Effect of Acute Fat Loads on Serum Lipids in Atherosclerosis

By Louis Horlick, M.D., F.R.C.P. (C)

Since the imposition of a lipid load might uncover latent defects in lipid absorption or transport, "normal" individuals, and those with atherosclerosis, were subjected to test meals of butter fat, and qualitative and quantitative aspects of serum lipids and lipoproteins were measured. In contrast to the metabolic defects in diabetes mellitus which can be uncovered by a glucose load, the metabolic faults in atherosclerosis are not readily influenced by single or repeated high fat meals.

In recent years, a large body of evidence relating lipid abnormalities to atherosclerosis in man has been accumulated. Whether one considers the cholesterol alone, the cholesterol phospholipid ratio, the serum lipoproteins determined chemically, electrophoretically, or by ultra centrifuge, a good case can be made for the relationship between abnormal lipid values and human atherosclerosis. While this holds true when groups of atherosclerotic individuals are compared with so-called "normals," because of the great overlap between the groups the same can not be said to be true where individuals are concerned. With the exception of relatively few individuals with gross lipid abnormalities, the majority of atherosclerotics manifest lipid levels which are not strikingly different from those of normals.

Most studies on the serum lipids in atherosclerosis have been conducted with subjects in the fasting state. It is possible that at such times the lipid absorption, transport and metabolic mechanisms would be under little or no stress, and borderline or latent deficiencies might be concealed. By analogy with the glucose tolerance test, it was thought that the imposition of a lipid load might result in the uncovering of hitherto latent defects. Accordingly, we have studied the effect of high fat meals on quantitative levels of serum cholesterol, phospholipid and neutral fat and on the distribution of lipoproteins. Certain qualitative aspects of lipoproteins were also studied, namely, their reactions with the alpha toxin (lecithinase) of Cl. welchii and with thymol before and after centrifugation and removal of the cream layer.

Some observers have reported that lipid tolerance tests may have clinical value in separating individuals with atherosclerosis from normals. Thus, Schwartz and co-workers, and Woldow and associates, have reported that following a meal high in butter fat, serum turbidity was greater in individuals with atherosclerosis and persisted for longer periods of time. Block found that the lipemic serum of patients with atherosclerosis was more resistant to clearing with heparin than was normal lipemic serum. Serum turbidity following a fatty meal is almost entirely a function of the amount of neutral fat circulating as chylomicrons. Chylomicrons have been shown to contain very little protein and cholesterol and to be essentially unrelated to changes in serum cholesterol, phospholipid, or to levels of lipoproteins below S: 400.

In a study by Pomeranze, Bienfield and Chessin, the serum cholesterol, phospholipids and total fatty acids were measured before, and at varying periods up to 24 hours after a high fat test meal in clinically normal males, clinically normal males with abnormal serum lipids, elderly males with clinical evidence of atherosclerosis, and obese males. In none of the groups was the serum cholesterol value altered in the acute experiment following the intake of 204 Gm. of fat. The phospholipids rose moderately in all groups with a coincident fall in the C/P ratio. The serum fatty acids rose moderately in the normal group and returned to fasting levels within 3 hours; in the hyperlipemic group, although the fasting fatty acids
were lower than in normals, they rose considerably higher and were maintained above the postabsorptive level longer than 12 hours. Prolonged elevation of fatty acids was demonstrated in the elderly group with atherosclerosis, and in those with extreme obesity. In the experiments reported below, we have obtained similar results.

Observations have also been made on ultracentrifugal lipoprotein levels before and after the ingestion of a fatty meal. Goldner and coworkers studied several times during a 2-week control period the total lipid content of the serum. There was a distinct quantitative difference between the higher density and the lower density aggregates. Sf 11-21 and 21-35 were affected to a much lesser degree than were Sf 35-100 and 100-400. These results are in accordance with those of Gofman and coworkers who noted only slight changes in Sf 12-20 macromolecules, but considerable changes in Sf 35-100 molecules following a high fat meal.

**METHODS**

**Determination Made**

**Serum Lipids.** Cholesterol, free and total, by Sperry and Webb's modification of the Schoenheimer-Sperry method. Lipid phosphorous by the Fiske-SubbaRow method, as modified by Hawk, Oser and Summerson. Total fatty acids by the hydroxamic acid procedure of Stern and Shapiro. Quantity Serum Lipoproteins. Separation of the alpha and beta-lipoproteins by electrophoresis, and determination of the relative amount of cholesterol in each fraction, was carried out as described by Boyd. Three tenths ml. of serum was spotted evenly on a strip of Whatman 3 mm. paper 6.0 cm. wide. After electrophoresis at 100 volts and 5 ma. for 12 to 15 hours, the papers were dried at room temperature and then heated in an oven at 100 C. for 20 min. A band 2 cm. in width was then cut off and stained with Oil Red O to reveal the location of the lipoprotein fractions. The remaining 4 cm. strip was marked in three main sections: alpha, beta-lipoprotein, and a blank. The strips were extracted with acetone alcohol at 66 C. for 1 hour. The extracts were then evaporated to dryness in a water bath. Cholesterol was determined by application of the direct Lieberman-Burchard color reaction without digitonin precipitation.

Ultracentrifugal separation of the lipoproteins was carried out for us by Dr. Robertson in the ultracentrifugal laboratory of the Department of Veterans Affairs at McGill University, Montreal. Serum samples were packed in ice in thermos flasks and shipped by air. They arrived at the laboratory on the following day and were processed at once.

**Stability of Serum Lipoproteins.** “Stability” was measured by the lecithinase technic previously described by us. The alpha toxin of Cl. welchii is a potent and specific lecithinase. When incubated with serum, it hydrolyzes lecithin to phosphorylcholine and a diglyceride. After a variable latent period, the serum suddenly becomes turbid and the turbidity increases rapidly to a peak between 48 and 72 hours of incubation. We have previously reported that the latent period is shorter in individuals with coronary disease. The final intensity, or degree, of turbidity also correlates with the total lipid content of the serum.

For the present study, the technic was modified as follows: 0.1 ml. of serum was added to 6.0 ml. of borate buffer containing 0.03 ml. of a 0.025 per cent solution of Cl. welchii alpha toxin. The tubes were incubated at 37 C. and the turbidity read in a Coleman (model 9) nephelometer at hourly intervals until a major change in nephelos had occurred. After 72 hours of incubation, suitable dilutions were made and nephelos were determined.

Thymol turbidity was determined by Shank and Hoagland's modification of the Hanger technic. Results are expressed in terms of optical density at 650 μ.

**CLINICAL MATERIAL**

**Normal Controls (27).** These were healthy young males (medical students, interns and staff) with no complaints referable to the cardiovascular system, and no significant previous illnesses. Physical examination was negative. Age range was 19 to 43 (mean 26.3).

**Atherosclerotic Group (22).** These were hospitalized because of clinical manifestations of atherosclerosis of the coronary, cerebral or peripheral arteries. Twenty had electrocardiographic evidence
EFFECT OF ACUTE FAT LOADS ON SERUM LIPIDS

TABLE 1.—Mean Serum Lipid Values Before and After a Standard High Fat Meal

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Control</th>
<th>S.D.</th>
<th>Arteriosclerosis</th>
<th>S.D.</th>
<th>p</th>
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<td></td>
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<td>Cholesterol (total)</td>
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<tr>
<td></td>
<td>12</td>
<td>180.67±31.90</td>
<td>181.80±33.35</td>
<td>181.94±32.61</td>
<td>184.01±28.61</td>
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<td></td>
<td></td>
<td>8.95±1.18</td>
<td>9.20±1.75</td>
<td>9.43±1.62</td>
<td>9.80±1.30</td>
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<tr>
<td></td>
<td></td>
<td>1.62±.17</td>
<td>1.59±.18</td>
<td>1.56±.16</td>
<td>1.51±.14</td>
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<tr>
<td></td>
<td>16</td>
<td>266.67±61.45</td>
<td>270.38±67.84</td>
<td>269.03±70.79</td>
<td>267.67±67.54</td>
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<td></td>
<td></td>
<td>10.66±2.20</td>
<td>11.29±1.91</td>
<td>11.38±1.92</td>
<td>11.54±1.92</td>
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<td></td>
<td></td>
<td>2.0±.23</td>
<td>1.89±.26</td>
<td>1.89±.24</td>
<td>1.81±.21</td>
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<td>Phospholipid</td>
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<tr>
<td></td>
<td></td>
<td>2.86±1.18</td>
<td>3.05±1.75</td>
<td>3.09±1.62</td>
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<td>1.62±.17</td>
<td>1.59±.18</td>
<td>1.56±.16</td>
<td>1.51±.14</td>
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<td></td>
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<td>1.89±.26</td>
<td>1.89±.24</td>
<td>1.89±.21</td>
<td>1.81±.21</td>
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<td>C/P Ratio</td>
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<tr>
<td></td>
<td></td>
<td>2.59±.89</td>
<td>18.29±8.99</td>
<td>8.99±4.86</td>
<td>8.99±4.86</td>
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<tr>
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<td>2.59±.89</td>
<td>18.29±8.99</td>
<td>8.99±4.86</td>
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*Significance of difference between the two groups (control and atherosclerotic).

Results

Quantitative Lипids. Tables 1 and 2 show that the mean level of serum cholesterol did not change appreciably in either group following the high fat meal, although there were considerable variations about the mean in individual cases. There was a slight increase in lipid phosphorous and a resultant fall in the C/P ratio. The serum fatty acids rose in both groups and appeared to decline a little faster in the control group than in the atherosclerotic group.

Three normal controls were subjected to several lipid tolerance tests while on a diet of their own choice (40 per cent of calories from fat) and again while eating an isocaloric diet yielding 54 per cent of the calories from butter fat for 3 weeks. There was no significant rise or fall in the serum cholesterol or lipid phosphorous following the test meals. There was a tendency for the fasting blood lipid levels to rise during the high fat period.

Lipoproteins. Electrophoretic studies shown in table 3 revealed that the percentage of cholesterol in the a-lipoprotein in the control group was 27.84 (±6.48 per cent), and in the atherosclerotic group 15.38 (±4.86 per cent). The ingestion of a high fat meal did not result in any significant changes in either group.

Ultracentrifuge determinations shown in table 4 revealed significantly higher levels of Sf 0-12 (p < 0.01) and 12-20 (p = 0.05) in the atherosclerotic group. Following the high fat meal there was no increase in the Sf 0-12, 12-20 and 20-100 classes, but there was a suggestive increase in Sf 100-400 class (not statistically significant). Both controls and atherosclerotics behaved similarly in this respect.

Lipoprotein Stability. Twenty-six controls and 17 atherosclerotics were studied. Time of onset of turbidity was definitely earlier in the atherosclerotic group (fig. 1A). Eighty-one and two-tenths per cent of the atherosclerotic group became turbid in less than 8 hours of...
TABLE 4—**Ultracentrifugal Separation**

<table>
<thead>
<tr>
<th>Class</th>
<th>No.</th>
<th>F</th>
<th>1 hr.</th>
<th>2 hr.</th>
<th>3 hr.</th>
<th>4 hr.</th>
<th>5 hr.</th>
<th>6 hr.</th>
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<tr>
<td>Class S, 0-12</td>
<td>13</td>
<td>279.3</td>
<td>316.4</td>
<td>269.6</td>
<td>314</td>
<td>277.3</td>
<td>308</td>
<td>270</td>
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<tr>
<td>Control</td>
<td></td>
<td>± 68.8</td>
<td>45.9</td>
<td>71.7</td>
<td>43.5</td>
<td>54.5</td>
<td>37.6</td>
<td>57.0</td>
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<td>Athrcl</td>
<td></td>
<td>±120.4</td>
<td>137.3</td>
<td>111.6</td>
<td>387.9</td>
<td>389.5</td>
<td>406.7</td>
<td>124.2</td>
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<td>Class S, 12-20</td>
<td>38.4</td>
<td>38.9</td>
<td>31.9</td>
<td>29.3</td>
<td>34.8</td>
<td>38.6</td>
<td>34.0</td>
<td>16.1</td>
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<tr>
<td>Control</td>
<td></td>
<td>± 16.5</td>
<td>14.6</td>
<td>15.6</td>
<td>12.5</td>
<td>12.5</td>
<td>12.1</td>
<td>16.1</td>
</tr>
<tr>
<td>Athrcl</td>
<td></td>
<td>± 29.0</td>
<td>35.1</td>
<td>31.4</td>
<td>27.3</td>
<td>30.4</td>
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<td>18.0</td>
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<td>Class S, 20-100</td>
<td>59.8</td>
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<td>± 93.0</td>
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<td>30.3</td>
<td>24.4</td>
<td>12.2</td>
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<tr>
<td>Athrcl</td>
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<td>± 95.6</td>
<td>100.5</td>
<td>99.8</td>
<td>80.9</td>
<td>80.9</td>
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<td>80.9</td>
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<tr>
<td>Class S, 100-400</td>
<td>20.6</td>
<td>27.3</td>
<td>26.7</td>
<td>36.0</td>
<td>31.1</td>
<td>40.5</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>± 12.5</td>
<td>14.5</td>
<td>23.7</td>
<td>29.9</td>
<td>27.7</td>
<td>28.6</td>
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<tr>
<td>Athrcl</td>
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<td>± 38.5</td>
<td>39.3</td>
<td>57.8</td>
<td>57.2</td>
<td>57.2</td>
<td>53.1</td>
<td>53.1</td>
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<tr>
<td>S.D.</td>
<td></td>
<td>± 33.5</td>
<td>35.8</td>
<td>57.8</td>
<td>57.2</td>
<td>57.2</td>
<td>53.1</td>
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</table>

**Fig. 1.** Lecithinase test results. A. Time of onset of turbidity in individual cases. Control normals, closed circles; patients with atherosclerosis, open circles. B. Final turbidity at 72 hrs. expressed in nephelos units for samples drawn in the fasting state, and at 2, 4 and 6 hrs. following the test meal. Controls, black bars; patients with atherosclerosis, white bars. C. Nephelometric readings obtained during incubation of serum and lecithinase. Sample F, fasting; samples 1-6, drawn 1 to 6 hours following high fat meal. Note: (a) relative lack of fluctuation of readings in fasting (F) specimen and abrupt nature of end point (rise in nephelos), and (b) intermediate rise or plateau effect seen in 3 and 4 hour specimens (3, 4). (c) The 1 and 6 hour specimens (1, 6) show only minimal plateau effect.

**Fig. 2.** Thymol turbidity test results demonstrating that the marked rise of TT in postprandial samples is mainly due to chylomicon material. Thymol turbidity expressed in units of optical density (ordinate) for samples drawn in the fasting state (F) and at 2, 4 and 6 hours thereafter (obesity). The values for the uncentrifuged and centrifuged (10,000 r.p.m. for 30 mins.) specimens are connected by diagonal lines, /// for normals, and \/// for arteriosclerotics.
incubation, as compared with only 26.9 per cent of the control group. Ingestion of a high fat meal resulted in higher initial turbidity readings, but did not influence the time of onset of turbidity. Preliminary centrifuging of the serum, with removal of the chylomicrons and loosely suspended fat, did not influence the time of onset of turbidity, although it reduced the final turbidity readings. Final turbidity readings were higher in the atherosclerotic group at all time intervals following the high fat meal, and represent the higher lipid content of the serum in the atherosclerotic group (fig. 1B).

Examination of a representative set of curves (fig. 1C) reveals an interesting phenomenon not hitherto described. We note first an initial rise in turbidity, followed by a plateau, and then a sharp rise in turbidity, representing the mass breakdown of the lipoproteins. The plateau effect also occurs, but to a lesser extent, after preliminary centrifuging. The plateau effect becomes much more marked following ingestion of a high fat meal, and probably represents the early breakdown of lipoproteins intermediate between chylomicrons and \( \beta \)-lipoproteins.

**Thymol Turbidity.** The thymol turbidity was greater in the fasting sera of the atherosclerotic group than in the controls, and in both groups there was a substantial rise in thymol turbidity following the high fat meal (fig. 2). Centrifuging the serum at 10,000 r.p.m for 30 min. in the cold, and removal of the cream layer, entirely eliminated the postprandial rise in thymol turbidity in the control group and reduced it very considerably in the atherosclerotic group.

**DISCUSSION**

It is now generally agreed that the serum lipids in man are more responsive to alterations in the neutral fat content of the diet than to alterations in the cholesterol content. Several observers have demonstrated that reduction of the fat content in diet leads to a fall in serum cholesterol, and that this can be promptly reversed by replacing the neutral fat.\(^{19-20}\) On the other hand, Keys has adequately demonstrated the insensitivity of the serum cholesterol to manipulation of the cholesterol in diet.\(^{19}\)

In this experiment, acute fat loading with neutral fat of animal origin failed to alter significantly the serum cholesterol level in normals and in atherosclerosics. In some individuals, rather marked variations occurred but, as a group, the changes were negligible. On the other hand, the phospholipids increased following the fat load, resulting in a concomitant fall in the molar C/P ratio. This is in agreement with the data reported by Man and Gildea\(^{21}\) and by Pomeranz and co-workers.\(^{18}\) Serum fatty acids rose markedly in both groups and showed a tendency to remain elevated longer in the atherosclerotic group. In essence, whereas the lipid levels were higher in the atherosclerotic group than in the normal group, the response of the lipid levels to a fat load was essentially similar in both groups. The lipoprotein estimations showed significant differences between the fasting values for the 2 groups, but failed to reveal any difference in response to a fat meal. A 3-week trial of a high fat diet by 3 normal volunteers likewise failed to result in any change in response to the lipid tolerance test.

Lipoprotein stability as measured by the lecithinase test was not altered by the ingestion of the high fat meal. We have been able to confirm our previous finding of a shorter time of onset of turbidity in individuals with atherosclerosis.\(^{17}\)

Thymol turbidity is known to be closely related to the serum lipids and particularly to the \( \beta \)-lipoproteins. Maclagan\(^{22}\) demonstrated that the precipitate resulting from the addition of the thymol reagent to serum was high in cholesterol and phospholipid. Subsequently Cohen and Thompson,\(^{23}\) Kunkel and Hoagland,\(^{24}\) and Recant and co-workers\(^{25}\) all showed that \( \beta \)-lipoprotein was responsible for the major amount of turbidity produced in the thymol reaction. The turbidity however was only closely related to the amount of lipoprotein when there were abnormal lipoproteins present, as in liver disease.\(^{24}\) Since thymol reacts physically with chylomicrons, producing intense turbidity, these would have to be removed first, as was done in this study. "Abnormal" lipo-
proteins produced by the fat load would then be expected to produce a rise in thymol turbidity. In our experiments no real increase in thymol turbidity occurred following a fat meal when the effect of chylomicrons was eliminated. From this we may deduce that there was no influx of "abnormal" lipoprotein into the bloodstream following the high fat meal.

All in all, we were unable to observe any real differences in response to a single fat meal between normals and atherosclerotics. Unlike the metabolic defects in diabetes mellitus, which can be uncovered by a glucose load, the metabolic faults in atherosclerosis are not readily influenced by single or repeated high fat meals.

**SUMMARY**

The response of serum lipids and lipoproteins to a high fat test meal (1.0–1.5 Gm. butter/Kg.) was studied in normals and in patients with clinical evidence of atherosclerosis.

There were minimal variations in serum cholesterol only. The lipid phosphorous showed an increase with concomitant fall in the C/P ratio. Serum fatty acids showed a marked rise with a tendency to remain elevated longer in the atherosclerotic group. Both groups behaved similarly in response to the high fat meal. A three-week trial on a high fat diet failed to result in alterations in lipid tolerance.

Serum lipoproteins measured chemically and by ultracentrifugation failed to show any response to the fat meal in both groups.

Lipoprotein stability as measured by lecithinase test was unaltered by the fat meal. Early development of turbidity was again observed in the atherosclerotic group.

There was no increase in thymol turbidity in either group when the chylomicrons resulting from the test meal were removed by centrifugation.

A high fat test meal failed to uncover or exaggerate lipid metabolic defects in individuals with atherosclerosis.

**SUMMARIO IN INTERLINGUA**

Le responsa del lipidos e lipoproteinas seral al ingestion experimental de alte quantitates de grassia (1,0 a 1,5 g de butyro per kg de peso corporee) esseva studiate in subjectos normal e in patientes con indicationes clinic de atherosclerosis.

Esseva constatate variationes de grado solmente minimal in le cholesterol del sero. Le phosphoro lipidic se monstrava augmentate. Le acidos grasse del sero accresceva marcatemente. In le gruppo atherosclerotic illos revelava le tendentia de mantenere se a nivellos elevate durante plus longe periodos de tempore. Le duo gruppos se comportava similemente in responsa al repasto ric in grassia. Tres septimanas de dieta ric in grassia non resultava in alteraciones del tolerancia lipidic.

Measurementes chimic e mesurationes per ultracentrifugation non revelava ulle responsa del lipoproteinas del sero al ingestion de grassia in o le un o le altere del duo gruppos.

Le stabilitate lipoproteinic, mesurate per le test a lecithinase, non esseva alterate per le repasto grasse. Un disveloppamento precoce de turbiditate esseva observate de novo in le gruppo atherosclerotic.

Nulle augmento de turbiditate a thymol esseva notate in o le un o le altere gruppo quando le chylomicroiies resultante del repasto experimental esseva eliminate per centrifugation.

Repastos ric in grassia non revelava e non exagerava defectos del metabolismo lipidic in individuos con atherosclerosis.

**REFERENCES**


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Effect of Acute Fat Loads on Serum Lipids in Atherosclerosis

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