Dietary Fish Oil Increases Conversion of Very Low Density Lipoprotein Apoprotein B to Low Density Lipoprotein

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Dietary fish oils, which are rich in omega-3 fatty acids, are known to produce a marked lowering of very low density lipoprotein (VLDL) triglyceride concentrations, but they have a less marked effect on low density lipoprotein (LDL) cholesterol. Our previous apolipoprotein (apo) B kinetic studies in miniature pigs demonstrated that conversion of VLDL apo B to LDL apo B accounted for 15% to 20% of total VLDL apo B catabolism. In addition, 75% to 80% of LDL apo B was derived independent of plasma VLDL or intermediate density lipoprotein (IDL) apo B catabolism. The present studies were carried out to determine if fish-oil diets influenced: 1) the conversion of VLDL to LDL, and 2) the pathways of LDL apo B synthesis. Autologous 125I-VLDL and 131I-LDL were injected into four pigs after both a corn-oil (30 g/day for 18 days) and a Maxepa (30 g/day for 18 days) dietary period. Analysis of apo B specific activity curves demonstrated that fish oil reduced the VLDL pool size by 38% (p<0.05) due to an increase in fractional catabolic rate (0.83±0.13 vs. 0.48±0.03 hr⁻¹), as the synthesis rate was unaffected. However, the proportion of VLDL apo B converted to LDL increased significantly (56±7% vs. 17±3%, p<0.01) whereas the proportion cleared directly decreased (46±5% vs. 83±3%, p<0.005). Fish oil reduced total LDL apo B synthesis (0.6±0.1 vs. 1.1±0.2 mg/hr/kg, p<0.05). LDL-B derived independent of VLDL catabolism was reduced by 90% (0.1±0.04 vs. 0.9±0.2 mg/hr/kg, p<0.01), whereas VLDL derived synthesis increased significantly (0.5±0.08 vs. 0.1±0.01, p<0.01). Although LDL apo B fractional catabolic rate decreased 22% (p<0.01), the pool size decreased 20% (p<0.05) due to the larger decrease in synthesis. Total lipid profiling revealed no major differences in the percent composition of the main lipid classes present in VLDL, IDL, and LDL. Thus, the fish-oil diet resulted in the secretion of a VLDL particle that is preferentially converted to LDL. This may explain the inconsistent and variable effects of fish oil on LDL concentrations observed in other studies. Whether LDL concentrations are increased would depend on other factors regulating LDL concentrations. In the present study, the LDL-B pool size was reduced, due entirely to the marked reduction in VLDL independent synthesis.


It is well documented that the intake of marine (fish) oils, which are rich in omega-3 polyunsaturated fatty acids, significantly reduces plasma very low density lipoprotein (VLDL) triglyceride concentrations.¹⁻⁴ This effect is greater than with equal amounts of omega-6-rich vegetable oils.¹⁻³⁴ Nestel et al. have shown in both in vivo² and in vitro⁵,⁶ studies that the reduction in VLDL concentrations associated with fish-oil consumption is due to reduced synthesis of VLDL triglyceride and apoprotein (apo) B.

Since the majority of low density lipoprotein (LDL) apo B in normal humans is derived from VLDL apo B,⁷,⁸ it might be expected that fish-oil treatment would decrease LDL cholesterol and apo B levels. However, the effect on LDL cholesterol and apo B is inconsistent.¹,⁴,⁹ Illingworth et al.¹⁰ demonstrated a reduction of LDL cholesterol and apo B with fish oil that was, indeed, due to a reduced rate of LDL synthesis. On the other hand, in studies in which triglyceride-lowering effects were observed, no change or increases in LDL cholesterol and apo B were reported.²,⁶ The reasons for this are not known, but they may be related to the variable effects of fish oils on the complex mechanisms regulating LDL concentrations.

In normal humans, the proportion of VLDL apo B flux converted to LDL is variable.⁷,¹¹ For example, in hypertriglyceridemic subjects, compared to normals,⁷,¹² the proportion converted to LDL is decreased and the proportion cleared directly is increased. Thus, a reduction in VLDL apo B synthesis (flux) by fish-oil treatment could result in a decreased conversion of VLDL apo B to LDL, a decreased direct removal of VLDL apo B, or both. It is possible that an effect of fish oil is a reduction in direct VLDL removal and that the rise in LDL observed in some studies²,⁶ was due to an increase in VLDL converted to LDL. This possibility has not been tested.
FISH OIL ENHANCES CONVERSION OF VLDL TO LDL  Huff and Telford

In several animal species, including primates, rabbits, and miniature pigs, a large proportion of VLDL apo B is cleared directly from the circulation without conversion to LDL. Using apo B kinetic studies, we have also demonstrated in miniature pigs, as others have shown in primates, the metabolic heterogeneity of LDL formation. We found that over 80% of LDL apo B was synthesized directly, the remainder being derived from VLDL conversion. The increased secretion of LDL from perfused animal livers after cholesterol feeding indicated that the source of direct LDL synthesis was hepatic and thus may be related to the availability of hepatic cholesterol for transport. Kinetic studies have demonstrated direct LDL synthesis in both familial hypercholesterolemic homozygous and heterozygous subjects, as well as in familial combined hyperlipoproteinemic subjects, and, to a lesser extent, in normal humans. Treatment of familial combined hyperlipoproteinemic subjects reduced direct LDL synthesis, and weight loss in hypertriglyceridemic subjects increased LDL direct synthesis, indicating that this pathway could be regulated. We have also demonstrated that the LDL direct synthesis pathway in miniature pigs could be regulated. Enhanced cholesterol excretion by cholestyramine treatment and inhibition of cholesterol synthesis by mevinolin treatment inhibited the direct LDL synthesis pathway.

The present experiments carried out in miniature pigs were designed to answer two questions. First, would diets containing fish oil inhibit VLDL apo B synthesis (flux) and subsequently influence the proportion of VLDL apo B derived from the liver? Second, since it is known that fish oil inhibits VLDL synthesis, we wanted to determine if fish oil would also inhibit the direct synthesis of LDL.

**Methods**

**Animals and Diets**

Miniature pigs (20 to 35 kg) were obtained from a local supplier (Hyde Park Farms, Hyde Park, Ontario). After a 1-week acclimatization, an indwelling silastic catheter (0.078" ID) was surgically implanted in each external jugular vein under halothane anesthesia. Ketamine was used as a pre-anesthetic. The catheters were tunneled under the skin and externalized in the middle of the back. Three-way stop cocks were attached and held in place by a bandage and elastic netting. The catheters, kept patent by filling them with 7% EDTA-Na2, allowed for ease of sample injection as well as blood sampling throughout the study in unanesthetized animals. This protocol was approved by the animal care committee of the University of Western Ontario.

A crossover design in which each of four pigs received a diet containing either corn oil or Maxepa (R. P. Scherer, Windsor, Canada) for 18 to 20 days before lipoprotein turnover studies was used. Each animal was then switched to the other diet (either Maxepa or corn oil) for a further 18 to 20 days, followed by a second turnover study. Each animal received 750 g/day of Purina pig chow (16% protein, 5% fat, Ralston Purina, Longueuil, Quebec) to which 30 g of corn oil or 30 g of Maxepa were mixed just before feeding. The total daily fat content was 9% wt/wt. The Maxepa diet provided 9.45 g/day of omega-3 fatty acids (5.04 g/day of eicosapentaenoic acid, 20:5 w-3; 3.63 g/day docosahexaenoic acid, 22:6 w-3), 3.54 g/day of omega-6 fatty acids (3.48 g/day linoleic acid, 18:2, w-6), and 110 mg/day of cholesterol (contained in Maxepa oil). The corn-oil diet provided 17.0 g/day of omega-6 fatty acids (16.8 g/day, linoleic acid 18:2, w-6) and no omega-3 fatty acids. Cholesterol was dissolved in the corn oil to equal that present in the Maxepa diet. Maxepa oil, which contained <100 IU vitamin A/g, no vitamin D, and toco-pherol (1 IU/g) as an antioxidant, was aliquoted in daily doses and stored under N2 in sealed vials at 4°C until just before feeding. Maxepa and corn oil were then mixed with the feed, which was consumed within 1 hour.

**Lipoprotein Turnover Studies**

Lipoprotein turnover studies were conducted essentially as described previously. Autologous VLDL (SF 20 to 400) and LDL (SF 0 to 12) for radiolabeling were isolated from 100 ml of plasma obtained after a 16-hour fast. Radioiodination was performed using the iodine monochloride method as modified by Fidge and Pouli. Lipoproteins were sterilized by the addition of 100 μg/ml gentamycin sulfate (Schering, Pointe Claire, Quebec) and checked for sterility and pyrogenicity. VLDL contained less than 2% free iodine, 24% to 28% of the label was bound to lipid, and 25% to 35% of the protein-bound iodine was bound to apo B. LDL contained less than 1% free iodine, 20% to 24% lipid labeling; 80% to 90% of the LDL protein labeled was bound to apo B. After each dietary period, each of four pigs received 20 μCi of autologous labeled VLDL apo B and 15 μCi of autologous labeled LDL apo B. Animals were fasted for 16 hours before each study. After injection, blood samples (12 ml) were obtained at 5, 15, 30, and 45 minutes and at 1, 1.5, 2, 3, 4, 6, 12, 24, 30, 48, and 72 hours. Pigs received no food until after the 12-hour sample, at which time one-half of the daily feed was given. Animals were given their full diet after the 24- and 48-hour samples. This procedure limited the contribution of intestinally derived particles, resulting in constant concentrations of VLDL, IDL, and LDL apo B. VLDL (d<1.006), IDL (d=1.006 to 1.019), and LDL (d=1.019 to 1.063) were separated from plasma obtained from blood samples collected in tubes containing EDTA-Na2 (1.5 mg/ml). In three of four animals, as part of both turnover studies, LDL was subfractionated into LDL1 (d=1.019 to 1.040) and LDL2 (d=1.040 to 1.083). The density of total LDL was lowered to d=1.019 by the addition of d=1.006. LDL1 was separated by ultracentrifugation in a Beckman 50 Ti rotor, 12°C, 50K, for 24 hours. The infranatant was raised to d=1.063 by the addition of d=1.34 and was recentrifuged under the same conditions to give LDL2. Apo B was isolated from each lipoprotein fraction by isopropanol precipitation. Specific activities were calculated after counting the washed apo B pellet and subsequent determination of its protein content by a modified Lowry procedure. The plasma concentration of apo B in each lipoprotein fraction was determined by subtracting the protein value of the first precipitation supernatant from the
Total lipids were extracted by using chloroform/methanol (Peridochrom, GPO-PAP) and cholesterol chloride. LDL cholesterol was calculated by the difference in total cholesterol minus the sum of VLDL cholesterol and HDL cholesterol.

### Kinetic Analyses

The transport of apo B in VLDL and LDL was calculated from the apo B specific activity curves. As found previously, both curves were best described by a bi-exponential curve, and curve parameters were calculated by a computer with a nonlinear, least squares technique. Kinetic parameters yielded values for irreversible fractional catabolic rate (FCR) and the mass of apo B (pool size) in the largest, most rapidly turning over pool. Transport rates or flux were calculated by multiplying the FCR by the apo B mass (pool size). Details of the calculations have been published previously. 

### Analyses

Twice during each dietary period (immediately before and 5 days after each turnover study) plasma was obtained for lipid analysis. Total cholesterol and triglyceride, VLDL cholesterol and triglyceride, and HDL cholesterol concentrations were determined. VLDL were obtained after ultracentrifugation at d < 1.006, and HDL was obtained after precipitation of other lipoproteins by heparin-manganese chloride. LDL cholesterol was calculated by the difference method.

### Results

Concentrations of plasma triglyceride and cholesterol were both significantly reduced by the fish-oil diet compared to the corn-oil diet (Table 1). The fall in plasma triglyceride concentration (48%, p < 0.05) was due primarily to a 65% (p < 0.05) drop in VLDL triglyceride concentrations. The decline in total cholesterol (17%, p < 0.025) was related to a significant drop in HDL cholesterol concentrations (p < 0.01). There was a trend toward reduced VLDL and LDL cholesterol concentrations. Apolipoprotein B concentrations were also significantly reduced by the fish-oil diet. VLDL and LDL apo B levels declined by 37% (p < 0.01) and 25% (p < 0.05), respectively.

### Table 1. Plasma Lipid and Lipoprotein Concentrations in Miniature Pigs Consuming Corn- and Fish-oil Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Triglyceride</th>
<th>Cholesterol*</th>
<th>Apolipoprotein B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total VLDL</td>
<td>Total VLDL</td>
<td>VLDL LDL HDL</td>
</tr>
<tr>
<td>Corn oil</td>
<td>33±6 18±5</td>
<td>111±4 2±0.9</td>
<td>48±3 59±1</td>
</tr>
<tr>
<td>Fish oil</td>
<td>17±2 7±4</td>
<td>92±5 1.5±0.9</td>
<td>43±4 48±2</td>
</tr>
</tbody>
</table>

*p<0.01 p<0.05 p<0.005 p<0.025 NS p<0.001 p<0.05 p<0.005

Values are expressed as mg/dl. Each value is the mean±SEM of eight determinations, two samples from each of four animals. Significance was determined by paired t-test. NS = not significant.
The fish-oil diet had a striking effect on the source of LDL apo B. The percent of LDL derived from VLDL increased from 13%±1.9% to 78%±6% during the fish-oil period (Table 3). The peak LDL apo B specific activity value occurred close to the point where it crossed the IDL, and subsequently, the VLDL apo B curves. We have interpreted this as indicating that the independent synthesis of LDL apo B was dramatically reduced, suggesting that most LDL apo B is derived from VLDL/IDL catabolism. The fish-oil diet significantly reduced the pool size of VLDL apo B by 38% (1.13±0.14 mg/hr/kg, p<0.05) despite a significant fall in the irreversible fractional catabolic rate (0.046±0.006 vs. 0.059±0.006 hr⁻¹, p<0.01).

The fish-oil diet had a striking effect on the source of LDL apo B. The percent of LDL derived from VLDL increased from 13%±1.9% to 78%±6% during the fish-oil period (Table 3). Also, the fraction of LDL derived from the direct synthesis pathway was markedly reduced in a reciprocal fashion (22%±6% vs. 87%±2%). This was calculated by the area under the curve method, which on average differed from the peak specific activity method by 4.8%. Knowing the fraction of total LDL apo B flux derived from VLDL and the total LDL apo B flux, we can calculate that the flux of LDL apo B not converted to LDL divided by the VLDL apo B pool size. This was interpreted as indicating that fish oil decreased the capacity for VLDL apo B direct removal. One would have expected that, in view of the decreased VLDL apo B pool size, an increased FCR would have been observed if the removal capacity had not been decreased.

Thus, compared to the corn-oil diet, fish oil did not affect total VLDL apo B flux (synthesis). However, the proportion of total flux converted to LDL was significantly increased, whereas the proportion cleared directly was decreased (Table 2). The fish-oil diet significantly reduced the entry of total apo B into plasma (1.05±0.18 mg of apo B/mg/kg vs. 1.84±0.35, p<0.05). The latter was calculated as: total VLDL apo B synthesis plus direct LDL apo B synthesis.

To determine if these marked changes in apo B metabolism were related to changes in lipoprotein particle composition, total lipid profiles of VLDL, IDL, LDL, and HDL were analyzed as shown in Table 4. No major differences between the fish-oil and corn-oil diet were observed for any of the parameters measured. The percentage of omega-3 fatty acids in phospholipids, cholesterol esters, and triglycerides increased on the fish-oil diet (data not shown). The amount of apo B as a percent of total protein in VLDL, IDL, and LDL was not altered. In three of the four animals, the effect of the fish-oil diet on the metabolism of LDL subfractions, LDL₁ (d=1.019 to 1.040) and LDL₂ (d=1.040 to 1.063) was determined as shown in Figure 2. The peak specific activity of both fractions occurred well before they crossed...
the regulation of LDL concentrations. 

However, direct conversion of VLDL to LDL must be a key component in plasma LDL, it has been assumed that the degree of plasma LDL concentrations by a diet rich in fish oil (omega-3) fatty acids was related to a decreased synthetic rate and to determine whether, in the miniature pig, the lowering of VLDL concentrations by a diet rich in fish oil (omega-3) was equivalent to the flux of LDL apo B derived from VLDL apo B. The latter is determined as described in the footnotes to Table 3. Values in brackets are a percent of the total flux.

The experiments reported in this paper were designed to determine whether, in the miniature pig, the lowering of VLDL concentrations by a diet rich in fish oil (omega-3) fatty acids was related to a decreased synthetic rate and whether this diet would influence the degree of conversion of VLDL to LDL. Since plasma VLDL is a precursor of plasma LDL, it has been assumed that the degree of conversion of VLDL to LDL must be a key component in the regulation of LDL concentrations. 

However, direct conversion of VLDL to LDL must be a key component in the regulation of LDL concentrations. 

their respective precursors, and the areas under the LDL1 and LDL2 curves were substantially lower than the VLDL curve suggesting dilution of both the LDL1 and LDL2 by unlabeled apo B. As calculated from the dilution factor, a mean of 17%±4% of LDL1 was derived from VLDL, and 73%±6% of LDL2 was derived from LDL1.

During the fish-oil period, the specific activities of LDL1 and LDL2 peaked closer to the point they crossed their respective precursors. As calculated from the dilution factors, LDL1 derived from VLDL increased to 85%±6% and LDL2 derived from LDL1 increased to 88%±4% of the total LDL2 flux. This indicates that the fish-oil diet significantly inhibited the independent synthesis of both LDL1 and LDL2. The mass of LDL2 apo B accounted for 85%±5% of the total plasma LDL apo B (which did not change with the diet). 

Discussion

The experiments reported in this paper were designed to determine whether, in the miniature pig, the lowering of VLDL concentrations by a diet rich in fish oil (omega-3) fatty acids was related to a decreased synthetic rate and whether this diet would influence the degree of conversion of VLDL to LDL. Since plasma VLDL is a precursor of plasma LDL, it has been assumed that the degree of conversion of VLDL to LDL must be a key component in the regulation of LDL concentrations.

Table 2. Metabolism of Very Low Density Lipoprotein Apolipoprotein B In Miniature Pigs Fed Diets Containing Corn Oil and Fish Oil

<table>
<thead>
<tr>
<th>Animal</th>
<th>Diet</th>
<th>Pool size* (mg/kg)</th>
<th>FCR† (hr⁻¹)</th>
<th>Flux total (mg/hr/kg)</th>
<th>Flux to LDL‡ (mg/hr/kg)</th>
<th>Flux direct removal§ (mg/hr/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>2.23</td>
<td>0.38</td>
<td>0.89</td>
<td>0.13</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.10</td>
<td>1.17</td>
<td>1.31</td>
<td>0.70</td>
<td>(54)</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>2.71</td>
<td>0.48</td>
<td>1.29</td>
<td>0.17</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.46</td>
<td>0.54</td>
<td>0.79</td>
<td>0.53</td>
<td>(77)</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>1.12</td>
<td>0.49</td>
<td>0.54</td>
<td>0.14</td>
<td>(26)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.80</td>
<td>0.85</td>
<td>0.68</td>
<td>0.33</td>
<td>(49)</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>1.20</td>
<td>0.55</td>
<td>0.66</td>
<td>0.11</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.16</td>
<td>0.74</td>
<td>0.86</td>
<td>0.38</td>
<td>(45)</td>
</tr>
<tr>
<td>Mean±SE</td>
<td>C</td>
<td>1.82±0.39</td>
<td>0.48±0.03</td>
<td>0.85±0.17</td>
<td>0.13±0.01</td>
<td>17.3±3</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.13±0.14</td>
<td>0.83±0.13</td>
<td>0.91±0.14</td>
<td>0.49±0.08</td>
<td>56.3±7</td>
</tr>
<tr>
<td>P&lt;</td>
<td></td>
<td>0.05</td>
<td>0.05</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Pool size refers to the mass of apo B in the largest pool (pool 1).
†FCR=irreversible fractional catabolic rate.
‡Calculated by subtracting the LDL apo B flux to LDL from the total VLDL apo B flux.
§Calculated by subtracting the VLDL apo B flux to LDL from the total VLDL apo B flux.

Table 3. Metabolism of Low Density Lipoprotein Apolipoprotein B In Miniature Pigs Fed Diets Containing Corn Oil and Fish Oil

<table>
<thead>
<tr>
<th>Animal</th>
<th>Diet</th>
<th>Pool size* (mg/kg)</th>
<th>FCR† (hr⁻¹)</th>
<th>Flux total (mg/hr/kg)</th>
<th>Flux from VLDL‡ (mg/hr/kg)</th>
<th>Flux direct synthesis§ (mg/hr/kg)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>19.6</td>
<td>0.080</td>
<td>1.56</td>
<td>0.13</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>15.2</td>
<td>0.064</td>
<td>0.95</td>
<td>0.70</td>
<td>(74)</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>26.7</td>
<td>0.055</td>
<td>1.45</td>
<td>0.17</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>12.9</td>
<td>0.044</td>
<td>0.56</td>
<td>0.53</td>
<td>(95)</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>15.9</td>
<td>0.053</td>
<td>0.84</td>
<td>0.14</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>13.2</td>
<td>0.035</td>
<td>0.46</td>
<td>0.33</td>
<td>(71)</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>13.6</td>
<td>0.051</td>
<td>0.68</td>
<td>0.11</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>13.2</td>
<td>0.040</td>
<td>0.52</td>
<td>0.36</td>
<td>(73)</td>
</tr>
<tr>
<td>Mean±SE</td>
<td>C</td>
<td>16.5±1.2</td>
<td>0.059±0.006</td>
<td>1.13±0.19</td>
<td>0.13±0.01</td>
<td>13±2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>13.3±0.6</td>
<td>0.046±0.006</td>
<td>0.62±0.11</td>
<td>0.49±0.08</td>
<td>78±6</td>
</tr>
<tr>
<td>P&lt;</td>
<td></td>
<td>0.05</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
<td>0.005</td>
</tr>
</tbody>
</table>
secretion of LDL 
triglyceride-poor particles with a density of LDL.

Our experiments have confirmed the triglyceride-lowering effects of omega-3 fatty acids (Maxepa) compared to omega-6 fatty acids (corn oil), reported in studies in humans 
and in rats. Although the effect was less marked, we observed lower LDL cholesterol and apo B concentrations with the fish-oil diet (Table 1). This is consistent with previous reports in normal humans, but differs from the rise or lack of change in LDL cholesterol in humans and in rats. Although the effect was somewhat less marked, we observed lower LDL cholesterol and apo B concentrations with the fish-oil diet. This was related to both LDL, and LDL2 density classes. The kinetic data indicate that fish oil inhibited the direct synthesis of VLDL remnants is thought to occur via this receptor. The lack of change in FCR for VLDL apo B removed directly, in spite of a lower VLDL apo B pool size, is consistent with this interpretation.

The increased conversion of VLDL apo B to LDL during the fish-oil diet could also be related to the production of a smaller VLDL particle of altered lipid composition. Packard et al. reported that smaller VLDL particles are preferentially converted to LDL. However, results from the lipid analyses by total lipid profiling (Table 4) do not support this idea. No change in estimated particle diameter or proportions of the major lipid classes were observed.

Sullivan et al. reported that men fed fish oil had reduced VLDL particle size; however, the control diet, unlike our experiments, was not based on polyunsaturated fatty acids of the omega-6 series. In our experiments, the increased proportions of omega-3 fatty acids in the major VLDL lipid classes may have enhanced the conversion to LDL. Although not determined in the present study, differences in VLDL small molecular weight apoprotein composition, such as reduced apo E content, may have been a factor. Yamada et al. have demonstrated in rabbits that VLDL particles poor in apo E are preferentially converted to LDL.

In the present studies, the increased conversion of VLDL apo B to LDL by fish oil did not result in an increased LDL apo B pool size. LDL pool size actually decreased by 20% (Table 3), which was due primarily to the decrease in LDL apo B direct synthesis being much larger than the increase in LDL apo B derived from VLDL. This net decrease in total LDL apo B synthesis is consistent with the work of Illingworth et al., who found a decrease in LDL apo B synthesis in normal men fed fish-oil diets. The observed fall in LDL apo B direct synthesis is not consistent with the idea proposed by Nestel et al. that a decreased production rate of VLDL triglyceride may lead to an increased secretion of triglyceride-poor particles with a density of LDL.

The direct synthesis of LDL in the corn-oil fed pigs was related to both LDL1 and LDL2 density classes. The kinetic data indicate that fish oil inhibited the direct synthesis of

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**Table 4. Effects of Fish-Oil Diet on Percent Lipid Composition of Plasma Lipoproteins**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>C</th>
<th>M</th>
<th>C</th>
<th>M</th>
<th>C</th>
<th>M</th>
<th>C</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unesterified cholesterol</td>
<td>3.5</td>
<td>3.6</td>
<td>9.1</td>
<td>7.6</td>
<td>9.9</td>
<td>9.8</td>
<td>4.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>11.5</td>
<td>12.1</td>
<td>23.6</td>
<td>27.6</td>
<td>54.9</td>
<td>51.1</td>
<td>41.7</td>
<td>46.5</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>25.8</td>
<td>24.0</td>
<td>33.1</td>
<td>33.0</td>
<td>32.4</td>
<td>34.8</td>
<td>52.7</td>
<td>47.5</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>58.8</td>
<td>60.1</td>
<td>34.0</td>
<td>31.5</td>
<td>3.2</td>
<td>4.6</td>
<td>1.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Cholesterol ester: triglyceride</td>
<td>0.261</td>
<td>0.273</td>
<td>0.91</td>
<td>1.28</td>
<td>47.9</td>
<td>41.2</td>
<td>28.7</td>
<td>21.1</td>
</tr>
<tr>
<td>Unesterified cholesterol: phospholipid</td>
<td>0.266</td>
<td>0.301</td>
<td>0.555</td>
<td>0.465</td>
<td>0.62</td>
<td>0.58</td>
<td>0.234</td>
<td>0.231</td>
</tr>
<tr>
<td>Apo B (percent of total protein)</td>
<td>23</td>
<td>20</td>
<td>21</td>
<td>17</td>
<td>84</td>
<td>89</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Estimated average diameter (Å²)</td>
<td>318</td>
<td>374</td>
<td>210</td>
<td>210</td>
<td>210</td>
<td>198</td>
<td>128</td>
<td>149</td>
</tr>
</tbody>
</table>

C = corn-oil diet, M = Maxepa (fish-oil) diet, NS = not significant. Values are the means from four animals.
both LDL₁ and LDL₂. The results with the animals fed corn oil differ somewhat from our previous studies. In pigs fed only chow, no direct LDL₁ synthesis was observed (Huff MW, Telford DE, unpublished observations). Although paired experiments have not been carried out to confirm this finding, it is possible that LDL synthesized directly in corn-oil-fed animals is lighter in density and thus a greater proportion enters the circulation as LDL₁. The physiological significance of an elevated direct synthesis of LDL apo B, particularly LDL₂, is not known. However, it is possible that the metabolism of directly synthesized LDL differs from that of LDL derived from VLDL and, if associated with high levels of plasma LDL, may contribute to the accelerated atherosclerosis observed in patients with hypercholesterolemia and familial combined hyperlipoproteinemia.\(^{17,18,19}\) We have proposed that the direct synthesis pathway of LDL formation is related to the amount of hepatic cholesterol relative to triglyceride destined for hepatic secretion.\(^{20}\) Previously, we demonstrated that this pathway was inhibited by cholestyramine and/or mevinolin treatment.\(^{21}\) It is possible that fish oil reduces hepatic cholesterol concentrations and reduces apo B synthesis. Rats fed fish oil have lower liver cholesterol concentrations, cholesterol synthesis, and an increased rate of biliary cholesterol excretion compared to rats fed safflower oil.\(^{34}\) Omega-3 fatty acids have been shown to inhibit the synthesis of apo B by cultured liver cells (Hep G2).\(^{35}\) It is possible that the inhibition of direct LDL synthesis observed in our studies with fish oil is due to the inhibition of apo B and cholesterol destined for assembly and secretion with LDL-like particles.

The concept of direct LDL apo B synthesis remains controversial even though other investigators have interpreted their kinetic data in terms of direct LDL apo B synthesis.\(^{3,12,14,17-22}\) An alternate explanation for direct synthesis of LDL is the secretion from the liver of VLDL-like particles that are rapidly converted to LDL, but whose plasma pool size within the VLDL density range is very small and thus are not labeled with plasma VLDL nor contribute to the VLDL apo B mass for specific activity determinations. It is possible that the fish-oil diet selectively inhibited the synthesis of this hypothetical VLDL subpopulation. Our studies could not rule out this possibility. It should be pointed out that in our studies total apo B synthesis (total VLDL plus LDL direct synthesis) was reduced by 41% with fish-oil treatment.

The observed decrease in LDL apo B FCR and the increased conversion of VLDL apo B to LDL could be interpreted in terms of a decrease in hepatic LDL receptor activity. Wong et al.\(^{39}\) have reported lower LDL (B/E) receptor activity in Hep G2 cells after preincubation with eicosapentaenoic acid. Roach et al.\(^{39}\) have demonstrated a decreased LDL receptor activity in liver membranes isolated from rats fed fish oil. Illingworth et al.\(^{40}\) demonstrated no consistent difference in LDL apo B FCR in normal men fed fish oil, although the FCR decreased in five of seven subjects studied.

Witztum et al.\(^{37}\) have demonstrated that alterations in LDL composition can result in a reduced LDL apo B FCR due to a poor interaction of LDL with altered composition with the LDL receptor. In the present experiments, plasma LDL composition during the corn-oil period, which was derived mainly from direct LDL synthesis, and LDL during the fish-oil period, which was derived mainly from VLDL catabolism, did not differ in terms of size or percent composition of lipid classes. Thus, once in the circulation, LDL derived from either source had similar composition of major lipids, although the percent of omega-3 fatty acids was higher on fish oil. However, the control monkeys were fed a lard diet rather than a diet rich in omega-6 fatty acids.

The findings in the present study clearly demonstrate that fish oil reduces VLDL apo B concentrations; however, the proportion converted to LDL is increased. LDL apo B concentrations are not elevated due to the greater reduction in the direct LDL synthesis pathway. The increased conversion of VLDL apo B to LDL apo B combined with reduced fractional catabolic rates for LDL apo B suggest a down-regulation of hepatic LDL receptor activity. Whether this mechanism is responsible for the variable and incon-
sistent effect of fish oil on LDL cholesterol concentrations in humans requires further investigation.

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M W Huff and D E Telford

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