Lipoprotein Metabolism in Nonresponders to Increased Dietary Cholesterol

Henry Ginsberg, Ngoc-Anh Le, Carol Mays, Joyce Gibson, and W. Virgil Brown

We have studied the effect of increased dietary cholesterol on production and degradation of plasma very low density lipoproteins and low density lipoproteins. We studied five unselected normal volunteers who were on weight-maintaining diets consisting of 45% carbohydrate, 40% fat, and 15% protein with a ratio of polyunsaturated to saturated fat of 0.4. Cholesterol content was 150 mg/1000 kcal in Period A and 500 mg/1000 kcal in Period B. Subjects were fed each diet for 4 to 5 weeks. The turnover of apoproteins B in very low density and low density lipoproteins and of triglyceride in very low density lipoproteins were measured during the last 2 weeks of each study period using $^{3}H$-labeled very low density lipoprotein and $^{13}C$-labeled low density lipoproteins and 2-$^{3}H$-glycerol, respectively. There was no significant change in plasma total cholesterol or triglycerides or in low density lipoprotein cholesterol levels during the high cholesterol versus the low cholesterol diet. Similarly, high cholesterol intake had no effect on the rate of production or clearance of apo B in very low density and low density lipoproteins or of triglyceride in very low density lipoproteins. These data indicate that the flux of very low density and low density lipoproteins does not change in nonresponders to high cholesterol diets. (Arteriosclerosis 1:463-470, 1981)

The relationship of dietary composition to arteriosclerosis has been the focus of intensive study for many years. Strong correlations have been found between arteriosclerotic cardiovascular disease (ASCVD) and consumption of both saturated fat and total fat. These dietary constituents are also associated with increased serum cholesterol and low density lipoprotein (LDL) cholesterol concentrations, two strong predictors for ASCVD. It is probably the effect of dietary fats on plasma total and LDL cholesterol levels that increases the prevalence of ASCVD in a population. The effect of dietary cholesterol on either serum cholesterol or ASCVD has been more difficult to demonstrate, with inconsistent or conflicting data resulting from several published studies. This controversy has been reflected in public policy; both the American Heart Association and the Senate Select Committee have recommended general restrictions in dietary cholesterol intake, whereas the Food and Nutrition Board of the National Academy of Science has recommended that dietary cholesterol not be limited in healthy individuals.

A major factor in this controversy is the variability in plasma cholesterol response to increased dietary cholesterol. This variability does not appear to be due to individual differences in absorption of dietary cholesterol. Studies of dietary cholesterol absorption have revealed a linear increase in absorption with increased dietary cholesterol content. The average absorption was 30% to 50% over a cholesterol range of 100 to 2000 mg/day.

Sterol balance studies have provided evidence for the hypothesis that
regulatory mechanisms involving secretion of biliary sterols and synthesis of endogenous cholesterol play a significant role in the individual response to increased dietary cholesterol. In the report by Nestel and Poyser,13 “nonresponders” (subjects who showed no change in plasma cholesterol during consumption of high cholesterol diets) had a significantly greater suppression of endogenous cholesterol synthesis than did "responders." In fact, increased biliary excretion of sterols and decreased endogenous cholesterol synthesis completely compensated for increased dietary cholesterol in the nonresponders. Responders, in contrast, did not fully compensate for the increased load of absorbed dietary cholesterol. The data of Quintao et al.14 are similar. However, since cholesterol is transported in the circulation as a lipoprotein component, alterations in the production and degradation of these macromolecules could also play an important role in the effect of dietary cholesterol upon plasma cholesterol levels. We chose, therefore, to study the turnover of LDL and their precursor in plasma, very low density lipoproteins (VLDL), in normal males who consumed amounts of cholesterol near the extremes of normal for American men.6

Methods

Study Subjects

Five healthy male volunteers were recruited from the faculty and staff of the Mount Sinai Medical Center (table 1). Criteria for inclusion in the study required the absence of disorders associated with lipoprotein abnormalities and a willingness to participate. All subjects were near ideal body weight, had no history of significant medical problems, and were not taking medications. Screening lipid profiles obtained while subjects consumed an unrestricted diet revealed normal total plasma triglyceride and cholesterol levels. Informed consent was given by each subject before admission to the study.

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Height (in)</th>
<th>Weight (kg) Period A</th>
<th>Weight (kg) Period B</th>
<th>IBW* (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5'9&quot;</td>
<td>64.0</td>
<td>63.3</td>
<td>92</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>6'0&quot;</td>
<td>77.0</td>
<td>75.2</td>
<td>101</td>
</tr>
</tbody>
</table>

*Percent ideal body weight as determined by the Metropolitan Life Insurance tables.

Procedure

The experiment included a baseline study, Period A, and a high cholesterol study, Period B. All subjects participated first in Period A, which preceded Period B by 3 to 8 months. During both study periods, subjects consumed weight maintaining solid food diets divided into three feedings and two snacks. During the 48-hour period of blood sampling for the VLDL turnover study, formula feedings described below were used. The solid food diets consisted of over 100 foods whose compositions have been listed in a computer program in our Clinical Research Center (CRC). Subjects received the same foods each day with occasional minor substitutions. All meals were prepared in the CRC; 70% to 80% of the meals were eaten at the Center, and subjects were given weighed, prepackaged meals at other times. All subjects were hospitalized in the CRC from the day preceding the injection of VLDL until the day after the LDL injection (as described later). The diets in Period A and Period B were identical except for the cholesterol content. They consisted of 45% carbohydrate, 40% fat, and 15% protein, with a ratio of polyunsaturated to saturated fat of 0.4. The cholesterol content was 150 mg/1000 kcal in Period A and 500 mg/1000 kcal in Period B. The additional cholesterol in Period B was given as egg yolk (no eggs were eaten in Period A), with dietary fat composition corrected for the fatty acid composition of egg yolk. Calories, initially supplied at a level of 35 kcal/kg body weight, were adjusted to maintain body weight.

In Period A all subjects received the lower cholesterol diet for a total of 4 consecutive weeks. After the first week of this diet, 500 ml of blood were removed and the red blood cells were returned to the subject. The plasma was used in the isolation of the VLDL and LDL for the turnover studies. An aliquot was also taken for determination of plasma lipids and lipoprotein cholesterol levels. At the end of the second week of the low cholesterol diet, subjects began the VLDL-LDL turnover study. For studying VLDL, we used a fat-free liquid diet, which has been described in detail elsewhere. Briefly, each subject received 50 μCi of 131I-VLDL and 300 μCi of 2-3H-glycerol by rapid intravenous injection. Over the next 48 hours, 18 blood samples were obtained, after which the baseline solid food diet was resumed. On the following morning after an overnight fast, subjects received 25 μCi of 125I-LDL via rapid intravenous injection. Six blood samples were obtained during the next 12 hours. The low cholesterol diet was resumed after the third sample (2 hours after injection) and continued during the final 2 weeks of the study. Daily fasting blood samples were obtained during this time.
In Period B, the high cholesterol diet was consumed for 4 to 5 consecutive weeks. After 1 week (two subjects) or 2 weeks (three subjects) of the high cholesterol diet, a 500 ml blood sample was obtained for isolation of VLDL and LDL. The remainder of the protocol was identical to that described for Period A except that the liquid feedings used for the VLDL turnover period were modified to include 500 mg cholesterol per 1000 kcal. This was supplied as pure cholesterol dissolved in corn oil (5% total calories). All subjects received 3 drops of a saturated solution of potassium iodide, three times a day, starting 1 day before injection of radiolabeled VLDL and continuing for 3 weeks.

**Lipoprotein Analyses**

Blood for determination of plasma lipids, lipoproteins, and specific radioactivity of apoprotein B and triglyceride was drawn into tubes containing 1.0 mg/ml ethylene diamine tetraacetate (EDTA). These tubes were kept on ice until separation of plasma by centrifugation at 2000 rpm at 4°C. Plasma samples were stored at 4°C until measurements were made. Blood obtained during the plasmapheresis was collected into a sterile bag containing 1.0 mg/ml EDTA.

VLDL were isolated at plasma density (d < 1.006) by ultracentrifugation in an SW 28 rotor at 23,000 rpm at 10°C for 24 hours in an L8-75 ultracentrifuge. This preparation was further purified and concentrated in an SW 40 rotor at 39,000 rpm at 10°C for 24 hours. LDL (d = 1.025–1.055) was isolated by three sequential ultracentrifugational steps beginning in a 60 Ti rotor at 59,000 rpm at d = 1.025. The infranate was then removed and after adjustment to d = 1.063 and concentrated by centrifugation in a 40.3 rotor at 39,000 rpm. Each ultracentrifugation was performed at 10°C for 20 to 24 hours. All procedures were carried out using aseptic technique and sterilized equipment. After several dialyses against 0.15M NaCl containing 0.1 mg/ml EDTA (pH 7.2), the lipoproteins were iodinated by a modification of the iodine monochloride method. Extensive further dialyses were carried out to remove free iodide. The lipoproteins were then diluted in 0.15M NaCl and human serum albumin (5 g/dl) and passed through Millipore filters (VLDL = 0.45 μm and LDL = 0.22 μm) before storage in sterile vials. 2-3H-glycerol (purchased from New England Nuclear, Boston, Massachusetts) was diluted to a concentration of 300 μCi/ml and passed through a Millipore filter (0.22 μm) before use.

VLDL were isolated from the 18 plasma samples obtained during the 48 hours following injection of 131I-VLDL and 3H-glycerol by ultracentrifugation in a 40.3 rotor at 39,000 rpm at 10°C for 16 to 20 hours. The specific radioactivity of apo B in this lipoprotein class was determined by the use of 1,1'3,3'-tetramethyl ura to separate apo B from the other apoproteins present. Each specific activity determination was carried out in duplicate or triplicate. The specific radioactivity of VLDL-3H-triglyceride was determined by the method of Grundy et al. This procedure was carried out several weeks after isolation of the VLDL, at a time when beta emissions from 131I were no longer measurable. LDL were isolated from plasma samples obtained after injection of 125I-LDL by sequential ultracentrifugation at d = 1.020 and then at d = 1.063 in a 40.3 rotor at 39,000 rpm at 10°C for 24 hours each. Apo B specific activity was then calculated using the protein determinations made by the method of Lowry et al.; gamma radioactivity was measured in a Packard Scintillation Counter.

Cholesterol and triglyceride concentrations were measured by specific enzymatic methods using an ABA-100 Analyzer. Lipoprotein cholesterol levels were determined after ultracentrifugation according to Lipid Research Clinic methodology, except that dextran sulfate and MCI were used to precipitate the lower density lipoproteins before measurement of high density lipoprotein (HDL) cholesterol.

Apo B in each lipoprotein class was measured by electroimmunoassay utilizing a specific anti-human, apo B antiserum prepared in a goat. Lipoproteins were subjected to incubation with purified bovine milk lipoprotein lipase before apo B measurement. This was necessary to unmask all antigenic sites on the lipoprotein particles. LDL were used as standard and samples were normalized using pools of normal serum (stored at −70°C) as internal controls on all assays. All of the samples from a single dietary period were measured on a single assay. The interassay coefficient of variation was 9%.

**Statistical Analyses**

Values for the fractional catabolic rates of VLDL apo B and VLDL-TG were obtained using compartmental models that we have recently described. VLDL apo B kinetics were described by two subpopulations of plasma particles, one with a fast turnover rate and the other with a significantly slower rate. The second population is assumed to be a catabolic product of the first population. VLDL-TG kinetics were described by two synthetic pathways into plasma and the two populations of plasma particles used to analyze VLDL apo B kinetics. The fractional clearance rate of LDL apo B was estimated using the analysis of Matthews. The parameters of each
compartmental system were estimated by a nonlinear least square routine using the facilities of the Columbia University Computer Center. The production rate for each lipoprotein component was obtained by multiplying the fractional catabolic rate by the mean steady state plasma pool size. Statistical analysis was carried out using Student's t test for paired data.

Results

Mean plasma total cholesterol and triglyceride concentrations and LDL and high density lipoprotein (HDL) cholesterol levels were determined using the values from three to five fasting samples obtained during the final 2 weeks of each diet period. These values were obtained after each subject had already consumed the lower cholesterol diet for 2 weeks and the high cholesterol diet for 2 or 3 weeks. The results demonstrated that increased dietary cholesterol had no effect on plasma total or LDL cholesterol levels in any subject (table 2). In addition, there was no change in the plasma total or LDL cholesterol levels in any individual, on either diet, from the first day to the last day of each diet period. For example, Subject 4, whose mean total and LDL cholesterol levels were lower at the start of Period B than at the beginning of Period A, maintained these lower levels throughout the study period, although cholesterol intake more than tripled. The group mean (±sd) for total plasma cholesterol was 169.3 ± 33.6 mg/dl in Period A and 167.9 ± 13.2 mg/dl in Period B. Similarly, group means for LDL cholesterol were 106.5 ± 21.8 and 107.5 ± 16.0 mg/dl in the two periods.

Plasma triglyceride and HDL cholesterol were also unaffected by the more than threefold increase in dietary cholesterol during Period B. Although some individual mean values in Period B differ from those in Period A, these differences were again due to variation in levels between studies and were clearly evident at the start of each period. No changes in plasma triglyceride or HDL cholesterol were observed from beginning to end of Period B. Finally, the high cholesterol diet had no effect on the ratio of cholesterol to triglyceride in VLDL (0.26 ± 0.04 vs 0.25 ± 0.04 in Period A vs B).

The concentrations of apo B in VLDL and LDL during Periods A and B are depicted in table 3.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total cholesterol</th>
<th>Total triglyceride</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>160.0 ± 11.5</td>
<td>160.8 ± 5.0</td>
<td>60.0 ± 8.0</td>
<td>55.6 ± 11.8</td>
</tr>
<tr>
<td>2</td>
<td>156.5 ± 18.0</td>
<td>153.3 ± 6.3</td>
<td>51.8 ± 5.6</td>
<td>68.0 ± 10.9</td>
</tr>
<tr>
<td>3</td>
<td>151.7 ± 5.0</td>
<td>166.0 ± 9.2</td>
<td>52.7 ± 3.8</td>
<td>49.7 ± 7.9</td>
</tr>
<tr>
<td>4</td>
<td>207.7 ± 4.1</td>
<td>188.3 ± 9.3</td>
<td>76.0 ± 7.9</td>
<td>119.5 ± 6.4</td>
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<tr>
<td>5</td>
<td>170.9 ± 6.8</td>
<td>171.3 ± 11.8</td>
<td>63.0 ± 10.5</td>
<td>64.3 ± 12.2</td>
</tr>
</tbody>
</table>

Each value is the mean of three to five values obtained during the final 2 weeks of the low cholesterol (A) and high cholesterol (B) diet periods: mg/dl ± sd. LDL = low density lipoprotein; HDL = high density lipoprotein.

<table>
<thead>
<tr>
<th>Subject</th>
<th>VLDL (µg/ml)</th>
<th>LDL (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>50.4 ± 3.5</td>
<td>56.8 ± 11.9</td>
</tr>
<tr>
<td>2</td>
<td>42.3 ± 5.6</td>
<td>65.6 ± 7.2</td>
</tr>
<tr>
<td>3</td>
<td>56.0 ± 10.4</td>
<td>42.7 ± 3.8</td>
</tr>
<tr>
<td>4</td>
<td>65.9 ± 11.1</td>
<td>89.6 ± 16.4</td>
</tr>
<tr>
<td>5</td>
<td>76.8 ± 13.7</td>
<td>48.6 ± 7.8</td>
</tr>
<tr>
<td>Mean</td>
<td>58.3 ± 13.5</td>
<td>60.7 ± 18.3</td>
</tr>
</tbody>
</table>

Each value is the mean of four to five samples obtained during the 48 hours of VLDL turnover (see Methods) during the low cholesterol (A) and high cholesterol (B) diet periods.

Table 2. Plasma Lipid and Lipoprotein Cholesterol Levels

Table 3. Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) Apoprotein B Concentrations
Consistent with the lack of effect of increased dietary cholesterol upon plasma lipid levels, mean VLDL apo B concentrations were nearly identical in each period (58.3 ± 13.5 μg/ml in Period A; 60.7 ± 18.3 μg/ml in Period B). Similarly, the mean LDL apo B level of 633 ± 161.5 μg/ml in Period B was not significantly different from the level of 570.4 ± 113.3 μg/ml during Period A. In addition, both the mean fractional catabolic rates and the mean production rates of both VLDL apo B and LDL apo B were essentially identical during the two diet periods (tables 4 and 5).

The mean plasma concentration and turnover of VLDL triglyceride were not affected by the increase in dietary cholesterol in Period B (table 6). Although one subject (Subject 2) had marked increase in both the fractional catabolic and production rates of VLDL-TG during Period B, his plasma concentration did not change significantly. It is interesting to note that although the VLDL apo B synthetic rates ranged more widely than did the synthetic rates for VLDL triglyceride, neither changed significantly with high cholesterol intake.

### Discussion

Plasma concentrations of total and LDL cholesterol are strong predictors of the risk of developing ASCVD. Although genetic lipoprotein disorders have been demonstrated in some individuals with hypercholesterolemia, the majority of subjects with elevated plasma cholesterol have no clearly defined metabolic defect. Some investigators have proposed that consumption of diets high in saturated fat and cholesterol has a major effect in elevating plasma cholesterol levels in this latter group. However, while epidemiologic surveys have supported the belief

### Table 4. Very Low Density Lipoprotein Apoprotein B Metabolism

<table>
<thead>
<tr>
<th>Subject</th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
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<td>14.2</td>
<td>22.1</td>
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<tr>
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<td>0.36</td>
<td>0.34</td>
<td>16.4</td>
<td>24.1</td>
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<tr>
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<td>0.36</td>
<td>0.43</td>
<td>21.8</td>
<td>19.8</td>
</tr>
<tr>
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<td>0.50</td>
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<td>35.8</td>
</tr>
<tr>
<td>5</td>
<td>0.36</td>
<td>0.45</td>
<td>29.9</td>
<td>23.6</td>
</tr>
<tr>
<td>Mean</td>
<td>0.37</td>
<td>0.39</td>
<td>23.6</td>
<td>25.1</td>
</tr>
</tbody>
</table>

A = low cholesterol diet period; B = high cholesterol diet period.

### Table 5. Low Density Lipoprotein Apoprotein B Metabolism

<table>
<thead>
<tr>
<th>Subject</th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.39</td>
<td>0.35</td>
<td>12.7</td>
<td>10.9</td>
</tr>
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<td>0.65</td>
<td>0.42</td>
<td>13.2</td>
<td>10.3</td>
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<tr>
<td>3</td>
<td>0.48</td>
<td>0.46</td>
<td>11.8</td>
<td>9.1</td>
</tr>
<tr>
<td>4</td>
<td>0.51</td>
<td>0.46</td>
<td>14.8</td>
<td>18.0</td>
</tr>
<tr>
<td>5</td>
<td>0.55</td>
<td>0.59</td>
<td>12.0</td>
<td>16.5</td>
</tr>
<tr>
<td>Mean</td>
<td>0.52</td>
<td>0.46</td>
<td>12.9</td>
<td>13.0</td>
</tr>
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</table>

A = low cholesterol diet period; B = high cholesterol diet period.

### Table 6. Very Low Density Lipoprotein Triglyceride Metabolism

<table>
<thead>
<tr>
<th>Subject</th>
<th>A</th>
<th>B</th>
<th>Fractional catabolic rate (hr⁻¹)</th>
<th>Production rate (mg/kg/hr)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>30</td>
<td>0.35</td>
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</tr>
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<td>2</td>
<td>38</td>
<td>45</td>
<td>0.30</td>
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<td>22</td>
<td>0.45</td>
<td>6.3</td>
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<td>53</td>
<td>55</td>
<td>0.27</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>43</td>
<td>40</td>
<td>0.30</td>
<td>8.2</td>
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<tr>
<td>Mean</td>
<td>41.0</td>
<td>38.4</td>
<td>0.33</td>
<td>6.0</td>
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</tbody>
</table>

*Mean of 18 samples obtained during the 48-hour turnover period. A = low cholesterol diet period; B = high cholesterol diet period.
that total and saturated fat intake correlates with plasma cholesterol concentration. Several large surveys have failed to demonstrate such a link between dietary cholesterol and plasma cholesterol levels. The studies of Kato et al. and Shekelle et al. are notable exceptions.

Smaller, better controlled studies of the effects of increasing dietary cholesterol on plasma cholesterol concentration have also produced conflicting results. Several conclusions may be drawn from these investigations. First, the most consistent results have been obtained when high cholesterol diets have been compared to diets containing no cholesterol. Studies in which moderate or "average" quantities of cholesterol were consumed during the baseline period often failed to demonstrate effects of increased dietary cholesterol. The National Diet-Heart Study Final Report is an exception. Second, even in studies where increased cholesterol consumption resulted in elevations of plasma cholesterol, a plateau effect was noted for intakes above 600 mg cholesterol per day. This was evident even in studies where extraordinary amounts of cholesterol (up to 5000 mg/day) were consumed. The study of Mattson et al. was an exception. Finally, variability in individual responses to dietary cholesterol has been clearly demonstrated in well-controlled metabolic studies, and individual differences in ability to absorb dietary cholesterol cannot account for the wide range of plasma cholesterol responses observed. Compensatory reductions in endogenous cholesterol synthesis, coupled with increases in biliary cholesterol secretion, seem to significantly affect the maintenance of stable plasma cholesterol concentrations in spite of increased dietary cholesterol consumption. Thus, if subjects are divided into those who respond and those who do not respond to increases in dietary cholesterol, the nonresponders appear to suppress endogenous cholesterol synthesis more effectively than do responders.

In the present study, we attempted to delineate the role of plasma lipoproteins in the regulation of individual responsiveness to changes in dietary cholesterol. The possible importance of these macromolecules becomes evident when we consider the following. After absorption, dietary cholesterol enters plasma on triglyceriderich lipoproteins, chylomicrons. These particles interact with endothelial cells in the extrahepatic tissues where lipoprotein lipase hydrolyzes most of the triglyceride. In animal studies, up to 40% of the chylomicron cholesterol may be delivered to extrahepatic tissues during the action of this enzyme. The remaining components (chylomicron remnants) are rapidly taken up by the hepatocytes, where the residual cholesterol in these particles significantly affects the regulation of hepatic cholesterol synthesis. However, the liver also secretes cholesterol into the bloodstream as a component of VLDL, and this cholesterol may originate from both exogenous and endogenous sources. In plasma, VLDL loses triglyceride and apoprotein components and is either removed from plasma directly or converted to LDL. Most of the VLDL cholesterol stays with the particle as it is converted to LDL. Finally, cholesterol can leave the plasma along with LDL as these lipoproteins interact with cell surface receptors for specific apoprotein components. Obviously, regulation of response to increased dietary cholesterol intake could involve changes in the production or catabolism of either VLDL or LDL. Therefore, we measured the flux of LDL and their precursors, VLDL, in plasma. Apoprotein B was used as a marker of the production and degradation of these lipoproteins because it is a stable structural component of the particles as they undergo other metabolic changes in the circulation. VLDL-TG turnover was measured as an additional marker of VLDL production and degradation.

The present study demonstrates that, in nonresponders, compensatory mechanisms involved in maintaining the plasma cholesterol concentration are effective before the transport of cholesterol on VLDL and LDL. Specifically, the maintenance of plasma cholesterol levels is accomplished without the need to change production and/or degradation of these lipoproteins. Thus, hepatic regulation of endogenous cholesterol synthesis and biliary acidic and neutral sterol secretion appear to adequately meet the challenge of increased dietary cholesterol load in this group. This hypothesis is supported by the works of Nestel and Poyser and Quintao et al. In the latter study, however, total body cholesterol was noted to increase in some subjects in spite of stable plasma cholesterol levels. Since neither lipoprotein flux, nor the ratio of cholesterol to protein in VLDL or LDL changed in our subjects (data not shown), any increase in body cholesterol content probably occurred in the liver. We cannot, however, rule out direct delivery of cholesterol to peripheral tissues by chylomicrons.

This study cannot address the effects of increased dietary cholesterol upon VLDL and LDL turnover in responsive subjects. There is no available data showing he percentage of a normal population that is responsive to this dietary manipulation; thus, it is difficult to find a reason for our inability to find one responder among the five subjects we studied. Inadequate duration of the high cholesterol diet is an unlikely reason for lack of response, since subjects consumed the diets for 4 to 5 weeks without any change in plasma cholesterol levels. Thus, although the VLDL turnover study was carried out after 2 to 3 weeks of each diet, the subjects were already in
steady state. The use of a low cholesterol diet containing 300 to 400 mg/day of cholesterol and the likelihood of a plateau in the response to dietary cholesterol above an intake of 600 mg/day, might have been factors which lessened our chances of demonstrating a response. We chose the cholesterol levels to correspond to the present intake in the American diet. It is possible that a cholesterol-free diet might result in alterations in plasma lipoprotein turnover.

In summary, these results indicate that plasma VLDL and LDL turnovers are unchanged in nonresponders to increased dietary cholesterol. The data suggest that events occurring before secretion of VLDL by the liver can fully compensate for the increased dietary cholesterol load absorbed in the small intestine. Kushwaha and Hazard noted a lack of change in VLDL apo B sorbed in the small intestine. Langer et al. reported no change in LDL apo B production in two Type II subjects, although plasma LDL cholesterol levels rose when dietary cholesterol intake was increased. In the latter study, however, saturated fat content of the diet was markedly increased at the same time as dietary cholesterol. Thus, it is unclear whether human subjects who respond to an increase in dietary cholesterol alone have alterations in VLDL and LDL apo B metabolism.

Acknowledgments

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References

30. Nichols AB, Ravenacrost C, Lemplheir DE, Ostrander
44. Cooper AD. The metabolism of chylomicron remnants by isolated perfused rat liver. Biochim Biophys Acta 1977; 488:464-474
45. Eisenberg S. Plasma lipoprotein conversions: The origin of low density and high density lipoproteins. NY Acad Sci 1980;348:30-47
47. Kushwaha RS, Hazzard WR. Metabolism of very low density lipoproteins in diet induced hypercholesterolemic pigtail monkeys (Macaca nemestrina). Biochim Biophys Acta 1980;619:142-155

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