18% in the alpha fraction; 13-35% with the mean of 21% in the pre-beta fraction and 25-48% with the mean of 36% in the beta fraction.

The oldest group (50-59 years) revealed the values as followed: 12-22% with the mean of 17% in the alpha fraction, 23-29% with the mean of 26% in the pre beta fraction and 19-43% with the mean of 34% in the beta fraction.

#### B. Cholesterol. (Table II)

The cholesterol values were 112-226 mg% (mean = 170 mg%) for 20-29 years; 128-279 mg% (mean = 218 mg%) for 30-39 years; 144-261 mg% (mean = 185 mg%) for 40-49 years; 147-216 mg% (mean = 176 mg%) for 50-59 years

#### C. Triglycerides. (Table II)

The values of the triglycerides were 43-202 mg% (mean = 100 mg%); 106-214 mg% (mean = 147 mg%); 94-336

mg% (mean = 190 mg%); 111 - 193 mg% (mean = 161 mg%) for the ages of 20 - 29, 30 - 39, 40 - 49 and 50 - 59 years respectively.

D. albumin-bound free fatty acids (AFA). (Table I)

Their values disclosed 7 - 41 % (mean = 26 %) for the group of 20 - 29 years of age; 11 - 43 % (mean = 29%) for 30 - 39 years; 14 - 39% (mean = 26%) for 40 - 49 years and 11 - 29% (mean = 24%) for 50 - 59 years.

E. Total lipids. (Table II)

The total lipids revealed 546 - 857 mg% (mean = 669 mg%) for the age group of 20 - 29 years; 545 - 1200 mg% (mean = 888 mg%) for 30 - 39 years; 400 - 1120 mg% (mean = 795 mg%) for 40 - 49 years and finally, 660 - 1120 mg% (mean = 873 mg%) for 50 - 59 years.

F. phospholipid (II) (Table III)

The serum phospholipid varied from 5.8 - 13.5 mg of lipid P. / 100 ml with the average of  $9.1 \pm 2.8$

### COMMENTS

The lipoproteins by paper electrophoresis according to Block's method (6) give the average of 40% in the alpha fraction and 60% in the beta lipoprotein, with the ranges of 30 - 50% in the alpha lipoprotein and 50 - 70% in the lipoprotein in young adults. The percent of B lipoprotein is usually higher in the aged. In

our data, the pre-beta lipoprotein has the reverse value, being higher in the young and lower in the old. The alpha lipoprotein seems to be nearly in the same rang for all age groups.

The value depends upon number and completeness of the separation bands which are due to specificity and sensitivity of the methods. As the values of serum lipoproteins obtained by various methods are different from each other; the authors will not rigidly intend to compare their results with those of others. The authors' aim, as mentioned previously; is only to establish the "REFERENCE VALUES" in their laboratory.

It is usually stated that the serum cholesterol levels for a given individual show little fluctuation from day to day, but recent evidence indicates that the statement is not always true since in a few otherwise normal individual, it may vary as much as 100 mg% from day to day. However for most individuals, it is relatively constant and is not affected by an ordinary meal. The range of normal serum values is wide and seems to depend somewhat on the method used. The normal values as estimated by Viranuvatti (7) in 1971 in Siriraj Hospital by using Gauss' method definitely are shown in table IV. The averages of serum cholesterol in our study are lower than that of the European

and of the Thais studied by Viranuvatti. It reaches its highest peak around the age of 40 and then gradually declines and seems not to increase in advancing ages. As compared to the studies done in the same area (12, 11) and of larger group of subjects, the results are within the same range. However, the number we have obtained from each age group is too small to be considered seriously. The difference in socio-economic status, therefore, may play an important role in this variation.

In general, the normal limits of serum cholesterol for healthy young adults may vary from 150-270 mg%. It may be less in the children. The cholesterol level increases with age and reaches its maximum value at the age of about 60 years. Those who are over 70 years, may have cholesterol levels approaching those of young adults, or even lower. Menopausal females have higher cholesterol than males of the same age. Tomkins and Chaikoff (8) had shown that caloric restriction for several days reduced cholesterol synthesis. In the liver, cholesterogenesis is under dietary control.

The rate of synthesis of hepatic cholesterol in animals has been shown to be greatly reduced by fasting and by high cholesterol diets. Bhattathiry and Sipers-tein (9) found that a diet supplement of eggs sufficient to provide 3 to 4 grams of

cholesterol per day drastically decreased the conversion of the labelled  $C^{14}$  acetate to cholesterol by the liver biopsy specimens. The mechanism of the blockage induced by dietary cholesterol has not been clarified. People leading fast life tend to have higher cholesterol. Emotion also increases serum cholesterol. There are still many conditions altering the biosynthesis of cholesterol.

By using the micromethod of Van Handel-Zilversmit, (4) the values of the triglycerides we have obtained are still within the same ranges as those of Fredrickson and Lee (10) shown in table V.

The free fatty acid are expressed in per cent ranging from 24-29% for the whole group.

The total lipids where their ranges and means of various age groups are summarized in table II, include cholesterol and its esters, phospholipids, triglycerides, as well as smaller quantities of the other compounds that are also classified as lipids. Accordingly, the total lipids will be elevated in conditions in which there are increases in cholesterol or triglycerides or both. However, the absolute or relative increase in a certain fraction of serum lipids is more meaningful than the total lipids themselves.

The values of serum phospholipid are within normal range by the method. Un-

fortunately all of the subjects were limited to the young adult group. As we have previously mentioned, the blood specimens obtained were not entire overnight fast and even all of them were taken from those who were "apparently healthy" but the number of the samples in some age groups were small. Therefore further additional data must be obtained before any conclusion can be made.

TABLE I.

"REFERENCE VALUES" OF SERUM LIPOPROTEIENS

| Age-yr. | No. | Lipoprotein Expressed in percent |      |          |      |       |      | Albumin-bound<br>free fatty acids% |      |
|---------|-----|----------------------------------|------|----------|------|-------|------|------------------------------------|------|
|         |     | alpha                            |      | pre-beta |      | beta  |      | Range                              | Mean |
|         |     | Range                            | Mean | Range    | Mean | Range | Mean |                                    |      |
| 20-29   | 17  | 12-23                            | 17   | 13-48    | 29   | 19-43 | 28   | 7-41                               | 26   |
| 30-39   | 8   | 14-23                            | 17   | 13-33    | 22   | 26-39 | 32   | 11-43                              | 29   |
| 40-49   | 7   | 13-23                            | 18   | 13-35    | 21   | 25-48 | 36   | 14-39                              | 26   |
| 50-59   | 3   | 12-22                            | 17   | 23-29    | 26   | 19-43 | 34   | 11-29                              | 24   |

N.B. Sex difference is ignored since almost all of the subjects are male.

TABLE II

|       | No. | Cholesterol mg% |      | Triglycerides |      | Total Lipids |      |
|-------|-----|-----------------|------|---------------|------|--------------|------|
|       |     | Range           | Mean | Range         | Mean | Range        | Mean |
| 20-29 | 17  | 112-226         | 170  | 43-202        | 100  | 546-857      | 669  |
| 30-39 | 8   | 128-278         | 218  | 106-214       | 147  | 545-1200     | 888  |
| 40-49 | 7   | 144-261         | 185  | 94-336        | 190  | 400-1120     | 795  |
| 50-59 | 3   | 147-216         | 176  | 111-193       | 161  | 660-1120     | 873  |

N.B. Sex difference is ignored since almost all of the subjects are male.

TABLE III.

## THE VARIATION OF SERUM PHOSPHOLIPID.

| Number | Sex   | Age   | Phospholipid as lipid P. mg% |               | choiesterol<br>mg% |
|--------|-------|-------|------------------------------|---------------|--------------------|
|        |       |       | range                        | Mean          |                    |
| 13     | male  | 20-24 | 5.8-13.5                     | $9.1 \pm 2.8$ | $209. \pm 48$      |
| 5      | Femal |       | 5.6-12.5                     |               |                    |

TABLE IV

## NORMAL VALUES OF CHOLESTEROL ACCORDING TO VIKUL'S (7) ESTIMATION

| Age-yr. | Male    |      | Female  |      |
|---------|---------|------|---------|------|
|         | Average | S.D. | Average | S.D. |
| 0-10    | 154.6   | 36.6 | 156.5   | 35.4 |
| 11-20   | 173.7   | 52.7 | 175.0   | 48.4 |
| 21-30   | 197.8   | 66.9 | 219.0   | 56.4 |
| 31-40   | 226.8   | 63.7 | 230.0   | 55.0 |
| 41-50   | 246.7   | 64.4 | 237.98  | 61.2 |
| 51-60   | 242.1   | 69.8 | 256.1   | 62.8 |
| 61-     | 223.7   | 63.1 | 244.4   | 64.7 |

TABLE V

## SUGGESTED "NORMAL LIMITS" OF TRIGLYCERIDES (10)

| Age-year | Triglyceride mg% |
|----------|------------------|
| 0-19     | 10-140           |
| 20-29    | 10-140           |
| 30-39    | 10-150           |
| 40-49    | 10-160           |
| 50-59    | 10-190           |

## REFERENCES

1. Fredrikson, D. S., Levy, R. I., Lees, R. S.: Fat transportation in lipoprotein in an integrated approach to mechanism and disorders. *New Eng. J. Med.*; 276: 37-41, 95-98, 1967.
2. Beckering, Jr., R. E.; Ellefson, R.D.: A rapid method for lipoprotein electrophoresis using cellulose acetate as support medium.: *Am. J. Clin. Pathol.*; 53: 84-88, 1970.
3. Abell, L. L.; Levy, B. B.; Brodie, B. B.; Kendall, F. E.: Simplified method for the estimation of total cholesterol in serum and demonstration of its specificity: *J. Biol. Chem.* 195: 357, 1952.
4. Van, E., Zilvermit, D. B.: *J. Lab. & Clin. Med* 50: 152, 1957.
5. Merkotest, N. Z.; Kirsch, K.Z.: *Exp. Med.* 135: 545, 1962.
6. Block, R.J.; Durrum, E.J., Zwerg, G.: *A manual of paper chromatography and paper electrophoresis*, New York, 1955 Academic Press, Inc., P. P. 348-375, 391-405
7. Viranuvatti, V.: Blood cholesterol levels and the question of normal values. *siriraj Hosp. Gaz.* 24: 1448, 1972.
8. Tomkins, G. M.; Chaikoff, I. L.: *J. Biol. Chem.*, 196, 569-573, 1952.
9. Bhattathiry, E.; Siperstein, M.: Feed Back control of cholesterol in man. *J. Clin. Invest.* 42: 1913, 1963.
10. Fredricson, D. S.; Levy, R. I.; Lees, R. S.: Fat transportation in lipoprotein in an integrated approach to mechanism and disorders. *New Eng. J. Med.*: 276: 151, 1967.
11. Chulalucksiriboon, P., Waiwatana, N. and Keoplung, M.: Serum phospholipid and cholesterol: The term paper in the degree of B.Sc. (Med. Tech.) 1973.
12. Sasasmit, P., Waiwatana, N. and Keoplung M.: Blood Cholesterol level in Northern Thailand. The term paper in the degree of B.Sc. (Med. Tech.) 1970.
13. Hemg, R. J.: *Clinical chemistry: Principles and technics* 1968, P. 843.