Effects of Dietary Protein on Composition and Metabolism of Plasma Lipoproteins in Rabbits

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Summary
Changes in the concentration and composition of serum VLDL, LDL, and HDL were studied in rabbits transferred from Chow diets to cholesterol-free, semipurified diets containing casein or isolated soy protein. During the first week on the casein diet, there was a marked increase in LDL-cholesterol and these higher levels were maintained during the subsequent 3 weeks of the study. Similar but less marked changes were obtained with the soy protein diet. When the percent composition of the particles was determined, both VLDL and LDL had a higher proportion of cholesterol. Turnover studies indicated that the FCRs for radiolabelled VLDL and LDL were reduced in casein-fed animals compared to those fed soy protein. The elevated LDL levels in casein-fed rabbits were primarily due to a reduction in receptor-mediated catabolism of LDL-apo B. Receptor-independent removal in the two groups was similar. These studies show that the hypercholesterolemia in casein-fed rabbits, compared to those fed soy protein, is associated with cholesterol enrichment of LDL and impaired receptor-dependent removal of LDL-apo B.

Key Words casein, soy protein, hypercholesterolemia, lipoprotein composition, lipoprotein metabolism, kinetics, rabbits

Introduction
Rabbits fed Chow diets have low levels of plasma cholesterol, whereas rabbits fed cholesterol-free, semipurified diet with casein as dietary protein have elevated levels of plasma cholesterol, mainly due to increases in LDL (1). The rise in plasma cholesterol occurs over several weeks and atherosclerosis develops when the diets are fed for longer periods of time. These can both be prevented by replacing the casein in the semipurified diet by soy protein (2).

It is still not clear why dietary casein leads to an increase in plasma cholesterol in rabbits. It has been shown that the increased plasma cholesterol is associated with a decreased rate of cholesterol turnover, decreased fecal bile acid and neutral steroid excretion, and increased cholesterol absorption in comparison to rabbits fed soy protein (3). In addition, the disappearance of radiolabelled VLDL and intermediate density lipoprotein from plasma was more rapid in rabbits fed soy protein than in those fed casein (4).

In order to elucidate the mechanism underlying the elevation in plasma cholesterol in casein-fed rabbits, we investigated the effects of dietary casein and soy protein on the composition and metabolism of lipoproteins.

Experimental
Studies were carried out in which young, male, New Zealand White rabbits weighing about 1.5 kg were first maintained on Laboratory Chow (Ralston Purina Co., St. Louis, MO) and then changed...
gradually over one week to the semipurified diets. Blood samples were taken from the marginal ear vein of fasted and unanesthetized animals.

Effects of diet on lipoprotein composition

At weekly intervals over a period of 4 weeks, rabbits were bled and serum lipoproteins were separated by discontinuous ultracentrifugation. VLDL (d<1.006 g/ml), LDL (1.006<d<1.063), and HDL (1.063<d<1.21) were obtained and analyzed for cholesterol, phospholipid and triacylglycerol using kits obtained from Boehringer-Mannheim. Protein was also determined using a modified Lowry procedure.

Effect of diet on lipoprotein metabolism

VLDL was isolated from the plasma of casein- and soy protein-fed rabbits by sequential 2-spin ultracentrifugation. It was labelled with \(^{125}\)I and injected into casein- and soy protein-fed animals. Blood samples were taken at timed intervals and lipoproteins isolated for the determination of apo B specific activities. The Procedures have been described in more detail elsewhere.

In a second study, LDL (1.109<d<1.063) was isolated and divided into 2 aliquots. One aliquot was labelled with \(^{125}\)I and the other with \(^{131}\)I. The \(^{125}\)I labelled tracer was methylated with NaBH\(_4\) and formaldehyde as described previously. This procedure modifies 8–20 lysine residues per apo B molecule, which abolishes high affinity LDL binding and delays the clearance of LDL from plasma. Casein- and soy protein-fed rabbits were injected with both tracers and blood samples were collected at timed intervals. Plasma apo B radioactivity was determined, disappearance curves were constructed and the FCR determined for both tracers. The FCR for \(^{131}\)I-LDL represents the sum of both receptor-dependent and -independent catabolism, whereas the FCR for the \(^{125}\)I-LDL represents receptor-independent catabolism. The difference is a measure of receptor-dependent catabolism.

Results

The time course of changes in lipoprotein cholesterol of rabbits fed the casein and soy protein diets is shown in Fig. 1. The increase in LDL cholesterol which occurred during the first week on diet, was significantly higher for casein than for soy protein-fed rabbits. The increase in LDL cholesterol was paralleled by increases in protein and phospholipid. Throughout the experiment, VLDL and HDL cholesterol remained relatively constant (Fig. 1).

When the changes in lipoprotein components are expressed as percent composition, the major change in VLDL and LDL is a relative increase in cholesterol and a corresponding decrease in triacylglycerol. Similar, but smaller changes in the

![Fig. 1](image-url)
Table 1. Percent composition of VLDL, LDL, and HDL components in rabbits fed casein or soy protein for 4 weeks.a

<table>
<thead>
<tr>
<th>Component</th>
<th>Casein</th>
<th>Soy protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>13.3 ± 0.8</td>
<td>16.5 ± 1.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>42.5 ± 2.2</td>
<td>26.1 ± 2.6</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>18.2 ± 2.5</td>
<td>32.8 ± 4.5</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>26.3 ± 0.6</td>
<td>24.5 ± 2.8</td>
</tr>
<tr>
<td>LDL</td>
<td>21.8 ± 1.0</td>
<td>24.5 ± 0.8</td>
</tr>
<tr>
<td>HDL</td>
<td>43.7 ± 1.7</td>
<td>46.8 ± 1.7</td>
</tr>
</tbody>
</table>

a Results are presented as mean ± SEM, n=6 per group. b Soy protein values with an asterisk are significantly different from their casein counterparts (p<0.05) using Student’s t-test.

Table 2. FCR (pools/day) of VLDL and LDL-apo B in rabbits fed casein or soy protein.a

<table>
<thead>
<tr>
<th>Component</th>
<th>Casein</th>
<th>Soy protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL (n=3)</td>
<td>3.60 ± 0.72</td>
<td>5.52 ± 0.24</td>
</tr>
<tr>
<td>LDL (n=4)</td>
<td>Receptor-dependent</td>
<td>0.21 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Receptor-independent</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.64 ± 0.11</td>
</tr>
</tbody>
</table>

a Data are expressed as mean ± SEM. Statistical analysis carried out using Student’s t-test. b Each observation is obtained from the pooled plasma of 3 animals. c Each observation is obtained from individual animals.

relative proportions of cholesterol and triacylglycerols were observed in rabbits fed soy protein (Table 1). HDL components showed little change.

The FCR for VLDL- and LDL-apo B was lower in casein-fed rabbits compared to those fed soy protein (Table 2). The removal of the methylated LDL tracer was slower than that of the unmodified tracer (Fig. 2). However, in the case of rabbits fed casein, the difference in the rate of removal of the two tracers was less marked than for animals fed...
soy protein. This resulted in a lower FCR for LDL-apo B via the receptor-dependent pathway in animals fed casein compared to those fed soy protein. Removal via the receptor-independent pathway was not significantly different between the two groups (Table 2).

Discussion

These results provide further evidence that dietary proteins influence serum total and lipoprotein cholesterol. Changes in the composition and metabolism of lipoproteins, particularly LDL, become evident after a short period on diet.

Despite changes in the composition of VLDL, there were no significant changes in FCR. However, LDL metabolism was drastically different between the two groups. In casein-fed animals the FCR of LDL-apo B was significantly lower than in animals fed soy protein. In conjunction with an increased rate of independent production of LDL-apo B (9), the decreased FCR for LDL-apo B resulted in a larger plasma pool of LDL compared to animals fed soy protein. Using a cross-over design, kinetic studies of LDL-apo B showed that despite the differences in its composition the FCR of LDL-apo B remained low in animals fed casein. This suggests that the effect is related to receptor saturation rather than changes in particle composition (13).

Results of the study presented here indicate that the decrease in FCR of LDL-apo B in animals fed casein is due to a decrease in receptor-dependent catabolism, compared to rabbits fed soy protein. These in vivo studies are supported by the in vitro findings of Chao et al. (14) who showed that LDL binding to liver membranes of casein-fed rabbits was greatly diminished compared to those fed Chow (15). In addition, our results show that it is the protein component of the cholesterol-free semipurified casein diet which causes impaired receptor-dependent catabolism in comparison to the semipurified soy protein diet when fed to rabbits.

The mechanism whereby dietary casein and soy protein exert their effects on cholesterol metabolism remains unknown. Previous studies have shown that the hypercholesterolemia of casein increases with the level of casein in the diet (16, 17). This suggests that casein contains a hypercholesterolemic factor such as a peptide component (18) or amino acid which may be responsible for the regulation of lipoprotein metabolism.

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REFERENCES


