Relationship between Sensitivity to Dietary Fat and Dietary Cholesterol

Peter M. Clifton, Mark Kestin, Mavis Abbey, Mary Drysdale, and Paul J. Nestel

A group of 56 hypercholesterolemic and normocholesterolemic men and women were given approximately 700 mg a day of egg yolk cholesterol in a double-blind, crossover study while they were on a background diet containing approximately 30% of energy as fat. Overall there was a 0.23 mmol/l rise in plasma cholesterol (3.7%, p<0.001) after 4 weeks, a 0.19 mmol/l rise in low density lipoprotein (LDL) cholesterol (4.9%, p=0.002), and a 0.07 mmol/l rise in high density lipoprotein (HDL) cholesterol (5.4%, p<0.001). Plasma triglycerides fell by 0.07 mmol/l (5.1%). Normocholesterolemic individuals (plasma cholesterol <5.2 mmol/l) experienced small, nonsignificant rises of 0.06, 0.02, and 0.05 mmol/l in total, LDL, and HDL cholesterol, respectively. Hypercholesterolemic subjects were classified on the basis of their response to a low fat diet. Diet-sensitive subjects were defined by a > 10% fall in plasma cholesterol on a 25% fat, low cholesterol (<200 mg/day) diet. These individuals were found to be more responsive to the effect of dietary cholesterol than were diet-insensitive subjects; the respective changes in the two groups were rises of 0.36 mmol/l versus 0.19 mmol/l in plasma cholesterol (p=0.06) and rises of 0.30 versus 0.15 mmol/l in LDL cholesterol (p=0.06). In addition to elevating HDL cholesterol by 0.09 mmol/l and 0.07 mmol/l, respectively, dietary cholesterol also produced an increase in the proportion of HDLα from 40% to 44% of HDL protein (p<0.001). The change in both LDL and HDL cholesterol with dietary cholesterol supplementation was related to the change with fat supplementation (r=0.35, p<0.05 and r=0.45, p<0.001, respectively). However, normocholesterolemic individuals who are not particularly responsive to dietary cholesterol may, nevertheless, also need to consider such restrictions, especially if they are at risk for atherosclerosis. (Arteriosclerosis 10:394–401, May/June 1990)

An increase in dietary cholesterol produces a small, but significant, increase in plasma cholesterol in most large experimental groups, but there is great individual variability.1–8 The response to a change in saturated fat intake tends to be much larger than the response to dietary cholesterol, and although individual variability occurs, few people are truly unresponsive.8–13 McNamara et al.13 in an extensive trial of free-living subjects, found that 70% of people could effectively compensate for an increase in dietary cholesterol by down-regulating cholesterol synthesis. A similar proportion of metabolic “compensators” had earlier been reported by Nestel and Poyer.14 In the former study, only 10% of the individuals had a significant (10%) increase in plasma cholesterol, and these individuals failed to down-regulate cholesterol synthesis and tended to have high cholesterol absorption rates. A possible genetic basis has been suggested by Kesäniemi et al.15 who demonstrated that subjects with the E4 allele for apolipoprotein (apo) E had a higher fractional cholesterol absorption than did subjects with the E2 allele. Individuals carrying an apo E4 allele had a much higher low density lipoprotein (LDL) cholesterol concentration than did those with the E2 allele.16

Katan et al.17 have recently shown that there may be a correlation between sensitivity to changes in dietary saturated fat intake and sensitivity to dietary cholesterol in normocholesterolemic individuals. However, hypercholesterolemic individuals with high habitual egg consumption failed to show this correlation. If responsiveness to these two dietary components is inherited independently, then dietary advice might be defined more precisely. We have examined this important question in a double-blind controlled trial of cholesterol supplementation in individuals identified as responsive or unresponsive to dietary fat manipulation. We have found that, while there was no individual correlation between responsiveness to these two dietary components, hypercholesterolemic individuals who were sensitive to dietary fat reduction showed, as a group, a significantly larger response to dietary cholesterol than did either fat-insensitive hypercholesterolemic subjects or normocholesterolemic individuals.

Methods

Subjects

A total of 56 healthy men and women were recruited either by advertisement to enroll normocholesterolemic
subjects or from a pool of hypercholesterolemic subjects diagnosed through routine screening. Subjects with very high cholesterol levels (>8 mmol/l) were excluded. The persons in the study group were not on any medication likely to affect lipid metabolism and were advised to maintain their exercise pattern. Their characteristics and plasma cholesterol levels on their habitual diets at first screening (before any dietary intervention) are shown in Table 1. Subjects with a total cholesterol level of 5.2 mmol/l or less (n=11) were defined as normocholesterolemic. Hypercholesterolemic subjects were selected and provisionally classified on the basis of a response to dietary advice to lower cholesterol, while a second group either experienced a rise or minimal change in plasma cholesterol after appropriate dietary advice. They were, therefore, classified as probable responders or nonresponders. An equal number of subjects was recruited into each group for the controlled phase of the study.

The normocholesterolemic subjects were significantly younger than the hypercholesterolemic subjects (42±9 vs. 54±10, p<0.01). Approval was obtained from the Human Ethics Committee of the CSIRO Division of Human Nutrition, and the subjects gave their informed consent.

**Study Design**

The background diet throughout the controlled phase of the study was planned to contain 25% of energy as fat, a ratio of polyunsaturated to saturated fatty acids of 1.0, and approximately 180 mg of cholesterol (Table 2). Subjects were definitively categorized as diet-sensitive or diet-insensitive after a 4-week baseline period on the background diet alone. Hypercholesterolemic subjects who experienced at least a 10% fall in plasma cholesterol on this diet were defined as diet-sensitive and the others as diet-insensitive. The next phase of the study was carried out in a double-blind crossover design and comprised two experimental periods of 4 weeks each, testing in random order one of two liquid supplements, one containing egg yolk and one containing a cholesterol-free fat mixture. The egg yolk supplement was planned to provide 70 mg of cholesterol per megajoule of energy per day (mean about 700 mg/day), while the cholesterol-free supplement was matched in energy, color, protein, carbohydrate, and fatty acid content with the yolk drink (β-carotene, egg white, and a mixture of vegetable fats: olive oil, palm oil, coconut oil, and soy lecithin) (Table 3). The supplements contributed approximately 5% of energy from fat to the daily intake.

Close dietary instruction and supervision were maintained throughout the study. Subjects were trained to count daily saturated fat intake from a simplified food composition table and to maintain a relatively constant intake over each week. Weighed food records were completed on four separate days in each experimental period for a total of 12 days over the entire experiment.

### Table 1. Characteristics of Test Subjects according to Final Classification as Sensitive or Insensitive to Diet

<table>
<thead>
<tr>
<th></th>
<th>Hypercholesterolemic group</th>
<th>Diet-sensitive (14 men, 9 women)</th>
<th>Diet-Insensitive (11 men, 11 women)</th>
<th>Normocholesterolemic group (8 men, 3 women)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td></td>
<td>53±10</td>
<td>55±9</td>
<td>42±9†</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
<td>65.7±0.9</td>
<td>66.2±0.9</td>
<td>71.7±0.9</td>
</tr>
<tr>
<td>Pre-baseline values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td></td>
<td>6.86±0.90</td>
<td>6.35±0.60*</td>
<td>4.68±0.30</td>
</tr>
<tr>
<td>Baseline values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td></td>
<td>5.89±0.83</td>
<td>6.19±0.56*</td>
<td>4.49±0.65</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td></td>
<td>3.82±0.83</td>
<td>4.33±0.56*</td>
<td>2.93±0.65</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td></td>
<td>1.36±0.41</td>
<td>1.33±0.38</td>
<td>1.11±0.13</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td></td>
<td>0.51±0.15</td>
<td>0.53±0.16</td>
<td>0.45±0.16</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td></td>
<td>1.41±0.58</td>
<td>1.39±0.42</td>
<td>1.25±0.55</td>
</tr>
</tbody>
</table>

The results are expressed as means±1 SD. The pre-baseline cholesterol value was obtained between 1 and 6 months before entry into trial while the subjects were on their usual diet. The baseline cholesterol and triglyceride values were obtained after 4 weeks on the baseline low-fat, low-cholesterol diet.

* p<0.05, diet-sensitive versus diet-insensitive. † p<0.01, hypercholesterolemic versus normocholesterolemic.

LDL=low density lipoprotein, HDL=high density lipoprotein, VLDL=very low density lipoprotein.

### Table 2. Dietary Intakes of All Individuals during Both Test Periods

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control supplement</th>
<th>Cholesterol supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ)</td>
<td>9.28±2.06</td>
<td>9.26±2.67</td>
</tr>
<tr>
<td>Protein (% en)</td>
<td>18.2±3.6</td>
<td>20.0±5.4</td>
</tr>
<tr>
<td>Carbohydrate (% en)</td>
<td>48.2±6.6</td>
<td>49.2±7.0</td>
</tr>
<tr>
<td>Soluble NSP (g)</td>
<td>6.3±1.2</td>
<td>6.8±1.5</td>
</tr>
<tr>
<td>Fat (% en)</td>
<td>29.1±7.4</td>
<td>27.8±8.2</td>
</tr>
<tr>
<td>PFA</td>
<td>6.2±3.1</td>
<td>5.8±2.3</td>
</tr>
<tr>
<td>MFA</td>
<td>10.0±2.9</td>
<td>9.7±2.6</td>
</tr>
<tr>
<td>SFA</td>
<td>10.4±3.0</td>
<td>9.7±2.9</td>
</tr>
<tr>
<td>Cholesterol (g)</td>
<td>185±89</td>
<td>866±95</td>
</tr>
<tr>
<td>Alcohol (% en)</td>
<td>2.3±3.2</td>
<td>2.6±3.9</td>
</tr>
</tbody>
</table>

The values are means±1 SD and are calculated from food diaries during the basal diet and supplement periods. en=energy; NSP=non-starch polysaccharide, PFA=polyunsaturated fatty acids, MFA=monounsaturated fatty acids, SFA=saturated fatty acids.
HDL3, this was used to calculate the area of HDL 2 and the coefficient of variation on two samples of plasma cholesterol was 3.0±3.6 (mean±1 SD, n=135), while the coefficient of variation of the assay, determined by heparin-manganese gradient gel electrophoresis as previously described, was 8.4% (two-tailed) on a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Nutley, NJ) by using standard enzymatic methods (Boehringer Mannheim, Mannheim, FRG). The intra-individual coefficient of variation on two samples of plasma cholesterol was 8.4% (two-tailed). Cholesterol and triglycerides were measured after precipitation of apo B containing lipoproteins with 5.6% trichloroacetic acid and cholesterol-free period and 880 mg/day in the egg yolk, high cholesterol period. Subjects were weighed at frequent intervals, and changes in weight exceeding ±0.4 kg were rectified successfully by minor across-the-board adjustments in food intake. Analysis of the diaries revealed excellent compliance.

**Lipid and Lipoprotein Measurements**

Fasting blood samples were collected in Na2 ethylenediaminetetraacetate tubes (1 mg/ml) on two occasions 5 days apart at the end of weeks 4, 8, and 12. Plasma was separated by low-speed centrifugation at 4°C and immediately frozen at −20°C until analysis. All samples were assayed together after completion of the trial. Very low density lipoprotein (VLDL) cholesterol was measured after ultracentrifugation at d=1.006 g/ml for 17 hours at 34 K in a 50.3 rotor (Beckman Instruments, Palo Alto, CA). High density lipoprotein (HDL) cholesterol was measured after precipitation of apo B containing lipoproteins with heparin-manganese. LDL cholesterol was calculated by difference. Cholesterol and triglycerides were measured on a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Nutley, NJ) by using standard enzymatic methods (Boehringer Mannheim, Mannheim, FRG). The intra-individual coefficient of variation on two samples of plasma cholesterol obtained on separate days was 3.0±3.6 (mean±1 SD, n=135), while the coefficient of variation of the assay (between and within run) was 1.6% (n=24 on eight occasions).

The subpopulation distribution of HDL was assessed by gradient gel electrophoresis as previously described. If there was a clear demarcation between HDL2 and HDL3, this was used to calculate the area of HDL2 and HDL3; otherwise, all particles smaller than 4.4 nm were designated HDL3.

**Apolipoprotein E Phenotype**

Apolipoproteins in VLDL (d<1.006 g/ml) were delipidated at −20°C by washing twice with acetone/ethanol (1:1 vol/vol) and once with diethyl ether. Delipidated apoproteins were dissolved in 5 M urea (Aristar, BDH Chemicals, Poole, England) containing 10 mM Tris-HCl and dithiothreitol (final concentration, 5 mg/ml). Samples were subjected to isoelectric focusing in prefocused 7.5% polyacrylamide 5 M urea slab gels (pH 4 to 7), and were fixed, stained with Coomassie brilliant blue R250, and destained as previously described. A group of 23 subjects was provisionally classified as diet-insensitive and 22 subjects as diet-sensitive on the basis of the changes in plasma cholesterol after general dietary advice to lower fat and cholesterol intake. Final classification was made after 4 weeks on the controlled baseline diet as shown in Table 1. Those hypercholesterolemic subjects who experienced a 10% or greater fall in plasma cholesterol on the baseline diet were classified as diet-sensitive. Three subjects who were provisionally classified as diet-insensitive were reclassified as diet-sensitive after the controlled baseline period, and two diet-sensitive subjects were reclassified as diet-insensitive. There was a highly significant correlation (r=+0.75; p<0.001) between the decrements in plasma cholesterol dieting and that obtained by adhering strictly to the low fat baseline diet.

The initial plasma cholesterol concentrations, when most subjects were consuming their habitual high-fat diet, were significantly higher for the diet-sensitive than for the diet-insensitive group, 6.86 versus 6.35 mmol/l (p<0.05). Subsequently, on the 25% fat baseline diet the diet-sensitive subjects showed a mean fall in plasma cholesterol of 17.1% (range, −10% to −35%), from 6.86 to 5.69 mmol/l, while the diet-insensitive subjects experienced a mean fall of 2.5% (range, 26% to −9%), from 6.35 to 6.19 mmol/l. The normocholesterolemic group showed a mean fall of 4.1% (range, 21% to −14%) in plasma cholesterol, from 4.68 to 4.49 mmol/l. At the end of the baseline period, both the plasma and LDL cholesterol concentrations were significantly lower in the diet-
sensitive than in the diet-insensitive group (5.69 vs. 6.19 and 3.82 vs. 4.33 mmol/l, respectively) (Table 1).

**Response to Addition of Dietary Fat**

For the entire population of 56 subjects, the addition of the cholesterol-free supplement, which contained 5 g of saturated fat, to the low-fat baseline diet produced after 4 weeks a mean rise of 0.19 mmol/l in plasma cholesterol (3.4%, p<0.001), 0.10 mmol/l in LDL cholesterol (2.6%, p<0.025), and 0.08 mmol/l in HDL cholesterol (6.2%, p<0.001). (Since both supplements provided an additional 5% energy from fat, the total energy from fat rose to 30%). There were differences between the three groups in their response to dietary fat. The normocholesterolemic subjects showed no significant change in total cholesterol, LDL cholesterol, or HDL cholesterol (Table 4). In contrast, the hypercholesterolemic group showed a significant rise in plasma cholesterol of 0.23 mmol/l (3.8%, p<0.001), in LDL cholesterol of 0.13 mmol/l (3.2%), and in HDL cholesterol of 0.12 mmol/l (7.7%).

Within the hypercholesterolemic group, there were differences between the diet-sensitive and diet-insensitive subgroups. The diet-sensitive group experienced larger rises in plasma cholesterol (0.32 vs. 0.13 mmol/l), in LDL cholesterol (0.20 vs. 0.06 mmol/l), and in HDL cholesterol (0.12 vs. 0.09 mmol/l), but none of these differences was statistically significant. However, there was a significant difference between the two hypercholesterolemic groups in the numbers of individuals experiencing at least a 5% rise in plasma cholesterol. This occurred in 15 of the 23 diet-sensitive subjects but in only five of the 22 diet-insensitive subjects (χ²=8.31, p<0.01). Thus, individuals who had earlier shown the greater falls in plasma cholesterol on the low-fat baseline diet were much more likely also to show a rise with a modest (5 g) supplement of saturated fat. Only one of the 11 normocholesterolemic subjects experienced at least a 5% rise in plasma cholesterol (χ²=4.712, p<0.05, df=1 compared with the hypercholesterololemic group).

**Response to Addition of Cholesterol**

The addition of 700 mg of dietary cholesterol on average per day to the 30% fat (25% baseline +5% from supplement), low cholesterol diet resulted in a significant increase for the whole group of 0.23±0.44 mmol/l (mean±SD) in plasma cholesterol (p<0.001) and of 0.19±0.41 mmol/l in LDL cholesterol (p<0.001). HDL cholesterol also increased significantly by 0.07±0.14 mmol/l (p<0.001), whereas plasma triglycerides decreased by 0.07±0.28 mmol/l. This represented a 3.6% increase in total cholesterol, a 4.8% increase in LDL cholesterol, and a 5.1% increase in HDL cholesterol (Table 5). The individual responses are shown in Figure 1.

Both normocholesterolemic subjects and diet-insensitive hypercholesterolemic subjects were insensitive to egg yolk cholesterol supplementation, so that no significant increases occurred in LDL cholesterol in these groups (Table 5). In contrast, hypercholesterolemic subjects sensitive to dietary fat reduction were also very responsive to dietary cholesterol supplementation, so

### Table 4. Effect of Dietary Fat Supplementation (5 g Saturated Fat) in Diet-sensitive and Diet-insensitive Hypercholesterolemic and Normocholesterolemic Subjects

<table>
<thead>
<tr>
<th>Hypercholesterolemic group</th>
<th>Diet-sensitive (n=23)</th>
<th>Diet-insensitive (n=22)</th>
<th>Normocholesterolemic group (n=11)</th>
<th>Combined (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol</td>
<td>0.32±0.41</td>
<td>0.13±0.34</td>
<td>0.03±0.31</td>
<td>0.19±0.38*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.20±0.40</td>
<td>0.06±0.31</td>
<td>-0.02±0.28</td>
<td>0.10±0.35†</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.12±0.15</td>
<td>0.09±0.18</td>
<td>0.01±0.14</td>
<td>0.08±0.174</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.01±0.13</td>
<td>-0.02±0.12</td>
<td>0.05±0.03</td>
<td>0.00±0.13</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>-0.02±0.34</td>
<td>-0.05±0.32</td>
<td>0.0±0.45</td>
<td>-0.03±0.35</td>
</tr>
</tbody>
</table>

Values of changes in concentration are mmol/l±SD.

Comparisons with baseline values: *p<0.001, †p<0.01, ‡p<0.05.

See the legend for Table 1 for an explanation of the abbreviations.

### Table 5. Effect of Dietary Cholesterol Supplementation (700 mg) in Diet-sensitive and Diet-insensitive Hypercholesterolemic and Normocholesterolemic Subjects

<table>
<thead>
<tr>
<th>Hypercholesterolemic group</th>
<th>Diet-sensitive (n=23)</th>
<th>Diet-insensitive (n=22)</th>
<th>Normocholesterolemic group (n=11)</th>
<th>Combined (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol</td>
<td>0.36±0.37</td>
<td>0.19±0.47†</td>
<td>0.06±0.47</td>
<td>0.23±0.44*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.30±0.36</td>
<td>0.15±0.46†</td>
<td>0.02±0.40</td>
<td>0.19±0.41*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.09±0.14</td>
<td>0.07±0.16</td>
<td>0.05±0.13</td>
<td>0.07±0.14*</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>-0.04±0.10</td>
<td>-0.03±0.12</td>
<td>-0.01±0.12</td>
<td>-0.03±0.11</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>-0.07±0.26</td>
<td>-0.10±0.29</td>
<td>0.02±0.31</td>
<td>-0.07±0.28</td>
</tr>
</tbody>
</table>

The values of changes in concentration are mmol/l±SD.

*p<0.001, comparisons with control values. †p=0.06, diet-sensitive versus diet-insensitive.

See the legend for Table 1 for an explanation of the abbreviations.
that LDL cholesterol increased by 8.5%, or 0.30 mmol/l, double the increase in LDL cholesterol seen in the diet-insensitive group. However, there was no difference between the three groups in the HDL cholesterol response to egg yolk cholesterol, with increases of 0.09, 0.07, and 0.05 mmol/l (5% to 6%) in the diet-sensitive, diet-insensitive, and normocholesterolemic groups, respectively.

There was also a difference in the number of responders in each group: 17/23 in the diet-sensitive, 9/22 in the diet-insensitive, and 4/11 in the normocholesterolemic group experienced a 5% or more rise in plasma cholesterol ($\chi^2=6.0, p<0.05, df=2$). However, the concordance between the subsets sensitive to fat and those sensitive to cholesterol was less than 50%. Nevertheless, combining these two subsets shows that half of the diet-sensitive group had a significant response to both fat and cholesterol compared with only 2/22 of the diet-insensitive group and none of the normocholesterolemic group.

On multiple regression, the two significant variables that predicted response to cholesterol were group designation (i.e., diet-sensitive, diet-insensitive, or normocholesterolemic) and change in LDL cholesterol with fat supplementation ($r=0.52, p<0.01$). These two variables explained 28% of the variation in response to dietary cholesterol. On univariate analysis, the change in HDL cholesterol with cholesterol supplementation was strongly related to the change seen with fat supplementation ($r=0.45, p<0.001$). The change in LDL cholesterol with egg yolk cholesterol was weakly related to the mean LDL cholesterol ($r=0.35, p<0.05$), and the change in HDL cholesterol was also related to the baseline HDL cholesterol ($r=0.38, p<0.01$). Despite a difference in response to dietary cholesterol between the diet-sensitive and diet-insensitive groups, there was not a significant correlation between the percentage decrease in plasma cholesterol on the baseline diet and the percentage increase in plasma or LDL cholesterol with dietary cholesterol in the 56 individuals.

Due to the proportionately greater effects on HDL cholesterol than on total cholesterol, the total cholesterol/HDL cholesterol ratio in the diet-sensitive group decreased from 4.18 to 4.06 with fat supplementation, and to 4.06 with dietary cholesterol. In the diet-insensitive group, the corresponding ratios were 4.65, 4.45, and 4.37, and in the normocholesterolemic group, 4.05, 4.04, and 3.91, respectively ($p>0.05$ for all comparisons).

High Density Lipoprotein Particle Size Distribution

HDL profiles were usually separable into HDL$_3$ and HDL$_2$ subpopulations, between 4.4 nm and 4.6 nm radius (Figure 2). Where no separate HDL$_{33}$ subpopulation was apparent, the profile was arbitrarily divided at 4.4 nm radius, and the areas of HDL$_2$ and HDL$_3$ were calculated. With egg yolk cholesterol, there was an increase in the proportion of HDL$_2$ from 40% to 44% (the difference was $2.7.8\%, p<0.001, n=56$) of total HDL protein. There was no relationship between the change in HDL subpopulation distribution and any other lipoprotein parameter.

Apolipoprotein E Phenotype

There was a significant difference between the apo E allele frequencies in the normocholesterolemic group and the hypercholesterolemic subjects (Table 6). E2 was present more frequently and E4 less frequently in the normocholesterolemic group ($\chi^2=14.5, p<0.001, df=2$), while E3 was present with the same frequency in both groups. In the hypercholesterolemic group, there was a difference between the diet-sensitive and the diet-insensitive groups in apo E phenotype. The E3/E3 phe-
The present study, half of all subjects experienced a rise from fat (polyunsaturated/saturated 1.0) and 180 mg/day fraction was run on a 3% to 27% nondenaturing polyacrylamide gel. The area of HDL₂b and HDL₂a and the total area were calculated to determine the HDL₂/HDL₃ ratio.

**Figure 2.** The change in HDL profile with cholesterol supplementation in one responsive individual. The plasma lipoprotein fraction was run on a 3% to 27% nondenaturing polyacrylamide gel for 3000 V/hr. Particle size was determined by comparison with high molecular weight standards on the same gel. The area of HDL₂a and HDL₂b, and the total area were calculated to determine the HDL₂/HDL₃ ratio.

**Table 6. Distribution of Apolipoprotein E Phenotype**

<table>
<thead>
<tr>
<th>Apo E phenotype</th>
<th>Hypercholesterolemic</th>
<th>Normocholesterolemic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Insensitive</td>
</tr>
<tr>
<td>2/3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3/3</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>3/4</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>4/4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

Values are numbers of subjects.

Apo E phenotyping was not carried out in all subjects.

notype was under-represented and the E3/E4 phenotype over-represented in the diet-insensitive group. \( \chi^2 = 9.55, p < 0.025, \text{df} = 3 \). When allele frequencies were calculated, E4 was present more often in the diet-insensitive group, but the difference was not significant. Overall, individuals with an E4 allele showed a rise of 0.12±0.36 mmol/l and individuals without an E4 allele, a rise of 0.27±0.37 mmol/l \((p > 0.05)\).

**Discussion**

This study confirms the results of several studies in free-living subjects in demonstrating a small, but significant, 0.23 mmol/l (3.7%) rise in plasma cholesterol when approximately 700 mg of egg yolk cholesterol was added to a background diet containing 30% of energy from fat (polyunsaturated/saturated 1.0) and 180 mg/day of cholesterol. Other studies in free-living subjects, including our previous study, failed to demonstrate significant rises in plasma cholesterol with supplements of 250 to 750 mg of cholesterol, although between 21% to 40% of subjects experienced at least a 5% rise. In the present study, half of all subjects experienced a rise of 5% or more in plasma cholesterol with dietary cholesterol.

In other studies, larger and more consistent responses to dietary cholesterol have been shown. Some of the discrepancies may be due to the base level of dietary cholesterol; the rise in plasma cholesterol is likely to be greater if the initial intake is very low.

Our study shows that in a population of modestly hypercholesterolemic individuals, only some respond readily to changes in dietary fat and in cholesterol, with corresponding changes in plasma cholesterol levels. Others responded minimally. Whether or not this represents a normal distribution of responses or specific metabolically regulated adaptations has been debated at length. There was clearly a degree of consistency in the responses among those who were classified as being diet-sensitive. First, in their habitual dietary environment, the diet-sensitive group's average plasma cholesterol was higher than among the diet-insensitive group. Second, the diet-sensitive group showed greater responses to dietary fat, to dietary cholesterol, and to both. Finally, on multiple regression, the two significant determinants of the response to dietary cholesterol were group designation and the change in LDL cholesterol with fat supplementation.

Interestingly, the normocholesterolemic group, which responded with a mean 4.1% fall in plasma cholesterol on the basal low fat diet, did not show a rise in plasma cholesterol with either the fat or the cholesterol supplement. It must be remembered, however, that the initial reduction in dietary fat was much greater than the fat supplement. In fact, for all subjects, the mean fall in plasma cholesterol on the low fat, low cholesterol diet (14%) was four times greater than the average rise with the cholesterol supplement. This suggests that the reduction in saturated fat on the baseline diet accounted for most of the fall in plasma cholesterol. This is consistent with many such previous conclusions.

Katan et al. have also presented suggestive evidence for a relationship between the response to a change in cholesterol intake and the response to a change in fat intake, although this did not occur in every one of their studies. The inconsistencies in their study may have been due to the high background fat intake. The low fat diet used in our study may have allowed a clearer expression of sensitivity to cholesterol.

Although we distinguished the sensitivity and insensitivity on the basis of individual responses to the baseline diet, there was only a weak trend for these changes to correlate with changes brought about by the supplements. Nevertheless, Keys has argued for the validity of grouping subjects in this manner and demonstrating significant group associations in the face of no individual correlations.

The mechanisms underlying the differences in responsiveness have been discussed elsewhere. Factors associated with both the production and the removal of lipoproteins are likely to be involved. Down-regulation of LDL receptors occurs with high cholesterol intakes; a similar response has been noted for saturated fat only in hamsters, and not in humans. Since excess dietary cholesterol induces increased hepatic secretion of IDL and of LDL, responsiveness may reflect the capacity of...
removal mechanisms to clear the influx of lipoprotein. Similarly, saturated fatty acids induce higher LDL secretion rates in humans and greater triglyceride synthesis in rat liver than do more unsaturated fatty acids. Responsiveness to dietary cholesterol has also been attributed to compensatory mechanisms involved in maintaining cholesterol homeostasis in the body. Plasma cholesterol levels rise more clearly when cholesterol synthesis is not adequately suppressed and cholesterol re-excretion not increased appropriately.

A further determinant of the plasma cholesterol response to dietary cholesterol and possibly fat may be the capacity of the HDL system to accept excess circulating lipid. Patsch et al. have shown that this is a critical factor in controlling postprandial lipemia. We have previously shown in subjects eating cholesterol an increase in HDL_2 cholesterol (as a proportion of total cholesterol), which we postulated might have prevented a rise in total cholesterol.

In the present study, we have observed in both the normocholesterolemic and diet-insensitive hypercholesterolemic groups a larger proportional rise in HDL than in LDL cholesterol with cholesterol supplementation. Consequently, the total/HDL cholesterol ratio decreased in these two groups. However, since the diet-sensitive group experienced a similar rise in HDL cholesterol, the increase in LDL cholesterol in this group cannot be attributed to a failure of HDL to accept cholesterol.

The increase in HDL_2 protein and cholesterol with dietary cholesterol has been noted previously by ourselves and others. In the present study, the increase was less than we have reported in a different group of subjects. Oh and Miller also noted a significant increase in the HDL_2/HDL_3 ratio without a change in HDL cholesterol in their hyperresponders. However, we have failed to confirm the observations of Oh and Miller and Beynen and Katan of a positive correlation between baseline HDL cholesterol and response to dietary cholesterol. Buzzard et al. noted an inverse association between initial HDL cholesterol and the change in total cholesterol with egg yolk feeding. The most significant association, especially in the diet-insensitive group which responded predominantly through HDL, was that between baseline HDL cholesterol and the change in HDL cholesterol with dietary cholesterol. This mirrored the weaker findings with LDL cholesterol.

The significance of the apo E phenotype in the present study is not clear. The presence of one E4 allele appeared to be associated with a lower sensitivity to dietary change. However, two individuals homozygous for E4 were quite sensitive, as were three subjects with an E2 allele. These results conflict with those of Kesäniemi et al. who showed that, in Finns, an E4 allele is associated with increased responsiveness to dietary cholesterol through increased cholesterol absorption. The number of subjects in our study may have been too low to draw any firm conclusions.

A final important consideration is the degree to which these hypercholesterolemic subjects were representative of the general population of mildly hypercholesterolemic individuals. Those with obviously predominant genetic disease were excluded. We selected equal numbers of subjects who, in the past, had responded satisfactorily or otherwise to advice from a nutritionist employed by the National Heart Foundation of Australia. It is general experience that at least half of healthy individuals found at screening to be hypercholesterolemic and subsequently counseled by a dietitian will respond with at least a 10% fall in plasma cholesterol. To this extent, this group is not grossly unrepresentative of subjects with polygenic hypercholesterolemia, although the proportion of unresponsive individuals may be a little high.

There are several public health implications to this study. Individuals who are found to be hypercholesterolemic but clearly responsive to a prudent (low saturated fat, low cholesterol) diet should avoid dietary cholesterol. By contrast, normocholesterolemic subjects appear less responsive to dietary cholesterol, and the need to restrict egg yolks, for instance, is less obvious. Nevertheless, this conclusion may be premature if dietary cholesterol predisposes to coronary atherosclerosis even when plasma cholesterol levels are not raised. This possibility has been suggested by Shekelle et al. on the basis of epidemiological surveys and may reflect the increased flux of lipoprotein cholesterol through plasma.

Acknowledgments

The authors thank Margaret Dewar, Lena Baldassare, and Paula Bothe for their excellent technical assistance.

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Index Terms: cholesterol • fats • diets • low density lipoproteins • high density lipoproteins
Relationship between sensitivity to dietary fat and dietary cholesterol.
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doi: 10.1161/01.ATV.10.3.394

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