TRIGLYCERIDES AND APOPROTEINS IN TOXEMIA OF PREGNANCY

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Synopsis In order to clarify the mechanism of hypertriglyceridemia caused by toxemia of pregnancy, apoprotein B, CII, CIII, and E in serum were determined by single radial immunodiffusion, and apoprotein CIII, CII, CIII1 and CIII2 in very low density lipoprotein (VLDL) fractionated by ultracentrifugation were quantified by analytical isoelectric focusing.

The increase in triglycerides in toxemia of pregnancy was estimated in VLDL and low density lipoprotein (LDL), mainly in VLDL. In contrast to the increase in triglycerides, apoprotein B and E, believed to interact with LDL receptors on cell membranes, remained unchanged from levels in normal pregnancy. The lack of correlation between triglycerides and these apoproteins may be related to the increase in triglyceride in VLDL.

In toxemia of pregnancy, the relative amounts of apoprotein CII, an activator of lipoprotein lipase, and CII, an inhibitor of lipoprotein lipase, in serum were similer to those in normal pregnancy, but those in VLDL decreased significantly. This finding suggests that the increase in triglyceride in VLDL was caused by inhibition of VLDL to LDL catalbsim.

These results suggest that one of the factors which causes hypertriglyceridemia in toxemia of pregnancy is impaired removal of triglyceride, in LDL fraction, but mainly in VLDL fraction.

Key words: Triglycerides • Toxemia of pregnancy • Apoproteins • Pregnancy • Isoelectric focusing

Introduction

The lipoproteins are complexes of lipids and apoproteins. The lipids are free and esterified cholesterol, phospholipids and triglycerides. And apoproteins (AI, AII, B, CII, CIII and E) play important roles in the structure and metabolism of lipoproteins. A number of studies have demonstrated that the plasma of pregnant women shows more than the usual levels of triglycerides, cholesterol and phospholipids in non-pregnant women[16][22][24][26]. The plasma cholesterol concentration in pregnancy rises by approximately 25~50% in the third trimester[8][12], while triglyceride content undergoes a steady increase and is two fold to three fold higher than non-pregnant level in the third trimester[10][19][26]. The onset of hypertension or toxemia of pregnancy leads to a further increase in triglycerides[13][17]. We previously reported the changes in serum cholesterol and apoproteins in toxemia of pregnancy[19]. In this study, I measured triglycerides in ultracentrifugated fractions and serum apoproteins (B, CII, CIII and E) by single radial immunodiffusion, and analyzed the apoprotein CII/CIII ratios obtained by isoelectric focusing in very low density lipoproteins in order to clarify the mechanism of hypertriglyceridemia induced by toxemia of pregnancy. These apoproteins and their ratios are important because they are believed to influence the removal of triglyceride-rich particles[8].

Materials and Methods

1. Subjects

Twenty-two non-pregnant women (20- to 28-years-old) who were healthy volunteers and sixteen normal pregnant women (24- to 32-years-old) who were between 32 and 39 weeks of gestation were studied as controls for lipid and apoprotein analysis. Twenty-nine patients with toxemia of pregnancy (21- to 38-years-old) who were in the 32 ~ 39 weeks of pregnancy comprised the study group. Each patient with toxemia of pregnancy was diagnosed according to the criteria specified by the Committee of Toxemia of Pregnancy of Japan Society Obstetrics and Gynecology. And the patients were divided into two groups according to the severity of toxemia of pregnancy (mild or severe type). All patients had proteinuria and/or hypertension and were judged healthy both prior to
and during early pregnancy. The mild and severe groups included 13 and 16 patients, respectively.

There were no statistically significant differences in maternal age or gestational weeks between the normal pregnancy and toxemia of pregnancy.

2. Lipid analysis
Following an overnight fast, blood samples were collected by venipuncture with most of the subjects in the supine position. Some samples were obtained in the sitting position, but additional studies showed that there were no significant differences in lipid analysis between samples obtained in the supine and sitting positions. The serum was separated by low speed centrifugation and kept at 4°C. Ultracentrifugal fractionation of lipoproteins was performed by the method of Hatch and Lees, and serum lipoproteins were separated into very low density (VLDL), low density (LDL), and high density lipoprotein (HDL). Chylomicrons were not fractionated in this study. Ultracentrifugation was performed using a Beckman L5-50B ultracentrifuge and rotor 50 at 4°C. Duplicate determinations of triglyceride in each fraction were performed by the enzyme method (Autoanalyzer Type 726, HITACHI). Recovery during the procedure was determined by comparing the sum of all triglyceride fractions with total triglyceride measured in the unfractiected serum. The mean recovery of triglyceride was 87%. After lipid analysis, each fraction was stored at −20°C.

3. Apoprotein analysis
Proteins in VLDL, LDL, and HDL were determined by the method of Lowry et al. using a bovine serum albumin standard. This determination is accurate only when the isolated fractions contain no contaminating plasma proteins. On the other hand, it has been reported that small amounts of albumin and IgG contaminate VLDL isolated by a single ultracentrifugation. However, the triglyceride and cholesterol levels of "washed" VLDL which were floated up through EDTA saline in a second ultracentrifugation were decreased by less than 10%, whereas levels of VLDL-protein decreased by 25±3%, and the decrements were proportional to the initial concentration [correlation of difference in protein (VLDL-protein—"washed" VLDL-protein) vs. VLDL-protein=0.93]. Therefore, in this study I used fractions isolated by a single ultracentrifugation in order to determine apoproteins and lipids. Apoprotein B, CII, CIII and E in serum were quantified by single radial immunodiffusion (DAIICHI PURE CHEMICALS CO., LTD.).

4. Delipidation and analytical isoelectric focusing (IEF) of VLDL
VLDL fraction dialyzed against 1mM EDTA was delipidated with ethanol-diethyl ether at −20°C and dried in a stream of nitrogen. The delipidation method is presented in Fig. 1. VLDL-apoproteins (50~150μg of protein) were solubilized with 100μl of 8M urea containing 0.01M Tris at pH 8 and were incubated at room temperature for 60 min. Slab gels for IEF were prepared by the following procedure. Acrylamide (24.25g), his-acrylamide (0.75g), Amberlite MB-6 (2.5g) and 250ml distilled water were mixed and stirred for one hour, and the Amberlite MB-6 in this stock solution was filtered.

![Diagram](image-url)
Ampholine (1.9ml) (Pharmalyte 4~6.5, Pharmacia Fine Chemicals) and glycerol (4ml) were added into 15ml of the stock solution, and the solution was made up to 30ml with distilled water and deaerated completely under vacuum. After 22.8mg/ml ammonium persulphate solution (200μl) was added to the solution, I cast the solution into slab gel forms 230×115×1.0mm. The anode electrode solution was 0.04M glutamic acid, and the cathode solution was 0.2M L-histidine. After prefocusing the solubilized VLDL-apoproteins were applied on the slab gel, and IEF was performed at constant power 20W with a final voltage of 2,000 volts. To determine the pH gradient, 5×10mm segments of unfixed and instained gels were transferred into a glass tube containing 1ml of distilled water. After 24 hours at 4℃, the pH of each segment was determined with the pH of the water. After the completion of focusing, the gel was fixed in 5% sulphanilic acid plus 10% trichloroacetic acid for 60 minutes and stained for 10 min in 0.2% Coomassie Brilliant Blue R-250 and destained in a solution of methanol: acetic acid: distilled water (3:1:6) until the background was clear, using several changes of the solution. For analysis of VLDL-apoprotein C, the gel was scanned at an optical density of 560nm. This procedure was performed almost exactly according to the manual of Pharmacia Fine Chemicals. The destaining gel and photometric scanning of VLDL-apoprotein C are shown in Photo. 1 and Photo. 2. The area under each apoprotein peak (CIIIα, CII, CIII, and CIIβ) was calculated. The results were expressed as the ratio of apoprotein CII/CIIIα+CIIIβ+CIIβ. Apparatus for IEF and photometric scanning included the IEF-2000 (Pharmacia Fine Chemicals), Electrophoresis Constant Power Supply ECPS 3000/150 (Pharmacia Fine Chemicals), and SHIMADZU CS-910 Photoscanner (SHIMADZU CORP.).

5. Statistical procedure
Statistical analysis was carried out by Student's t-test.

Results
1. Triglycerides
The triglyceride levels in serum and lipoprotein fractions are shown in Table 1. Serum triglycerides increased significantly, about 3.9 fold, in normal pregnant women compared with non-pregnant women. This triglyceride increase occurred in each fraction. In VLDL, LDL, and HDL the triglyceride increased, respectively, about 4.3, 5.0 and 3.5 fold. In toxemia of pregnancy, the triglyceride concentration in the serum rose by about 46% compared with normal pregnant women. Serum triglycerides in severe toxemia of pregnancy showed higher concentrations than those in mild toxemia of pregnancy, but the difference was not significant. In the ultracentrifuged fractions from serum of
patients with toxemia of pregnancy, triglyceride content increased statistically in the VLDL and LDL, but especially in VLDL. The LDL-triglyceride in toxemia of pregnancy increased about 1.3 fold, and VLDL-triglyceride about 1.8 fold compared with normal pregnancy. In contrast to the findings in the VLDL and LDL, no significant difference was found in triglycerides in HDL between normal pregnancy and toxemia of pregnancy. These analyses of lipoprotein fractions showed that hypertriglyceridemia of toxemia of pregnancy resulted mainly from triglyceride increases in VLDL.

Table 2 shows the distribution of triglyceride and protein in each fraction. The values are expressed as the percentage of the total amount of triglyceride and protein. The ratio of triglyceride to protein in each fraction in normal pregnancy increased compared to those in the non-pregnancy, however, no difference was observed in the proportions of triglyceride and protein between normal pregnancy and toxemia of pregnancy.

2. Apoproteins in serum

Apoproteins determined by single radial immunodiffusion are presented in Table 3. The apoprotein B concentration in normal pregnancy increased about 2.3 fold compared to non-pregnancy, however there was no differences between normal pregnancy and toxemia of pregnancy. Apoprotein CII and CIII concentration in normal pregnancy also rose about 2.2 and 2.4 fold compared to non-pregnancy. However these two apoproteins showed no significant difference between normal pregnancy and toxemia of pregnancy. This change was similar to that in apoprotein B. Only in severe toxemia of pregnancy apoprotein CII and CIII levels were statistically higher than those in normal pregnancy. There was no significant change in apoprotein E between non-pregnancy, normal pregnancy and toxemia of pregnancy.

3. Apoproteins in VLDL

Slab gel obtained by analytical IEF of VLDL-apoproteins, mainly apoprotein C and E, is shown in Photo. 2. The four principal bands at pH 4.4 ~ 5.0 were identified as apoprotein CIIIa, CII, CIII, and CII by the IEF pattern of apoprotein C and by each isoelectric point (pI). In addition to apoprotein C bands, the gel demonstrated a few bands

<table>
<thead>
<tr>
<th>Type of Pregnancy</th>
<th>Serum (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnancy</td>
<td>59.5 ± 16.2</td>
<td>22.4 ± 10.4</td>
<td>14.8 ± 4.0</td>
<td>9.0 ± 2.3</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>233.0 ± 63.0</td>
<td>95.0 ± 45.7</td>
<td>75.4 ± 20.3</td>
<td>32.5 ± 6.5</td>
</tr>
<tr>
<td>Toxemia of pregnancy</td>
<td>339.3 ± 114.4</td>
<td>170.7 ± 87.3**</td>
<td>96.1 ± 31.8**</td>
<td>32.9 ± 9.9</td>
</tr>
<tr>
<td>Mild toxemia of pregnancy</td>
<td>315.0 ± 115.9*</td>
<td>166.5 ± 91.2**</td>
<td>84.0 ± 24.8</td>
<td>32.8 ± 6.9</td>
</tr>
<tr>
<td>Severe toxemia of pregnancy</td>
<td>359.1 ± 112.9**</td>
<td>186.4 ± 86.1**</td>
<td>105.6 ± 34.3*</td>
<td>34.1 ± 9.5</td>
</tr>
</tbody>
</table>

Table 2. Proportions of triglyceride and protein in VLDL, LDL and HDL. Each value represents per cent of triglyceride and protein in each fraction. No significant difference is between normal pregnancy and toxemia of pregnancy.

<table>
<thead>
<tr>
<th>Type of Pregnancy</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG (%)</td>
<td>Protein (%)</td>
<td>TG (%)</td>
</tr>
<tr>
<td>Non-pregnancy</td>
<td>66</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>74</td>
<td>26</td>
<td>35</td>
</tr>
<tr>
<td>Toxemia of pregnancy</td>
<td>74</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>Mild toxemia of pregnancy</td>
<td>75</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Severe toxemia of pregnancy</td>
<td>74</td>
<td>26</td>
<td>34</td>
</tr>
</tbody>
</table>
TRIGLYCERIDES AND APOPROTEINS

Table 3. Apoprotein B, CII, CIII and E concentration in serum. Each value represents mean±S.D. Significant difference between normal pregnancy and toxemia of pregnancy : *, p<0.05

<table>
<thead>
<tr>
<th></th>
<th>Apoprotein B (mg/dl)</th>
<th>Apoprotein CII (mg/dl)</th>
<th>Apoprotein CIII (mg/dl)</th>
<th>Apoprotein E (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnancy</td>
<td>n=22</td>
<td>81±22</td>
<td>2.7±0.9</td>
<td>7.8±1.9</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>n=16</td>
<td>186±52</td>
<td>5.9±2.2</td>
<td>19.1±4.5</td>
</tr>
<tr>
<td>Toxemia of pregnancy</td>
<td>n=29</td>
<td>198±53</td>
<td>6.9±2.5</td>
<td>22.8±4.8</td>
</tr>
<tr>
<td>Mild toxemia of pregnancy</td>
<td>n=13</td>
<td>186±45</td>
<td>5.9±2.5</td>
<td>22.1±5.0</td>
</tr>
<tr>
<td>Severe toxemia of pregnancy</td>
<td>n=16</td>
<td>208±58</td>
<td>9.0±4.4*</td>
<td>25.3±4.9*</td>
</tr>
</tbody>
</table>

which had different pl than apoprotein C. Detail analysis was not performed, but probably these were apoprotein E in the pH range 5~6.5. The pl of apoprotein CIIIe, CII, CIII, and CIII were 4.99, 4.80, 4.70 and 4.50, respectively. Besides the apoprotein E bands, two minor bands were observed with pl values of 4.58 and 4.39.

The result obtained by scanning of apoprotein CIII isoforms is as follows. The distributions of apoprotein CIIIe, CII, and CIII were 8.0±2.0%, 40.3±2.1%, and 51.0±1.0% in non-pregnancy; 10.9±3.6%, 51.5±4.5%, and 37.8±3.4% in normal pregnancy; and 12.0±5.7%, 44.2±7.3%, and 39.4±5.6% in toxemia of pregnancy of the total apoprotein CIII densitometric area. The two areas of the minor bands were not measured. The proportion of apoprotein CIIIe in normal pregnancy was similar to that in non-pregnancy, but the proportion of apoprotein CII was larger and that of apoprotein CII was smaller in normal pregnancy compared with non-pregnancy. However, there was no significant difference in the distributions of apoprotein CIIIe, CII, and CIII in normal pregnancy and toxemia of pregnancy, either mild and severe group.

4. Ratios of apoprotein CII/CIII

Table 4 presents the ratios of apoprotein CII/CIII in serum and VLDL. Apoprotein CIII in VLDL was equal to the amount of apoprotein CII, CIII, and CIII obtained by photometric scanning after IEF and staining. The apoprotein CII/CIII ratios in serum showed no significant difference between non-pregnancy, normal pregnancy and toxemia of pregnancy. However, in VLDL, the apoprotein CII/CIII ratio in normal pregnancy was slightly higher than that in non-pregnancy, but the ratio in toxemia of pregnancy was statistically lower than that in normal pregnancy. There was no difference between mild and severe toxemia of pregnancy.

**Table 4.** Ratios of apoprotein CII/CIII in whole serum and VLDL. Each value represents mean±S.D. Significant difference between normal pregnancy and toxemia of pregnancy : *, p<0.05

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnancy n=22</th>
<th>Normal pregnancy n=16</th>
<th>Toxemia of pregnancy n=29</th>
<th>Mild toxemia of pregnancy n=13</th>
<th>Severe toxemia of pregnancy n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole serum (SRID)</td>
<td>0.32±0.09</td>
<td>0.32±0.09</td>
<td>0.30±0.08</td>
<td>0.26±0.09</td>
<td>0.32±0.06</td>
</tr>
<tr>
<td>VLDL (IEF)</td>
<td>0.21±0.06</td>
<td>0.26±0.06</td>
<td>0.20±0.07*</td>
<td>0.19±0.08*</td>
<td>0.20±0.06*</td>
</tr>
</tbody>
</table>

Discussion

It is well known that normal pregnancy in human leads to an elevation in serum triglycerides. Many reports have demonstrated that serum triglyceride levels reach a maximum in the third trimester, remaining elevated until delivery at two to three fold compared to non-pregnant women. Svanborg and Vikrot et al. demonstrated a positive correlation between serum triglyceride levels and the duration of gestation. My investigations confirmed that the triglyceride increase in normal pregnancy was associated with triglyceride eleva-
tions in the major lipoprotein classes: VLDL, LDL and HDL. In this study, I found that toxemia of pregnancy resulted in a greater increase in serum triglycerides than occurred in normal pregnancy. Many other studies have reported this finding. In toxemia of pregnancy, the serum triglyceride increase was affected mainly by increases in VLDL and LDL triglycerides, and the triglyceride increase was greater in VLDL than in LDL. These findings suggest that hypertriglyceridemia in toxemia of pregnancy is caused by an increase in secretion of VLDL into plasma and/or suppression of catabolism of VLDL to LDL relative to normal pregnancy.

The apoproteins which are contained in the lipoproteins of human serum play a variety of roles in lipid transport and metabolism. Apoprotein B, the dominant apoprotein in LDL and one of the major apoproteins in VLDL, shares with apoprotein E the ability to interact with high affinity receptors on cell membranes. However, it has remained poorly characterized to date, primarily because of its marked insolubility in aqueous solution. In this study, the concentration of apoprotein B in serum of patients with toxemia of pregnancy was similar to that in normal pregnancy. This data supports the hypothesis that toxemia of pregnancy does not cause hypercholesterolemia but hypertriglyceridemia, because there are constant relationships between serum cholesterol and apoprotein B, not only in normal subjects, but in patients with disorders of lipoprotein metabolism.

Apoprotein E concentration in serum, mainly contained in VLDL and HDL, showed almost same value in normal pregnancy and toxemia of pregnancy, in contrast to the triglyceride concentration in VLDL. This suggests that the proportion of apoprotein E to triglyceride in VLDL decreased in toxemia of pregnancy. If this decrease occurs in the VLDL-remnant degraded from VLDL by lipoprotein lipase, the affinity of the VLDL-remnant for the LDL receptor will be lower in toxemia of pregnancy than in normal pregnancy. In toxemia of pregnancy, the increase in the VLDL-remnant, included in the LDL fraction in this study, was reported previously by Oogoshi et al. I subsequently confirmed this increase in my further studies.

Apoprotein C, found mainly in VLDL and HDL, is implicated in the activation of tissue lipoprotein lipase and the removal of triglyceride-rich lipoprotein. Specifically, apoprotein CII is believed to be the cofactor necessary for the activation of extrahepatic lipoprotein lipases, and apoprotein CIII is believed to be a specific inhibitor of lipoprotein lipase. Therefore, the apoprotein CII to CIII ratio is extremely important in the metabolism of triglyceride-rich lipoprotein. From this point of view, it is interesting that in serum, both apoprotein CII and CIII increased significantly in severe toxemia of pregnancy compared to normal pregnancy, but the apoprotein CII/CIII ratio, which influences the catabolism of triglyceride-rich lipoproteins, was unchanged. Apoprotein C is contained not only in VLDL, but in HDL, so it is more important to investigate the apoprotein CII/CIII ratio in VLDL to clarify VLDL metabolism. Therefore, for further analysis of apoprotein C in VLDL, apoprotein CII and the polymorphic forms of apoprotein CIII were isolated by IEF. The pl values of the major four peptides, CIIIα, CIIIβ, CIIIγ, and CIIIδ, were similar to those reported by Albers and Scanu. The pl values of the two minor bands, observed with pl values of 4.58 and 4.39, were in good agreement with the data reported by Catapano et al. These two peptides have not been well categorized. The ratio of apoprotein CII/CIII in VLDL in normal pregnancy was higher than that of non-pregnancy, but in toxemia of pregnancy the ratio remained at the non-pregnant level. This high ratio of normal pregnancy does not agree with some published data. These data in my study suggest that tissue lipoprotein lipase was inhibited in toxemia of pregnancy compared to normal pregnancy.

In conclusion, physiologic hypertriglyceridemia during pregnancy may be important for the fetus and the mother's energy requirement and result from increased production of triglyceride-rich lipoprotein. The elevated ratio of apoprotein CII/CIII in VLDL activates lipoprotein lipase allowing greater utilization of VLDL during pregnancy. The increase of triglycerides in LDL and HDL is believed to be the result of accelerated VLDL catabolism. Hypertriglyceridemia in toxemia of pregnancy is different than physiologic hypertriglyceridemia of normal pregnancy. The decrease in the apoprotein CII/CIII ratio in VLDL indicates suppression of tissue lipoprotein lipase in the en-
dothelial lining of the capillary wall. Although in toxemia of pregnancy there is a significant increase of triglyceride in VLDL, the concentrations of apoprotein E and B are similar to those in normal pregnancy. This causes the inhibition of VLDL and VLDL-remnant catabolism. It was suggested that triglyceride catabolism in triglyceride-rich lipoproteins was affected by disorder of apoprotein CII, CIII, B, and E in toxemia of pregnancy. Recently, various pathogenic factors of toxemia of pregnancy have been proposed. However, the cause of the disturbance in apoprotein metabolism is remained to be not explained in this study.

In the future, further studies will be required to understand more in detail the metabolic disorders of serum triglycerides in toxemia of pregnancy with respect to tissue lipoprotein lipase activity and the LDL receptor.

Acknowledgments

The author wishes to thank Prof. Y. Yagami and Assoc. Prof. K. Mizuno for their helpful suggestions throughout the course of this work. I am also grateful to Dr. Nakaya, Dr. Oshima, Assist. Matsumura and all his colleagues for the apolipoprotein determinations.

References


概要 妊娠中毒症における高トリグリセライド血症の成因を解明する目的でリポ蛋白の構成および代謝に関与するアポ蛋白の面より研究を行った。妊娠中毒症症婦の血中トリグリセライドの増加は、VLDL分画中のトリグリセライドの増加が主因であったが、LDL分画でも認められた。妊娠中毒症妊娠の血清において、一元免疫拡散法により測定したアポ蛋白の分析で、正常妊娠に比しトリグリセライドが有意に増加しているにもかかわらず、LDLレセプターに親和性のあるアポ蛋白BおよびEの増加はみられなかった。このことは、VLDLおよびVLDL-remnantの異化障害を示すものと考えられた。また、VLDL分画のアポ蛋白Cの等電点電気泳動による分析において、リポ蛋白リバーゼの活性化因子とされるアポ蛋白CIIと抑制因子とされるCIIIの相対比（CII/CIIIa+CIII+CIIIb）が、正常妊娠では、非妊娠より増加することを知り得た。このことは正常妊娠で、リポ蛋白リバーゼ活性が亢進し、活発にVLDLの異化が行われているのに対して、妊娠中毒症妊娠では、その相対比の増加がみられず、VLDLからLDLへの異化が障害されていると考えられた。すなわち、妊娠中毒症の高トリグリセライド血症はTriglyceride-richリポ蛋白の異化障害によるものであり、それはアポ蛋白の代謝異常が一因であると考えられた。