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

Murray W. Huff and Dawn E. Telford

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.

FISH OIL ENHANCES CONVERSION OF VLDL TO LDL  Huff and Telford 59

In several animal species, including primates,12 rabbits,13 and miniature pigs,14 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,14 as others have shown in primates,12 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 feeding15,16 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 homozygous17 and heterozygous subjects,18 as well as in familial combined hyperlipidemic subjects19 and, to a lesser extent, in normal humans.20 Treatment of familial combined hyperlipoproteinemic subjects reduced direct LDL synthesis,19 and weight loss in hypertriglyceridemic subjects increased LDL direct synthesis,21 indicating that this pathway could be regulated. We have also demonstrated that the LDL direct synthesis pathway in miniature pigs could be regulated.22 Enhanced cholesterol excretion by cholestyramine treatment and inhibition of cholesterol synthesis by mevinolin treatment inhibited the direct LDL synthesis pathway.22

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 contained in LDL, compared to that removed directly? 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 for ease of sample injection as well as blood sampling throughout the study in unanesthetized animals. This protocol was designed to answer two questions. First, would diets containing fish oil or Maxepa (R. P. Scherer, Hyde Park Farms, Hyde Park, Ontario) inhibit LDL direct synthesis in both familial hypercholesterolemic homozgyous22 and heterozygous subjects,18 as well as in familial combined hyperlipoproteinemic subjects19 and, to a lesser extent, in normal humans.20 Treatment of familial combined hyperlipoproteinemic subjects reduced direct LDL synthesis,19 and weight loss in hypertriglyceridemic subjects increased LDL direct synthesis,21 indicating that this pathway could be regulated. We have also demonstrated that the LDL direct synthesis pathway in miniature pigs could be regulated.22 Enhanced cholesterol excretion by cholestyramine treatment and inhibition of cholesterol synthesis by mevinolin treatment inhibited the direct LDL synthesis pathway.22

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 contained in LDL, compared to that removed directly? 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 for ease of sample injection as well as blood sampling throughout the study in unanesthetized animals. This protocol was designed to answer two questions. First, would diets containing fish oil or Maxepa (R. P. Scherer, Hyde Park Farms, Hyde Park, Ontario) inhibit LDL direct synthesis in both familial hypercholesterolemic homozgyous22 and heterozygous subjects,18 as well as in familial combined hyperlipoproteinemic subjects19 and, to a lesser extent, in normal humans.20 Treatment of familial combined hyperlipoproteinemic subjects reduced direct LDL synthesis,19 and weight loss in hypertriglyceridemic subjects increased LDL direct synthesis,21 indicating that this pathway could be regulated. We have also demonstrated that the LDL direct synthesis pathway in miniature pigs could be regulated.22 Enhanced cholesterol excretion by cholestyramine treatment and inhibition of cholesterol synthesis by mevinolin treatment inhibited the direct LDL synthesis pathway.22

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 contained in LDL, compared to that removed directly? 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 for ease of sample injection as well as blood sampling throughout the study in unanesthetized animals. This protocol was 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 contained in LDL, compared to that removed directly? 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 for ease of sample injection as well as blood sampling throughout the study in unanesthetized animals. This protocol was 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 contained in LDL, compared to that removed directly? 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.
Total lipids were extracted by using chloroform/methanol (Peridochrom, GPO-PAP) and cholesterol for lipid analysis. Total cholesterol and triglyceride, VLDL analyses were obtained by ultracentrifugation\(^22\) and were analyzed for lipids by total lipid profiling by using gas-liquid chromatography.\(^30\) LDL cholesterol was calculated as 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.\(^24\) As found previously,\(^22\) both curves were best described by a biexponential curve, and curve parameters were calculated by a computer with a nonlinear, least squares technique.\(^27\) 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.\(^3,7,28\) The 125\(^I\)-labeled apo B specific activity curves for VLDL, IDL, and LDL were compared, which allowed for the calculation of precursor-product relationships between lipoprotein fractions. Precursor-product relationships were assessed by two methods: 1) the peak product specific activity method described by Zilversmit\(^29\) and applied to apo B kinetics as reported previously,\(^7,14,22,28\) and 2) the area under the specific activity curves as described by Goldberg et al.\(^12\) and applied previously to apo B kinetics.\(^12,14,22\) The rationale and assumptions involved in using these methods has been discussed previously.\(^22\)

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.\(^14\) LDL cholesterol was calculated by the difference. Enzymatic methods described in kits obtained from Boehringer-Mannheim (Montreal, Canada) were used for triglyceride (Peridochrom, GPO-PAP) and cholesterol (CHOD-PAP C-system) analyses. In addition, VLDL, IDL, LDL (\(d=1.019\) to 1.063) and HDL (\(d=1.063\) to 1.21) were isolated by ultracentrifugation\(^22\) and were analyzed for lipids by total lipid profiling by using gas-liquid chromatography.\(^30\)

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</td>
<td>VLDL</td>
<td>Total</td>
</tr>
<tr>
<td>Corn oil</td>
<td>33±6</td>
<td>18±5</td>
<td>111±4</td>
</tr>
<tr>
<td>Fish oil</td>
<td>17±2</td>
<td>7±4</td>
<td>92±5</td>
</tr>
<tr>
<td>Significance</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.025</td>
</tr>
</tbody>
</table>

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.

VLDL cholesterol was determined after ultracentrifugation at \(d<1.006\). HDL cholesterol was determined after precipitation of apo-B-containing lipoproteins from plasma. LDL cholesterol was calculated by the dilution factor: 1 - (area under the LDL specific activity curve/area under the VLDL specific activity curve). This latter method does not require estimation of the exact point of peak specific (2:1, vol/vol) after dephosphorylation of the choline containing phospholipids by phospholipase-C.\(^30\) Dietary fatty acid composition was determined by gas-chromatography using a 2 meter column (SP 2330, liquid phase) on a Varian 6000 gas chromatograph. Lipoprotein protein was determined by the method of Markwell et al.\(^24\) Differences between control and treatment values were analyzed by paired \(t\) analysis.

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.

Autologous iodinated VLDL and LDL were simultaneously injected into each pig at the end of each dietary period. Apo B specific activity curves for VLDL, IDL, and LDL from one animal after injection of VLDL are shown in Figure 1. Examination of precursor-product relationships during the corn-oil period (Figure 1A) demonstrated that the maximal value for LDL apo B was reached well before it crossed the specific activity curves of its precursors, IDL and VLDL. We have interpreted this as indicating that a substantial proportion of LDL was derived independent of IDL and VLDL catabolism. The fraction of LDL synthesis derived from the catabolism of VLDL and LDL was determined by two methods as described previously.\(^22\) In the first method, described initially by Zilversmit\(^29\), this fraction was derived by calculating the ratio of the peak LDL specific activity value to the LDL specific activity value at the same time. Subsequently, the ratio of the peak LDL specific activity to the VLDL specific activity value was calculated. In the second method described by Goldberg et al.\(^12\) the fraction of LDL not derived from VLDL or IDL was determined by calculating the dilution factor: 1 - (area under the LDL specific activity curve/area under the VLDL or IDL specific activity curve). This latter method does not require estimation of the exact point of peak specific
activity values. We have previously applied this method to apo B metabolism in miniature pigs.\textsuperscript{22}

Figure 1B depicts apo B specific activity curves obtained from the same animal during the fish-oil dietary period. 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 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 vs. 1.82±0.38 mg/kg, $p<0.05$) as shown in Table 2. This was primarily due to an increase in irreversible fractional catabolic rate (0.83±0.13 vs. 0.48±0.03 hr\(^{-1}\), $p<0.05$) as the total flux or production rate was not altered (0.95±0.14 vs. 0.85±0.17 mg/hr/kg). Compared to the corn-oil diet, the fish-oil diet also reduced the LDL apo B pool size, but to a lesser extent than for VLDL (Table 3). LDL apo B declined by 20\% (13.3±0.6 vs. 16.5±1.2 mg/kg, $p<0.05$). This was primarily due to a decrease in flux or production rate by 40\% (0.62±0.11 vs. 1.13±0.19 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 derived from VLDL increased significantly (0.49±0.08 vs. 0.13±0.01 mg/hr/kg, $p<0.01$) on the fish-oil diet (Table 3). In contrast, the independent synthesis of LDL apo B was significantly reduced (0.13±0.04 vs. 0.99±0.19 mg/hr/kg, $p<0.025$). Of the total VLDL apo B flux, we know the amount converted to LDL, since this is equivalent to the amount of LDL apo B derived from VLDL. Subtracting this value from the total VLDL apo B flux, we determined the flux of VLDL apo B leaving the circulation directly (Table 2). Fish oil reduced the flux of VLDL apo B removed by this pathway by 40\%, but due to the variability between animals this was not significant. However, as a percent of total VLDL apo B flux, the amount removed by this pathway was reduced by 44\%, $p<0.005$. The fractional catabolic rate of VLDL apo B cleared directly did not change with the fish-oil diet (0.39±0.16 vs. 0.40±0.05 hr\(^{-1}\)). This was calculated as: the flux of VLDL 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, LDL\(_1\) (d=1.019 to 1.040) and LDL\(_2\) (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...
by guest on May 4, 2017 http://atvb.ahajournals.org/ Downloaded from

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>0.76</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>0.61</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>1.12</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>0.26</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>0.40</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>0.35</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>0.55</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>0.48</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 VLDL apo B flux to LDL apo B derived by direct synthesis from total LDL apo B flux.
C=com-oil diet, M=Maxepa (fish-oil) diet. Probability determined by paired t test.

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>
</tr>
</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>1.43</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>0.25</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>1.28</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>0.03</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>0.70</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>0.13</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>0.57</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>13.2</td>
<td>0.040</td>
<td>0.52</td>
<td>0.38</td>
<td>0.14</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>0.05</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 (from the 125I-LDL apo B curve) derived by direct synthesis from total LDL apo B flux.
C=com-oil diet, M=Maxepa (fish-oil) diet. Probability determined by paired t test.

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.21 However, direct
secretion of LDL,11-17 might also regulate steady-state plasma LDL concentrations. We have shown that the miniature pig possesses a substantial direct LDL synthesis pathway,14,22 which allowed us to assess the effect of fish oil on its regulation.

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 humans1-4,9,10 and in rats.5,9 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,4,10,31 but differs from the rise or lack of change in LDL cholesterol and apo B reported by others.4,12,39

The reduced VLDL triglyceride concentrations on the fish-oil diet in the present study were associated with a reduced VLDL apo B pool size (Table 2). This was related to an increased fractional catabolic rate, since flux or synthesis was not changed. Nestel et al.3 also observed that the VLDL apo B FCR was markedly increased in normal men fed fish oil, but in contrast to our results, they showed a substantial reduction in VLDL apo B synthesis. The reason for this difference is not known, but may be due to the fact that the already low VLDL apo B synthetic rates during the corn-oil period, (which were lower than observed in control animals previously) could not be lowered further by the fish-oil diet.

In the present study, we determined that fish oil has a marked effect on the catabolic fate of VLDL apo B compared to the corn-oil diet. The proportion of VLDL apo B converted to LDL increased fourfold, whereas the proportion removed directly decreased by 50% (Table 2). To our knowledge, the effect of fish oil on the metabolic channeling of VLDL apo B has not been reported. Our unique observation may explain why in some studies in humans, LDL concentrations are not changed or may actually rise,1,2,3,9 even though VLDL apo B production was reduced.3

The shift from VLDL removal in favor of conversion to LDL in our studies may be related to a lower hepatic LDL (B/E) receptor activity (as discussed below), since hepatic uptake of VLDL remnants is thought to occur via this receptor.32 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.33 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.3 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.13 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.,10 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.3 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
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 LDL. 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.22 Previously, we demonstrated that this pathway was inhibited by cholestyramine and/or mevinolin treatment.22 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.35 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.10 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 during the corn-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.

Acknowledgments

We thank Kim Woodcroft for expert technical assistance, Jay Engle and Chris Lipohar for performing the surgery, and Lynn Thomson for manuscript preparation. We are grateful to W. Carl Breckenridge, Dalhousie University, Halifax, Nova Scotia, for performing the total lipid profiling.

References


35. Wong S, Nestel PJ. Eicosapentaenoic acid inhibits the secretion of triglyceride and of apoprotein B and the binding of LDL in Hep G2 cells. Atherosclerosis 1987;64:139–146


Index Terms: fish oils • Maxepa • VLDL • LDL • apolipoprotein B metabolism
Dietary fish oil increases conversion of very low density lipoprotein apoprotein B to low density lipoprotein.

M W Huff and D E Telford

doi: 10.1161/01.ATV.9.1.58

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/9/1/58

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/