Original Article

Changes in Serum High-Density Lipoprotein Subfraction Cholesterol in the First Four Weeks of Life

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ABSTRACT: We evaluated the developmental change in serum total cholesterol and cholesterol concentration in high-density lipoprotein subfractions (HDL2-C and HDL3-C) during the neonatal period. Eighteen term newborn infants without any major perinatal complications were venipunctured before the initial feeding, and at the second and fourth weeks of life. HDL2 and HDL3 were separated by the micromethod for combined precipitation-ultracentrifugation. To measure the paired samples from a single subject in one assay, we verified that the concentrations of HDL2-C and HDL3-C were unaffected by 4 weeks of storage when the sera were kept at -70°C. The level of (total—HDL) cholesterol increased markedly in the first week of life and was unaltered thereafter. On the other hand, the HDL2-C level was unaltered in the first week and then increased at 4 weeks of age. The HDL3-C was increased at 1 week, but returned toward the initial level at 4 weeks. The overall increase in HDL-C was less than that in the rest of the fractions. Thus, a LDL predominant pattern was established during the neonatal period. These changes in the HDL3-C are unique to newborns, because changes in response to nutritional intervention occur solely in the HDL2 fraction in later life. These results suggest the existence of non-nutritional factors related to the evolution of lipoproteins in the neonatal period.

Key words: lipoproteins, lipid metabolism, high-density lipoproteins, cholesterol, newborn infant.

High-density lipoprotein (HDL) of human plasma is a heterogeneous group of particles of differing size within the density range 1.063–1.21 g/ml. On the basis of floatation rate, two major subfractions have been identified, HDL2 and HDL3, corresponding to the densities of 1.063–1.125 g/ml and 1.125–1.21 g/ml, respectively. It is well established that a high serum concentration of HDL cholesterol (HDL-C), and more specifically HDL2-C, is associated with a lower risk of coronary heart disease. We have previously demonstrated that serum HDL2-C level in children is modified by endocrine and nutritional factors, while the HDL3-C level has been found to be relatively stable.

It is well known that cord serum is relatively rich in HDL, and that a transition of lipoprotein profile to the postnatal low-density lipoprotein (LDL) predominant pattern takes place in the first four weeks of life. Several previous studies observed a rapid increase in the cholesterol concentration in both LDL and very low-density lipoprotein (VLDL) in the neonatal period. However, little is known about the changes in the levels of HDL2-C and HDL3-C in this stage of life.

Separation of HDL subfractions by con-
Conventional ultracentrifugation requires a large sample volume and is time-consuming, and therefore not suitable for studies in children. Methods based on precipitation with polyanions have been developed\(^{10}\), and can be applied to the study of newborns. However, optimal conditions for the specific precipitation of one of the two major HDL subfractions has not yet been determined\(^{11}\). The micromethod for combined precipitation-ultracentrifugation described by Eyre et al.\(^{12}\) separates operationally defined HDL\(_2\) and HDL\(_3\) in a small volume (<0.5 ml) of serum sample, and in a relatively short time (3.5 h). Lack of data on the optimal conditions for the storage of lipoprotein sample limited the application of this method to a time course study.

In the present study, we verified that serum levels of HDL\(_2\)-C and HDL\(_3\)-C were unaffected by 4 weeks of storage when the samples were kept frozen as sera. The changes in the serum lipoprotein cholesterol profile in the first 4 weeks of life were evaluated.

**Materials and Methods**

**Newborn Infants**

Eighteen term newborn infants (9 males and 9 females) were studied. The birth weight of all infants were appropriate for their respective gestational ages as shown in Table 1. All infants were born after uneventful delivery, their AGPAR scores being >7 points. They had no feeding problems. They did not suffer from respiratory distress, as requiring O\(_2\) administration, and neither such metabolic disorders as hypoglycemia nor hypocalcemia. Venous blood was obtained before enteral feeding was started, and at the second and fourth week of life. The sera were separated within 4 hours after blood sampling then stored as indicated by the result of the study on sample storage (vide infra).

**Biochemical Analyses**

Cholesterol was assayed manually by an enzymatic method, with the Cholestezyme C assay reagent kit (Eiken Chemical Co., Tokyo). Separation of HDL\(_2\) and HDL\(_3\) was performed according to the method of Eyre et al.\(^{12}\) with modifications described previously\(^{4}\). Briefly, apo B-rich lipoproteins were precipitated with heparin-MnCl\(_2\). The supernatant (175 ul) was transferred to a cellulose propionate Airfuge tube (Beckman Instruments, Palo Alto, CA) containing 31.5 mg of solid potassium bromide, and mixed thoroughly to give a final density of 1.125 g/ml. Ultracentrifugation was performed at 160,000xg for 3.5 hours in a Beckman Airfuge with an A-100/18 rotor. The infranatant after ultracentrifugation was aspirated and the cholesterol concentration in the whole supernatant was measured. The HDL\(_3\)-C was obtained by subtraction of HDL\(_2\)-C from HDL-C. To avoid the introduction of between-assay variations in the comparison of values in the time course study, the paired samples from each newborn subject were evaluated in a duplicate assay.

**The Study on Sample Storage**

Sera were obtained from 10 healthy women in the child-bearing age because of their reputed abundance of both HDL\(_2\) and HDL\(_3\). Each sample was divided into 5 aliquots. One was precipitated with heparin-MnCl\(_2\) and ultracentrifuged within 4 hours after blood sampling, and then stored frozen until cholesterol was assayed; this served as control. Two aliquots were stored frozen at -70°C for either 1 or 4 weeks, and then HDL subfractions were separated. The samples were stored frozen again until cholesterol assay (S\(_1\) and S\(_4\)). The remaining two were precipitated with heparin-MnCl\(_2\), stored frozen for 1 or 4 weeks, and then ultracentrifuged (H\(_1\) and
Lipoprotein Cholesterol in Newborns

Table 1. Clinical Cases (Newborns)

<table>
<thead>
<tr>
<th>Family Name (Initial)</th>
<th>Gestational Age (week)</th>
<th>Birth Weight (g)</th>
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<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>38</td>
<td>2868</td>
</tr>
<tr>
<td>Sg</td>
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<td>I</td>
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<tr>
<td>Nm</td>
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<td>Kw</td>
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</tr>
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<tr>
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</tr>
<tr>
<td>Y</td>
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</tr>
<tr>
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</tr>
<tr>
<td>K</td>
<td>40</td>
<td>3310</td>
</tr>
</tbody>
</table>

Table 2. Effect of freezing on the level of HDL-C and HDL2-C

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>S1b</th>
<th>S2b</th>
<th>H1b</th>
<th>H2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C (mg/dl)</td>
<td>56±5</td>
<td>55±4</td>
<td>57±5</td>
<td>61±6</td>
<td>60±5</td>
</tr>
<tr>
<td>HDL2-C (mg/dl)</td>
<td>81±3</td>
<td>30±3</td>
<td>30±3</td>
<td>30±3</td>
<td>30±3</td>
</tr>
</tbody>
</table>

Data are the means ± SEM (n=10). Control sera were precipitated with heparin-MnCl2 and ultracentrifuged within 4 hours after sampling, and then stored frozen until cholesterol was assayed.

a. p<0.001 (vs. controls).

b. S1: sera were stored frozen at -70°C for a period (weeks) indicated by the subscript, and then precipitated with heparin-MnCl2 and ultracentrifuged. The samples were frozen and stored again until cholesterol assay.

H1: sera were precipitated with heparin-MnCl2, stored at -70°C for the indicated period, and then ultracentrifuged. The samples were stored frozen again.

Data are expressed as the means ± SEM. Statistical methods used were the paired t-test and paired Wilcoxon test.

Results

Effect of Sample Storage on HDL-C and HDL2-C

Table 2 lists the level of HDL-C and HDL2-C in the 10 women, whose serum samples were treated and stored in different ways. The HDL-C levels of S1 and S2 were similar to the control, while those of H1 and H2 were significantly higher than the control. On the other hand, HDL2-C levels in both S and H groups were similar to the control. Correlation coefficients between the HDL-C levels in the control and those of S1, S2, H1 and H2 were r=0.994, 0.957, 0.967 and 0.979 (p<0.005 for all), respectively. The r values for HDL2-C between the control and S1, S2, H1 and H2 were 0.996, 0.998, 0.983 and 0.995 (p<0.005), respectively. These results indicated that the cholesterol concentration in HDL subfractions was unchanged for as long as 4 weeks when the samples were stored frozen as sera, but not when heparin-MnCl2 step was followed freezing.

Transition of Lipoprotein Cholesterol Profile in the Neonatal Period

The levels of total cholesterol (T-C) and (T-HDL)-C in newborn infants at three different times are summarized in Fig. 1. The T-C increased markedly in the first
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Fig. 1. Serum total cholesterol (left) and (total minus high-density lipoprotein) cholesterol (right) in newborn infants. Data are means ± SEM (n=18). Abbreviations: T-C, total cholesterol; (T-HDL)-C, (total minus high-density lipoprotein) cholesterol (= low-density lipoprotein + very low-density lipoprotein cholesterol).

Fig. 2. Serum high-density lipoprotein cholesterol in newborn infants. Abbreviation: HDL( subfraction) C; high-density lipoprotein (subfraction) cholesterol. Total HDL-C (left), HDLα-C (middle) and HDLβ-C (right).

week. This was followed by a gradual increase thereafter, but the difference between the levels at week one and 4 was not significant. Similarly, the (T-HDL)-C (i.e. VLDL-C+LDL-C) was increased markedly at 1 week of age and unaltered thereafter. On the other hand, the HDLα-C level was unaltered in the first week but was increased at 4 weeks of age (Fig. 2). The HDLα-C was increased at 1 week, but returned to the initial level at 4 weeks. As the sum of HDLα-C and HDLβ-C, the HDL-C was increased at 1 week and continued to increase gradually thereafter. The overall increase in VLDL-C+LDL-C in the newborn period was 1.5-fold, while that in HDL-C was 1.4-fold.

Discussion

Lipoprotein concentrations are most accurately measured in fresh samples, because storage reportedly damages the conformation of these complex molecules^{13)}. Matthew et al.^{14} reported that storage of serum for 1 week at either 4°C or -20°C resulted in a significant increase in HDLα-C concentration measured enzymatically after dextran sulfate-MgCl2 precipitation procedure. In the present study, the concentrations of both HDLα-C and HDLβ-C were unaltered after even 4 weeks of storage when the sera were kept at -70°C, while the HDLα-C was increased when the heparin-MnCl2 step preceded the freezing. These results indicate that the temperature at which the sample is stored is critically important, and that HDL is unstable after exposure to polyanions and/or metal ions.

During fetal life, nutrients are supplied via the placenta, with the principal caloric source being carbohydrate^{15}. Shortly after birth, the major route for nutrient intake is switched from placental supply to enteral feeding, with the caloric source containing substantial amounts of lipid (i.e. breast milk or formula). This rapid change in nutritional milieu induces the transition of serum lipoprotein pattern as a part of the metabolic adaptation to extrauterine life^{8}).

The onset of enteral feeding is reported to trigger a rapid increase in the syntheses of apolipoprotein B and C_III, and also the transfer of apo E from HDL to VLDL^{9}).
This results in the establishment of the flow of TG-rich lipoproteins as a caloric substrate into the blood from the intestine. The activation of apo B synthesis, in turn, leads to the increase in LDL-C. As the result of an abrupt metabolic alteration favoring the utilization of a lipid substrate as the caloric source, the catabolism of TG-rich lipoproteins by lipoprotein lipase is increased. The activation of this metabolic pathway facilitates the transfer of apo A1 and AII from the remnant of TG-rich lipoproteins to HDL and contributes to the increase in HDL-C.

Previous studies have observed a rapid increase in cholesterol concentration in LDL and VLDL or VLDL+LDL fractions in the first week of life, as was also found in the present study. They also consistently found an increase in HDL-C during the neonatal period. However, the increase in HDL-C became evident only in the late neonatal period (i.e. from 7 to 30 postnatal days) in some previous studies. In the present study, both (LDL+VLDL)-C and HDL-C significantly increased in the first week of life, but the increment of the former (from 55 to 76 mg/dl) was twice as large as that of the latter (from 36 to 47 mg/dl). Thus, a LDL predominant pattern was established during this period.

The transitional profile of HDL subfractions in the first 4 weeks of life has been evaluated in only two previous studies. These studies used different methodologies for separating HDL2 and HDL3 than we did; gradient gel electrophoresis in one study and isopycnic ultracentrifugation in the other. The former group reported a transient increase in HDL3-C at 1 week of age followed by an increase in HDL2-C, as was found in the present study. On the other hand, both HDL2-C and HDL3-C were increased but the HDL2-C/HDL3-C ratio was unchanged in the latter study, however the authors recognized that the resolution of HDL subfractions was less than complete by their method. The present results imply that cholesterol is accumulated in the HDL3 initially and then the HDL2 is converted to HDL2 afterwards. Such change in HDL3-C appears to be unique to the neonatal period, because the alteration of cholesterol concentration in HDL in response to nutritional intervention occurs invariably in the HDL2 fraction in later life. These results suggest the existence of some non-nutritional factors related to the evolution of lipoproteins in the neonatal period, as postulated by others.

REFERENCES


