Serum Triglyceride Levels of Normal Subjects*

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Fasting serum triglyceride levels of 263 normal subjects at 2 to 79 years of age were studied in relation to age, sex and obesity. The overall mean of serum triglyceride levels of normal subjects was 70±1.3 mg%, with a range from 30 to 110 mg%. Serum triglycerides increased with age, reaching the maximum in the fifth decade, and then decreased gradually in later decades. The difference in the levels between every two successive age groups was statistically significant.

On the other hand, the mean triglyceride level of subjects at 90 to 113 years of age had no significant difference when compared with that of the normal seventies. Sex difference was not found except for significantly higher triglyceride levels in females of the fifties and the sixties. These higher levels in females might be associated with menopause. The serum triglycerides tended to increase with the degree of obesity, and a clear positive correlation was found between relative body weight and serum triglycerides.

Serum lipids consist of cholesterol, phospholipids, triglycerides, free fatty acids, and lipid soluble materials such as vitamin A, D, E, K, etc. The first two lipids have been measured routinely in clinical laboratories. Since the methods for the determination of serum triglycerides were established by Albrink, Carlson and Wadström, Michaels, Randrup, and Van Handel et al., there has been increasing interest about the possible role of triglycerides in the pathogenesis of arteriosclerosis. However, precise studies on the physiological changes in serum triglycerides of normal subjects have scarcely been made. The aim of the present study is to measure the serum triglyceride levels of normal subjects and to examine its relation to age, sex and obesity.

Materials and Methods

Two-hundred-sixty-three subjects (148 males and 115 females), hospitalized in Iwate Medical University Hospital, showed no signs of organic, especially cardio-vascular disease, or metabolic disorder. Their ages ranged from 2 to 79 years. Furthermore, 32 subjects (9 males and 23 females) of advanced ages, ranging from 90 to 113 years, were also studied, who were all dwelling in a seaside town, Kamaishi City, in Iwate Prefecture.

The degree of obesity was calculated by the following formula:

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* Clinical Studies of Serum Triglyceride Levels in Diabetes Mellitus, I.

The outlines of this study were presented at the 10th Annual Meeting of the Japan Diabetic Society, Nagoya, April, 1967.
Degree of obesity (%) = \frac{\text{Actual body weight (kg)} - \text{Standard body weight (kg)}}{\text{Standard body weight (kg)}} \times 100.

Standard body weight (kg) = (\text{Height in cm} - 100) \times 0.9.

The subjects were classified into three groups; obese subjects heavier than +10%, normal subjects of +10 to -10%, and lean subjects lighter than -10% of the standard weight. Relative body weight was expressed as the difference between actual and standard body weights in kg.

Blood samples were obtained in the early morning after overnight fasting of at least 12 hours, and cellular elements were removed promptly by centrifugation at 3,000 rpm for 10 minutes at room temperature.

Prior to the study of serum triglycerides of normal subjects, it seemed to be important to establish the method of serum triglyceride determination. Therefore, serum triglycerides were measured by the method of Van Handel and Zilversmit\textsuperscript{5} with the following modifications:

**Reagents**

1. Tripalmitin standard solution (50 mg%): 50 mg of reagent grade tripalmitin were dissolved in reagent grade chloroform and made to 100 ml in a glass-stoppered volumetric flask.
3. Parmzit (80–100 mesh): Activated by heating for 4 hours at 125\(^\circ\)C. Kept in a closed bottle.
4. Absolute ethyl alcohol: Redistilled reagent grade.
5. 2.5% KOH: Reagent grade.
6. 6% Acetic acid: Reagent grade.
7. 1 N Sulfuric acid: Reagent grade.
8. 0.02 M Periodate solution: 1.07 g of reagent grade sodium metaperiodate was dissolved in 250 ml of redistilled water.
9. 0.2 M Sodium arsenite solution: 1.8 g of reagent grade NaOH and 4.0 g of reagent grade arsenic trioxide were dissolved in 200 ml of redistilled water.
10. Chromotropic acid reagent: 0.2 g of reagent grade sodium salt of chromotropic acid was dissolved in redistilled water and made to 20 ml. Separately, 60 ml of reagent grade concentrated sulfuric acid were added to 30 ml of redistilled water, then cooled in ice. After cooling, the chromotropic acid solution was added to the diluted sulfuric acid, filtering through a fat-free paper and avoiding the direct sun. Prepared fresh at each determination.
11. Acetone-washed paper.

**Procedure**

The assay procedure of serum triglycerides, as shown in Fig. 1, comprises the following six steps: (1) extraction of serum lipids, (2) removal of phospholipids by shaking the extract with paraxit, (3) saponification, (4) periodate oxidation of the liberated triglyceride glycerol, (5) color development by the reaction of chromotropic acid with formaldehyde, and (6) determination of the optical density.

Place two 1 g portions of paraxit in glass-stoppered 50 ml centrifuge tubes and add 1 ml of chloroform to each tube. Place 0.5 ml of serum on paraxit in one tube and place 0.5 ml of tripalmitin standard solution in the other, and add 9 ml of chloroform to each. Stopper the tubes and shake vigorously for 15 minutes. Filter through acetone-washed paper and pipette two 1 ml portions of each filtrate into test tubes. Evaporate the solvent from the tubes. To each one of the two standards and the two unknowns add 1 ml of absolute ethyl alcohol and 1 drop of 2.5% KOH (saponified samples), and to the others add 1 ml of absolute ethyl alcohol and 1 drop of 6% acetic acid in order to avoid the saponification (unsaponified samples). Keep all four tubes at 60\(^\circ\)C for 30
Fig. 1. Determination of serum triglycerides.

 minutes, and to the saponified samples add two drops of 6% acetic acid to stop the saponification. Place all tubes in a boiling water bath for 30 minutes to remove alcohol. After cooling, add 2 ml of 1 N sulfuric acid to all tubes and mix. To 0.3 ml of solution in each tube add 0.1 ml of 0.02 M periodate solution and, after 10 minutes, add 0.1 ml of 0.2 M sodium arsenite to stop oxidation. After 5 minutes, add 2.5 ml of chromotropic acid reagent and mix. Heat for 30 minutes at 100°C and, after cooling, determine the optical density at 570 μμ.

Calculation of results

The concentration of serum triglycerides was estimated with the following formula:

\[
\text{Concentration of serum triglycerides} = \frac{S_2 - U_2}{S_1 - U_1} \times 50 \text{ mg\% tripalmitin.}
\]

\(S_1\): Optical density of saponified standard
\(U_1\): Optical density of unsaponified standard
\(S_2\): Optical density of saponified unknown
\(U_2\): Optical density of unsaponified unknown
Removal of phospholipids

Chemical analysis for phospholipid phosphorus showed that no measurable amount of phospholipids was observed in the filtrate after shaking the extract with paraffin. Paraffin, therefore, might be well available to separate phospholipids from triglycerides.

Standard curve

The relationship between the optical density and the amount of tripalmitin as standard was shown in Fig. 2. A good straight line was obtained from 0 to 150 mg% of tripalmitin.

Recovery

A recovery test was performed on 1 ml of serum by addition of tripalmitin. As shown in Table 1, the recovery of tripalmitin added to serum ranged from 96.0 to 100.0% with a mean value of 98.5%.

Reproducibility

The reproducibility of serum triglycerides was studied in the same serum samples (A–J). As shown in Table 2, the standard deviation ranged from ±2.51 to ±4.69, and the coefficient of variation ranged from ±2.09 to ±8.80% with a mean value of ±4.08%. These values were considered to be within the acceptable error of the analytical technique.

![Fig. 2. Standard curve.](image)

| TABLE 1. Recovery test for tripalmitin added to 1 ml of serum sample |
|--------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| mg/ml              | Experimental | A    | B    | C    | D    | E    | Mean | Recovery (%) |
| 0.25               | 0.26          | 0.24  | 0.23  | 0.22  | 0.23  | 0.24  | 96.0           |
| 0.50               | 0.50          | 0.52  | 0.47  | 0.52  | 0.45  | 0.49  | 98.0           |
| 1.00               | 0.98          | 1.00  | 1.00  | 0.98  | 1.03  | 0.99  | 99.0           |
| 1.50               | 1.53          | 1.50  | 1.46  | 1.50  | 1.51  | 1.50  | 100.0          |
| 2.00               | 2.03          | 2.01  | 2.01  | 1.95  | 1.94  | 1.99  | 99.5           |

Mean of recoveries—98.5%
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TABLE 2. Reproducibility of serum triglycerides

| Coefficient of variation (C. V.) = standard deviation \( \times 100 \)/mean
| Mean value of C. V. = 4.08% |

From these results, the author found that the modification of Van Handel and Zilversmit's method was simple to perform and yielded a good reproducibility as well as a good recovery for the determination of triglycerides. The method, therefore, was used in the present study.

RESULTS

Serum triglyceride levels of normal subjects against age and sex

The results were summarized in Table 3 and Fig. 3. The mean level of serum triglycerides in 263 normal subjects was 70±1.3 mg% (mean ± S.E.).

In males, the serum triglyceride levels rose with age, reaching the maximum in the age group of 40-49 years and thereafter the levels tended to decrease with age. There was found a significant difference in the levels between two successive age groups. The most marked increase occurred between age groups of 30-39 and 40-49 years.

Table 3. Serum triglyceride levels of normal subjects against age and sex

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>Serum triglycerides in mg% (mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>0-9</td>
<td>30±2.1(6)</td>
</tr>
<tr>
<td>10-19</td>
<td>41±1.8(11)</td>
</tr>
<tr>
<td>20-29</td>
<td>53±1.5(25)</td>
</tr>
<tr>
<td>30-39</td>
<td>65±1.7(23)</td>
</tr>
<tr>
<td>40-49</td>
<td>97±2.7(25)</td>
</tr>
<tr>
<td>50-59</td>
<td>83±1.8(22)</td>
</tr>
<tr>
<td>60-69</td>
<td>73±2.1(23)</td>
</tr>
<tr>
<td>70-79</td>
<td>64±2.3(13)</td>
</tr>
<tr>
<td>All ages</td>
<td>69±1.7(148)</td>
</tr>
</tbody>
</table>

Number of cases in parentheses.
In females, the mean triglyceride levels rose with the increase of age and reached the maximum in the forties. The most marked increase occurred between the thirties and forties. The levels, however, decreased slightly between the forties and fifties, and made a plateau. After the fifties, the levels showed a decrease with age. The mean levels between two successive age groups were significantly different.

Table 4 showed the relationship between menopause and serum triglyceride levels in 115 females. The mean level of serum triglycerides was significantly higher in postmenopausal subjects ($p<0.001$).

In comparison of the serum triglyceride levels between both sexes of the same age groups, there was found no significant sex difference with the exception of significantly higher levels in females of the fifties and the sixties ($p<0.02$, $p<0.05$, respectively).

**Table 4. Serum triglyceride levels of females as related to menopause**

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Number of cases</th>
<th>Serum triglycerides mg% mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-menopausal (5–52 years of age)</td>
<td>67</td>
<td>$61±2.2$</td>
</tr>
<tr>
<td>Post-menopausal (46–79 years of age)</td>
<td>48</td>
<td>$86±2.1$</td>
</tr>
<tr>
<td>$p$</td>
<td></td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

*Frequency distribution of serum triglyceride levels in normal subjects*

The results were shown in Fig. 4. Since a curve approximately corresponding to a normal distribution was obtained, the ‘normal’ range of serum triglycerides including 95% of all normal subjects was found to be $70±40$ mg% (mean±1.96 s.d.). The author, therefore, regarded the levels over 120 mg% as indicating hypertriglyceridemia.
Fig. 4. Frequency distribution of serum triglyceride levels in normal subjects.

Table 5. Relationship between obesity and serum triglyceride levels in normal subjects

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>Lean subjects</th>
<th>p</th>
<th>Normal subjects</th>
<th>p</th>
<th>Obese subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>21±1.5(3)</td>
<td>&lt;0.02</td>
<td>30±1.9(4)</td>
<td>&gt;0.5</td>
<td>32±3.2(3)</td>
</tr>
<tr>
<td>10-19</td>
<td>35±0.7(5)</td>
<td>&gt;0.05</td>
<td>39±1.4(8)</td>
<td>&lt;0.05</td>
<td>45±1.1(6)</td>
</tr>
<tr>
<td>20-29</td>
<td>46±3.4(4)</td>
<td>&lt;0.005</td>
<td>51±1.0(30)</td>
<td>&lt;0.001</td>
<td>61±1.8(7)</td>
</tr>
<tr>
<td>30-39</td>
<td>54±2.4(4)</td>
<td>&lt;0.05</td>
<td>63±1.4(27)</td>
<td>&lt;0.02</td>
<td>70±2.0(10)</td>
</tr>
<tr>
<td>40-49</td>
<td>67±3.3(7)</td>
<td>&gt;0.1</td>
<td>94±2.5(26)</td>
<td>&lt;0.05</td>
<td>104±4.2(11)</td>
</tr>
<tr>
<td>50-59</td>
<td>78±1.9(4)</td>
<td>&lt;0.001</td>
<td>82±1.9(23)</td>
<td>&lt;0.001</td>
<td>94±1.5(15)</td>
</tr>
<tr>
<td>60-69</td>
<td>77±2.9(7)</td>
<td>&lt;0.05</td>
<td>75±1.9(24)</td>
<td>&lt;0.01</td>
<td>84±2.3(12)</td>
</tr>
<tr>
<td>70-79</td>
<td>60±2.3(4)</td>
<td>&gt;0.2</td>
<td>64±1.8(14)</td>
<td>&lt;0.05</td>
<td>73±3.8(5)</td>
</tr>
<tr>
<td>All ages</td>
<td>60±3.4(38)</td>
<td>&lt;0.02</td>
<td>69±1.5(156)</td>
<td>&lt;0.001</td>
<td>79±2.6(69)</td>
</tr>
</tbody>
</table>

Number of cases in parentheses.

Fig. 5. Relationship between obesity and serum triglyceride levels in normal subjects.
Relationship between obesity and serum triglyceride levels in normal subjects

The results were summarized in Table 5 and Fig. 5. The mean levels of serum triglycerides tended to be significantly higher in obese subjects of all age groups with the exception of the age group of 0–9 years, and significantly lower in the lean subjects of all age groups with the exception of the age groups 10–19, 40–49, and 70–79 years, when compared with the mean levels of the subjects with normal body weight.

The relationship between relative body weight and serum triglyceride levels was shown in Fig. 6. A good positive correlation was found ($n=263, r=+0.85$, $p<0.01$). If $x$ represents relative body weight in kg and $y$ represents mg% triglycerides, the following equation for the regression line was obtained: $y=2.17x+61.33$. Therefore, an overweight of 10 kg corresponded to an increase of 22 mg% triglycerides.

Serum triglyceride levels in subjects of advanced age over 90 years

The results were summarized in Table 6. In males, the mean triglyceride level of all subjects (9 cases) was $66\pm11.3$ mg% (mean±s.e.). The subjects in the age group of 90–91 years had a slightly lower level than those in the age group of 70–79 years, but there was no significant difference in the mean ($p>0.9$). A slight increase in the mean level of the subjects in the age group of 92–113 years was not significant as compared with those in the age group of 90–91 years ($0.6<p<0.7$). The serum triglyceride level of the oldest person (male) in Japan, 113 years of age, was 82 mg%.

In females, the mean level of all subjects (23 cases) was $76\pm4.8$ mg% (mean ±s.e.). The subjects in the age group of 90–91 years had a slightly higher level than those in the age group of 70–79 years, but no significant difference was found in the mean ($0.2<p<0.3$). A slight decrease in the mean level of the subjects in
TABLE 6. Serum triglyceride levels in subjects of advanced age over 90 years

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>Serum triglycerides mg% (mean±s.e.)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>p</td>
<td>Female</td>
<td>Male + female</td>
</tr>
<tr>
<td>90–91 p</td>
<td>63±13.3(7)</td>
<td>&gt;0.2</td>
<td>78±6.8(15)</td>
<td>73±6.3(22)</td>
</tr>
<tr>
<td>92–113</td>
<td>75±7.1(2)</td>
<td>&gt;0.8</td>
<td>72±5.8(8)</td>
<td>72±4.3(10)</td>
</tr>
<tr>
<td>All ages</td>
<td>66±11.3(9)</td>
<td></td>
<td>76±4.8(23)</td>
<td>73±4.5(32)</td>
</tr>
</tbody>
</table>

Number of cases in parentheses.

the age group of 92–113 years was not significant as compared with those in the age group of 90–91 years (0.5 < p < 0.6).

In comparison of the serum triglyceride levels between both sexes in the same age groups there was found no significant difference according to sex. Furthermore, all aged subjects (32 cases, both sexes) from 90 to 113 years had higher triglyceride levels than all normal subjects (263 cases, both sexes) from 0 to 79 years, but there was no significant difference in the mean (0.4 < p < 0.5).

DISCUSSION

Triglycerides have been determined by direct or indirect method. However, the latter is currently not used for serum on account of troublesome calculation of subtracting fatty acids of cholesterol esters and phospholipids from total fatty acids¹ and also on account of large error of the analytical technique.

The methods for the direct determination of plasma or serum triglycerides, established by Carlson and Wadström,² Michaels,³ Randrup,⁴ and Van Handel et al.,⁵⁶ are based on the quantitative measurement of the glycerol moiety produced by saponification of triglycerides. The present author found that a modification of Van Handel and Zilversmit’s method⁵ was relatively simple in procedure and yielded precise results in the determination of triglycerides. The method was, therefore, considered to be suitable for a routine use in the clinical examination.

In the present study, serum triglyceride levels of 263 normal subjects in the fasting state were found to range from 30 to 110 mg% with a mean level of 70±1.3 mg% (mean±s.e.). Therefore, serum triglyceride levels over 120 mg% may be regarded as indicating hypertriglyceridemia. In the estimation of plasma or serum triglycerides in normal subjects, the mean level has been reported by some foreign investigators as follows; 79 mg% by Van Handel and Zilversmit,⁵ 76 mg% by Carlson and Wadström,² and 95 mg% by Michaels.³ In Japan, the mean level has been reported to be 85 mg% by Yoshida et al.⁷ 87.2 mg% by Katsuki et al.⁸ and 56 mg% by Ishitoya et al.⁹ All these values are based on the glycerol determination. The mean value in this study is best corresponding to the levels reported by Van Handel and Zilversmit, and Carlson and Wadström. The results of other investigators were more or less different from ours. However, no direct
comparison is possible since the serum triglyceride levels may be well affected by such factors as age, sex, obesity and dietary habits.

Many investigations as to the influence of age on serum triglycerides have been demonstrated. Hayes and Neill\textsuperscript{10} reported that serum triglyceride levels increased with age, reaching the maximum in the fifth decade, and then decreased slightly in subsequent decades. A similar result was obtained in this study. Furthermore, the mean triglyceride levels between every two successive age groups were found to show a significant difference.

No report of the serum triglycerides in the aged subjects has hitherto appeared. The author found that the mean triglyceride level of the subjects 90 to 113 years of age had no significant difference as compared with that of the seventies.

As regards the relation of sex to serum triglycerides, Naito \textit{et al.}\textsuperscript{11} noted that the mean triglyceride level was higher in males than in females but the difference was not significant. The present author found no significant difference between both sexes with the exception of significantly higher levels in females of the fifties and sixties. Furthermore, the author noted that a significant increase of serum triglyceride levels occurred in females after menopause. Similar findings have been obtained by Antonis and Bersohn\textsuperscript{12} and Hayes and Neill.\textsuperscript{10} Therefore, the sharp rise in the triglyceride levels of females may be associated with menopause. These results suggest a possible role of endocrine factors in physiological changes and differences in the serum triglyceride levels with age and sex.

A close relation between serum triglyceride levels and various measures of obesity has been demonstrated by Albrink \textit{et al.}\textsuperscript{13,14} In the present study, it was also found that the mean levels of serum triglycerides tended to be significantly higher in the obese than in the normal, and the lowest serum triglycerides were found in the lean. Furthermore, a good positive correlation between the relative body weight and serum triglycerides was found. A similar result has been reported by Sailer \textit{et al.}\textsuperscript{15} and Braunsteiner \textit{et al.}\textsuperscript{16}

The elevation of serum triglycerides in relation to obesity may be associated with higher levels of plasma free fatty acids in the obese,\textsuperscript{17} and with Steinberg's fatty acid cycle.\textsuperscript{18}

From the above-mentioned results, it seems that serum triglycerides have close relation to age, sex and obesity, but the conclusion awaits further investigations.

\textbf{Summary}

1) A modification of Van Handel and Zilversmit's method was found to be suitable for routine clinical analysis of serum triglycerides because of its simplicity and sufficient accuracy.

2) The serum triglyceride levels in the fasting state of 263 normal subjects from 2 to 79 years of age were measured and examined in relation to age, sex and obesity.

a) The normal range of serum triglyceride levels was found to be $70 \pm 40$ mg\%
Serum Triglycerides of Normal Subjects

Serum triglycerides of normal subjects (mean ± 1.96 S.D.) with the mean of 70 ± 1.3 mg% (mean ± S.E.). Therefore, the levels over 120 mg% might be regarded as indicating hypertriglyceridemia.

b) The serum triglyceride levels of both sexes rose with increasing age and reached the maximum in the forties. Thereafter, the levels decreased with age. The mean levels were significantly different between every two successive age groups.

c) No significant sex difference was found with the exception of significantly higher triglyceride levels of females in the fifties and sixties. The levels of females were significantly higher after than before menopause.

d) Serum triglycerides tended to be higher in obese subjects than in subjects with normal body weight, and the lowest triglyceride levels were found in lean subjects. There was found a good positive correlation between relative body weight and serum triglycerides.

3) The serum triglyceride levels of 32 subjects of advanced ages over 90 years were also studied.

a) The mean triglyceride level of the subjects at 90 to 113 years of age had no significant difference as compared with that of the normal seventies.

b) Sex difference was not found in these aged subjects.

Acknowledgments

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References


