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.


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.¹⁻³,⁴ 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,⁷,⁸ 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 studies⁷,⁸ was due to an increase in VLDL converted to LDL. This possibility has not been tested.
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 hyperlipidemic 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 LDL apo B converted to 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 with 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 dietary 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 tocopherol (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 Poulis as described previously for pigs. 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 food was given. Animals were given their full diet after the 24- and 48-hour samples. This procedure limited the contribution of intrahepatically derived particles, resulting in constant concentrations of VLDL, LDL, and LDL apo B. LDL (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 1.063). 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 for lipid analysis. Total cholesterol and triglyceride, VLDL analyses were performed after precipitation of other lipoproteins by heparin-manganese (Boehringer-Mannheim (Montreal, Canada) were used for the determination of apo B-containing lipoproteins from plasma. 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. 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. The 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 and applied to apo B kinetics as reported previously, and 2) the area under the specific activity curves as described by Goldberg et al. and applied previously to apo B kinetics. The rationale and assumptions involved in using these methods have been discussed 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 apo B-containing lipoproteins from plasma. LDL cholesterol was calculated as total cholesterol minus the sum of VLDL cholesterol and HDL cholesterol.

### 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.

Apolipoprotein B concentrations were assessed by two methods: 1) the peak product specific activity method described by Zilversmit and applied to apo B kinetics as reported previously, and 2) the area under the specific activity curves as described by Goldberg et al. and applied previously to apo B kinetics. The rationale and assumptions involved in using these methods have been discussed previously.

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

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total Cholesterol</th>
<th>VLDL Cholesterol</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>33±6</td>
<td>18±5</td>
<td>111±4</td>
<td>59±1</td>
</tr>
<tr>
<td>Fish oil</td>
<td>48±3</td>
<td>15±0.9</td>
<td>43±4</td>
<td>48±2</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 as total cholesterol minus the sum of VLDL cholesterol and HDL cholesterol.**

Differences between control and treatment values were analyzed by paired t analysis.
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Figure 1. Apoprotein B precursor-product relationships between VLDL, IDL, and LDL fractions after the injection of radiolabeled VLDL. The results are from animal #1. A was obtained during the corn-oil dietary period and B, during the fish-oil (Maxepa) period.

Activity values. We have previously applied this method to apo B metabolism in miniature pigs. 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±0.19 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 LDL 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, 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 latter was determined from the 131 I-LDL apo B specific activity curve. Values in brackets are a percent of total flux.

During the fish-oil period, the specific activities of LDL, and LDL2 increased to 88%±4% of their respective precursors, and the areas under the VLDL, 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 diets).

### 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>(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>(28)</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>
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<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>
</tbody>
</table>

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>
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<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±2</td>
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<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±6</td>
</tr>
</tbody>
</table>

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 might also regulate steady-state plasma LDL concentrations. We have shown that the miniature pig possesses a substantial direct LDL synthesis pathway, 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 humans and 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 and in rats, but differs from the rise or lack of change in LDL cholesterol in some studies in normal men fed fish oil, but in contrast to our results, they observed a decrease in total LDL apo B synthesis. Sullivan et al. reported that men fed fish oil had reduced LDL 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 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. 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 VLDL apo B pool size actually rose, even though VLDL apo B production was reduced.

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. The lack of change in FCR for VLDL apo B converted to LDL 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 lipids were observed.

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

<table>
<thead>
<tr>
<th></th>
<th>VLDL</th>
<th></th>
<th>IDL</th>
<th></th>
<th>LDL</th>
<th></th>
<th>HDL</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>apo B (percent of total protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Estimated average diameter (Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>apo B</td>
</tr>
<tr>
<td>C</td>
<td>318</td>
<td>374</td>
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<td>210</td>
<td>210</td>
<td>198</td>
<td>128</td>
<td>149</td>
</tr>
<tr>
<td>M</td>
<td>23</td>
<td>20</td>
<td>21</td>
<td>17</td>
<td>84</td>
<td>89</td>
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C = corn-oil diet, M = Maxepa (fish-oil) diet, NS = not significant. Values are the means from four animals.

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
Recently, we demonstrated that this pathway was inhibited by cholestyramine and/or the accelerated atherosclerosis observed in patients with hypercholesterolemia and familial combined hyperlipidemia. We have proposed that the direct LDL apo B synthesis (total VLDL plus LDL direct synthesis) is related to the 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. Omega-3 fatty acids have been shown to inhibit the synthesis of apo B by cultured liver cells (Hep G2). 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. 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 LDL 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. have reported lower LDL (B/E) receptor activity in Hep G2 cells after preincubation with eicosapentaenoic acid. Roach et al. have demonstrated a decreased LDL receptor activity in liver membranes isolated from rats fed fish oil. Illingworth et al. 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. 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 the fish-oil diet (Table 4). Parks et al. reported changes in plasma LDL composition and size in monkeys fed 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 incom-
sistant effect of fish oil on LDL cholesterol concentrations in humans requires further investigation.

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References


4. Harris WS, Connor WE, McMurry MP. The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil vs vegetable oils. Metabo-

lism 1983;32:179–184

5. Wong SH, Reardon M, Nestel PJ. Reduced triglyceride formation from long-chain polyenacyc fatty acids in rat hepato-

cytes. Metabolism 1985;34:900–905


10. Illingworth DR, Harris WS, Connor WE. Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. Atherosclerosis 1984;4:270–275


12. Goldberg IJ, Le NA, Ginsberg HN, Paterniti JR Jr, Brown VW. Metabolism of apoprotein B in cynomolgus monkey: evidence for independent production of low-density lipopro-

tein apoprotein B. Am J Physiol 1983;244:E196–E201


14. Huff MW, Telford DE. Direct synthesis of low-density lipopro-

tein B in the miniature pig. Metabolism 1985;34:36–42


17. Soutar AK, Myant NB, Thompson GR. Simultaneous mea-


18. Janus ED, Michilli A, Wootton R, Turner PR, Magilli PJ, Lewis B. Quantitative studies of very low density lipopro-

tein conversion to low density lipoprotein in normal controls and primary hyperlipoproteinemic states and the role of direct secretion of low density lipoprotein in heterozygous familial hypercholesterolemia. Eur J Clin Invest 1990;10:149–159


rosis 1984;4:614–624


21. Ginsberg HN, Le NA, Gibson JC. Regulation of the pro-

22. Huff MW, Telford DE, Woodcroft K, Strong WLP. Mevlin-

colin and cholestyramine inhibit the direct synthesis of low density lipoprotein apolipoprotein B in miniature pigs. J Lipid Res 1986;26:1175–1186

23. Flodge NH, Poulla P. Studies on the radiodiode generation of very low density lipoprotein obtained from different mammalian species. Clin Chim Acta 1974;52:15–26

24. Markwell MAK, Haas MS, Bleier BL, Tolbert NE. A modifica-
tion of the Lowry procedure to simplify protein determina-
tion in membrane and lipoprotein samples. Anal Biochem 1978;87:206–210

25. Egusa G, Brady DW, Grundy SM, Howard BV. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipopro-


27. Band Y. Nonlinear parameter estimation. New York: Aca-
pemic Press, 1974:1–145

28. Nestel PJ, Reardon MF, Flodge NH. Very low density lipoprotein B-apoprotein kinetics in human subjects. Relations-


29. Zilversmit DB. The design and analysis of isotope experi-


33. Peckard CJ, Munro A, Lortimer AR, Gatto AM, Shepherd CJ. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceri-


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


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