Administration of n-3 Fatty Acids in the Diets of Rats or Directly to Hepatocyte Cultures Results in Different Effects on Hepatocellular ApoB Metabolism and Secretion

Anna-Marie Brown, Jenni Castle, Abdel-Malek Hebbachi, Geoffrey F. Gibbons

Abstract—Hepatocytes derived either from rats fed a diet enriched in n-3 fatty acids or from rats fed a low-fat diet and cultured with an n-3 fatty acid (eicosapentaenoic acid, EPA) in vitro were used to distinguish between the dietary effects and the direct effects of n-3 fatty acids on hepatocellular apolipoprotein (apo) B metabolism and secretion. ApoB-48 and apoB-100 synthesis, degradation, and secretion as large (d<1.006) and small (d>1.006) particles were determined after a pulse label with [35S]methionine. These effects were compared with changes in triacylglycerol (TAG) synthesis and secretion and with changes in de novo fatty acid synthesis (using 3H2O incorporation) under identical conditions. When n-3 fatty acid was given via the dietary route, apoB-48 very low density lipoprotein (VLDL) secretion was inhibited, but there was no effect on the secretion of apoB-100 VLDL. There was no effect on the secretion of either apoB-48 or apoB-100 as small, dense particles (d>1.006). Cellular TAG synthesis was significantly inhibited under these conditions, and fatty acid synthesis de novo was inhibited by 80%. By contrast, after direct addition of EPA to hepatocytes from normal rats, the secretion of both apoB-48 and apoB-100 VLDL was suppressed. The secretion of apoB-48, but not of apoB-100, as dense particles was also inhibited. However, there was little or no effect on TAG synthesis nor on fatty acid synthesis de novo. In addition, whereas dietary administration of n-3 fatty acid gave rise to decreased net synthesis and degradation of apoB-48, direct administration in vitro resulted in increased degradation with no effect on net synthesis. We conclude that the effects of n-3 fatty acids on hepatic lipid and apoB metabolism differ according to whether they are administered in vivo, via the dietary route, or in vitro, via direct addition to hepatocyte cultures. (Arterioscler Thromb Vasc Biol. 1999;19:106-114.)

Key Words: apolipoprotein B ■ hepatocytes ■ VLDL ■ dietary fish oil ■ fatty acid synthesis

The hypotriglyceridemic effect of dietary fish oils (FOs) has been recognized for some time,1,2 and it is generally believed that this effect is caused, in large part, by a suppression of hepatic VLDL secretion mediated by the constituent n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).3-6 The mechanism underlying the inhibition of VLDL output is, however, the subject of some controversy. Several studies using primary hepatocytes from rats and humans and also with the human hepatoma cell line HepG2 have shown that compared with oleate, the presence of EPA in the cell medium inhibits the secretion of apoB. This decrease was accompanied by a decline in the rate of secretion of triacylglycerol (TAG). In some of the above studies, the changes in apoB and TAG output occurred in the absence of any decline in the rate of TAG synthesis from EPA, compared with that observed when oleate was the TAG precursor.7,8,11 An unchanged TAG synthesis is not, however, a universal finding.9,12 Nevertheless, it was concluded7,8 that a lack of TAG per se for VLDL assembly provided an unlikely explanation for the decreased apoB output observed in the presence of EPA. On the other hand, Wang and coauthors8 have shown that addition of EPA to the culture medium of primary rat hepatocytes gave rise to an increased rate of degradation of newly synthesized apoB-48 and apoB-100. EPA had no effect on the rates of synthesis of these 2 forms of apoB.

In some respects, notably the decreased secretion of hepatic TAG and apoB, dietary studies provided some support for the above findings in vitro.13-16 However, when n-3 fatty acids were fed as part of the diet, no enhancement of the rate of hepatocellular apoB degradation was observed when isolated cells from these rats were compared with those from the low fat (LF)--fed animals.16 In this case, there was also a decreased synthesis of TAG,14 a finding that confirmed previous reports of the effects of dietary n-3 fatty acids17-19 and an effect that differed from the one observed in some of the above experiments in which EPA was added directly to the culture medium.7,8

It might well be expected, of course, that different effects on hepatic metabolism would arise as a result of differences in the experimental model used to expose the liver to n-3 fatty acids. The most obvious consequences of these experimental
differences are the following: (1) The much longer period of time over which hepatocytes are exposed to fatty acids delivered during long-term feeding compared with that achieved via direct addition in vitro. In the former case, n-3 fatty acids have sufficient time to fulfill their role as potent inducers of peroxisomal proliferation, an effect that over a period of time might be expected to have a major influence on hepatocellular lipid metabolism. (2) Differences in the major route by which n-3 fatty acids are delivered to the liver; in the case of dietary administration, this would involve chylomicron remnants as well as plasma nonesterified fatty acids. (3) The possibility of indirect effects resulting from dietary administration. For instance, n-3 fatty acids have been reported to have a hypoinsulinemic effect and to cause an increase in insulin sensitivity.

The present approach to understanding the causes of the observed effects of n-3 fatty acids on VLDL secretion was designed to take account of the above factors, and it differs from previous experimental approaches in the following ways. First, it allows a direct comparison of the effects of long-term n-3 fatty acid feeding with those resulting from short-term exposure of liver cells to EPA. Second, exposure of hepatocytes from n-3 fatty acid–fed donor rats to EPA in vitro permits a more physiological simulation of the hepatic environment of these animals in vivo. Third, the distribution of apoB between large (VLDL) and smaller, more dense particles of apoB between large (VLDL) and smaller, more dense particles (d=1.006) was studied. Previous work has not discriminated between these different types of secreted apoB-containing particles in hepatocytes from animals fed a diet rich in n-3 fatty acids. This is an important aspect that may provide information relevant to the dietary regulation of the proposed stepwise transfer of lipid to apoB during VLDL assembly. Finally, the magnitude of the above changes was compared, in each of the 2 different experimental designs, with those of TAG synthesis and de novo lipogenesis, both of which appear to be positively correlated with hepatic VLDL output. The comparative approach adopted in the present work has identified major differences in the response of liver cells to n-3 fatty acids administered either as part of the diet or directly in vitro. The results suggest that the direct, short-term effects of n-3 fatty acids on hepatic apoB metabolism and secretion of large and small particles cannot be entirely explained by changes in intracellular fatty acid or TAG synthesis.

**Methods**

**Materials**

All reagents were of analytical grade and were obtained from Sigma, with the exception of the following: MaxEPA was kindly supplied by Seven Seas Ltd (Kingston-on-Hull, UK). Waymouth’s medium and Dulbecco’s PBS were obtained from Gibco Ltd. [35 S]Methionine, [3H]oleate, [3H]H2O, and NCS-80 tissue solubilizer were from Amersham International. Acrylamide/bisacrylamide 40% (wt/vol) was purchased from Bio-Rad. Autoradiography film (blue-sensitive x-ray film) was obtained from Genetic Research Instrumentation, and anti-rat apoB antisemur was raised in rabbits as described previously.

**Preparation of Diets and Dietary Regimen**

Rat and Mouse No. 3 Breeding Diet from Special Diet Service was used as the LF control diet (LF diet) containing 4.3% fat, 51.2% carbohydrate (mainly starch), 22.3% protein, 4.5% fiber, and 7.7% ash. The 20% vol/wt MaxEPA (FO) was prepared by mixing 200 mL of the oil with 800 g of powdered LF diet and 400 mL of deionized water as described previously. This was then kept frozen at −20°C until use. The LF diet contained 3.66 cal/g whereas the FO diet contained 4.69 cal/g. The fatty acid composition (in terms of percentage of total) of MaxEPA was as follows: C16:0, 26.02%; C18:1, 17.2%; C20:5, 15.35%; C16:1, 10.95%; C14:0, 9.74%; C22:6, 7.67%; C18:0, 5.63%; C18:2, 1.94%; C22:5, 1.71%; C20:4, 1.14%; C17:0, 0.87%; C18:3, 0.74%; and C20:1, 0.58%.

Male rats (Wistar strain) were fed the above diets for 14 days ad libitum. Room temperature was kept constant at 20±2°C with a 10-hour light/14-hour dark cycle (lights on at 8 AM). The experimental animals were fed at 200±10 g (n=12 total), and there was no significant difference in weight gain (final weights were 275±8 g and 268±10 g for the FO and LF groups, respectively). Animal care and procedures were regulated by the Animals (Scientific Procedures) Act, 1986.

**Hepatocyte Isolation and Culture Conditions**

Hepatocytes were prepared under sterile conditions and plated out onto rat tail collagen–coated dishes in Waymouth’s medium MB752/1. Falcon Primaria (60×15-mm) dishes were used for culture, and rat tail collagen was obtained as described by Michalopoulos and Pitoi. The plating medium contained 10% FCS, penicillin (90,000 U/L), streptomycin (90,000 U/L), and added amino acids (3.60 mmol/L glutamine, 0.36 mmol/L alanine, and 0.45 mmol/L serine). Initial cell viability, as assessed by trypan blue exclusion, was 88% to 96% (n=20). There were no differences in hepatocyte viability between the 2 groups. Plating densities were 1.68±0.1 and 1.68±0.2×10^6 cells/dish for LF and FO groups, respectively. After 4 hours, the serum-containing medium was removed and the cell monolayer washed twice with Dulbecco’s PBS. Supplemented, serum-free Waymouth’s medium (3 mL) lacking methionine was then added to each dish. At this point, DNA levels were 20.1±3.2 and 20.9±2.4 μg/dish and protein levels were 1.2±0.3 and 1.6±0.2 mg/dish for LF and FO, respectively. Since the original plating densities were identical and the postincubation DNA levels were identical, plating efficiencies were equivalent between the 2 dietary groups. All experimental data were expressed per dish of cells because the DNA per dish was no different between the 2 dietary groups.

**Pulse-Chase Experiments**

The procedure for measurements of apoB synthesis, degradation, and secretion was essentially as described previously. Cells were cultured in supplemented Waymouth’s medium lacking methionine for 24 hours. At this point, [35 S]labeled methionine (100 μCi; 1000 Ci/mmol) and either oleate (0.75 mmol/L) or EPA (0.75 mmol/L) were added complexed to albumin (0.5% wt/vol), and the cells were pulsed for 1 hour. The cells were harvested from some of the dishes for measurement of [35S]methionine incorporation into apoB. To the remaining dishes was added 3.0 mL of supplemented medium containing unlabeled methionine (10 mmol/L) and either oleate (0.75 mmol/L) or EPA (0.75 mmol/L). The medium was removed and the cells and medium harvested after periods of 0.25, 0.5, 1, 2, and 24 hours. At the end of the pulse period, the amount of immunoprecipitable label in the medium was 1% to 3% of the amounts associated with the cells, irrespective of which fatty acid had been present. Throughout the pulse and chase periods, α-tocopherol (50 μmol/L) in dimethyl sulfoxide (15 μL) was present in each dish to protect against oxidative damage. Checks were carried out on their viabilities after the cells were cultured in the presence of oleate and EPA, with or without α-tocopherol, for 6 hours. Those cultured with oleate were found to be 81.7±1.4% viable with or without α-tocopherol present. Those cultured in the presence of EPA were found to be 100±1.9% viable without and 77.1±1.5% viable with α-tocopherol.

**Immunoprecipitation of ApoB and SDS-Polyacrylamide Gel Electrophoresis**

Immediately after harvesting, the cells were washed twice with PBS and solubilized by the addition of 0.5 mL of hot (75°C) 1% SDS-radioimmunoprecipitation (RIPA) buffer. Labeled apoB was
immunoprecipitated from the cells and from the VLDL and d>1.006 fractions of the medium with a rabbit polyclonal IgG antiserum as described previously. After 14 to 18 hours, the apoB-antibody complexes were recovered from the samples by using 40 μL of a protein A–Sepharose bead suspension (1 g/8 mL 0.05% SDS-RIPA).

After immunoprecipitation, the beads were combined with 40 μL of sample buffer and 10 μL of a 1 mg/mL Triton X-100-treated rat plasma VLDL solution (to aid visualization of the apoB-containing bands). The samples were then heated at 75°C for 20 minutes and run on a 3% to 20% gradient polyacrylamide gel. The residual beads typically contained ≤5% of the original immunoprecipitated label. The gel was electrophoresed at 15 mA/gel for 18 hours. Staining and destaining were carried out using Coomassie Brilliant Blue R, as described previously.

**Autoradiography and Scintillation Counting of apoB-48 and apoB-100**

Gels were dried, and the labeled bands containing apoB-48 and apoB-100 were located by autoradiography. These areas of the gels were excised and solubilized before addition of "Optiphase" scintillation fluid. The recovery of radioactivity from the gels was greater than 95%. The immunoprecipitated apoB-48 and apoB-100 were also solubilized from the beads before scintillation counting. The apparent recovery of label from the immunoprecipitated apoB-48 and apoB-100 was determined using an assay kit supplied by Boehringer Ingelheim (GPO-PAP) as described previously. In some experiments unlabeled EPA was used as the extracellular TAG precursor, and TAG synthesis was determined from the difference in total TAG mass in cells and VLDL, cultured in the presence and absence of this fatty acid (see the legend to Table 1).

**Measurement of De Novo Fatty Acid Synthesis**

Freshly isolated cells from the LF- and FO-fed rats were suspended in serum-free, supplemented Waymouth's medium (62.5 mg fresh weight per mL). Three milliliters of each suspension was incubated in the presence of albumin-bound oleate (0.75 mmol/L), albumin-bound EPA (0.75 mmol/L), or albumin alone (0.5%) for 3 hours. After incubation, the cells were sedimented by centrifugation, the medium removed, and the cell pellet washed twice with PBS. The washed cell pellet was sonicated in 1.0 mL PBS, and 50 μL was taken for protein determination. The remainder was saponified using ethanolic KOH, and the nonsaponifiable lipid fraction was extracted as described previously. The aqueous phase remaining after nonsaponifiable lipid fraction extraction was acidified to pH 2.0, and the saponifiable fraction, containing the labeled fatty acids, was extracted as previously described.

**Other Analytical Methods and Statistical Tests**

Cellular protein was determined colorimetrically by the method of Lowry et al. Preparation of oleate (0.75 mmol/L) and EPA (0.75 mmol/L) bound to albumin (0.5%) was carried out as described in Reference 38. Measurement of cellular DNA was carried out using a colorimetric assay, after phenol/chloroform extraction as described in Reference 39. The DNA concentration was estimated by optical density using a Pharmacia Gene Quan. All statistical tests (2-factor ANOVA with replication and 2-sample, 1-way t tests under the assumption of equal variances) were performed using the data analysis package in Microsoft Excel for Windows 95, Version 7.0. Significance was determined at the 95% confidence interval, ie, P<0.05.

**Results**

**Comparison of the Effects of Dietary n-3 Fatty Acids and of Direct n-3 Fatty Acid Addition on apoB Secretion as Large (d<1.006) and Small (d>1.006) Particles**

In the presence of extracellular oleate, the secretion of apoB-48 and apoB-100 into the unfractionated medium was similar in hepatocytes from the LF-fed rats and the FO-fed rats (Figure 1). However, when extracellular oleate was replaced by extracellular EPA, apoB output decreased in both types of hepatocyte preparation. This decrease was more apparent for the secretion of apoB-48 (by ANOVA; for FO, P<0.02; for LF, P<0.002) than for apoB-100, in which the
change became statistically significant only in the hepatocytes from the rats fed the LF diet (ANOVA \( P < 0.05 \)).

When the whole cell medium was separated into fractions containing either VLDL (\( d < 1.006 \)) or more dense particles (\( d > 1.006 \)), a more complex picture emerged. For instance, when hepatocytes were cultured in the presence of oleate, those from donor animals fed the FO diet secreted significantly less apoB-48 VLDL over the 24-hour period than did those from animals fed the LF diet (ANOVA \( P < 0.05 \)).

When the whole cell medium was separated into fractions containing either VLDL (\( d < 1.006 \)) or more dense particles (\( d > 1.006 \)), a more complex picture emerged. For instance, when hepatocytes were cultured in the presence of oleate, those from donor animals fed the FO diet secreted significantly less apoB-48 VLDL over the 24-hour period than did those from animals fed the LF diet (Figure 2). There was no change in the secretion of newly synthesized apoB-100. However, replacement of oleate with EPA gave rise to a very large decrease in the output of both apoB-100 (ANOVA, \( P < 0.001 \)) and apoB-48 (ANOVA, \( P < 0.002 \)) VLDL when compared with LF in the presence of oleate. EPA added to the FO diet significantly suppressed the output of apoB-48 (ANOVA, \( P < 0.02 \)) when compared with FO in the presence of oleate.

Analysis of the \( d > 1.006 \) fraction of the medium containing heavier, relatively lipid-poor apoB particles showed little or no effect of FO feeding when cells were cultured in the presence of oleate (Figure 3). However, replacement of oleate by EPA in the medium of the cells from both the LF- and FO-fed animals led to a significant decrease (\( P < 0.002 \) and \( P < 0.05 \), respectively, by ANOVA) in the secretion of small, dense, apoB-48–containing particles at all time points. This decline, however, was not so pronounced as that observed after feeding n-3 fatty acids in the diet.

It was of some interest to characterize the particles secreted into the \( d > 1.006 \) infranatant in terms of their size and density distribution and also to determine whether their distribution was affected by EPA. To do this, in some experiments hepatocytes from rats fed the LF diet were pulsed and then chased for 2 hours in the presence of either EPA or oleate. In this case, the \( d > 1.006 \) infranatant, obtained as described above, was further centrifuged to obtain fractions containing (IDL plus LDL) and HDL. The results are shown in Figure 4. Most of the label appearing in the \( d > 1.006 \) infranatant appeared to be associated with dense HDL-like particles of density \( > 1.063 \). Both labeled apoB-48 and apoB-100 were secreted into this density range, and compared with oleate, EPA tended to decrease secretion into this density range.
About 25% of the labeled apoB of d>1.006 was associated with IDL and LDL-like particles of density 1.006 to 1.063. Again, compared with oleate, EPA tended to decrease secretion of apoB into this density range, and there was also a trend toward a decreased apoB-48 to apoB-100 ratio. Sparks and Sparks\(^40\) and Higgins and colleagues\(^41,42\) have demonstrated the presence of metabolically distinct pools of apoB within the cell. Because in the present work we studied only the metabolic fate of newly synthesized apoB, it is possible that changes in other, unlabeled pools could have contributed to the effects observed.

**Intracellular ApoB Metabolism: Effects of Dietary n-3 PUFAs and of EPA Added In Vitro**

When hepatocytes were cultured in the presence of oleate, those from the FO-fed donors incorporated less \(^{[35}S\)methionine into apoB-48 than did those from the donor rats fed the LF diet \((P<0.02)\) (Figure 5). There was no difference in the rates of net accumulation of apoB-100. However, in each type of hepatocyte preparation, when oleate was replaced by EPA, there was no change in the maximum incorporation of label into apoB-48. The net accumulation of apoB-100 also remained unchanged. It appeared, therefore, that whereas administration of FO fatty acids via the dietary route selectively inhibited the net accumulation of apoB-48, this effect could not be reproduced simply by the addition of EPA to the medium of normal hepatocytes. The ineffectiveness of EPA added in vitro on the synthesis of apoB-48 and apoB-100 has also been demonstrated recently by Wang et al.\(^8\)

To determine whether the route of n-3 PUFA administration affected hepatocellular apoB degradation differently, the sum of the amount of apoB secreted into the medium and that remaining within the cell at any given postchase time point was compared with the peak level of \(^{[35}S\)methionine incorporation into apoB. These calculations of recovery were carried out for both apoB-48 and apoB-100. Figure 6 shows that when hepatocytes were cultured in the presence of oleate, those from the donors fed the LF diet degraded more apoB-48 at 24 hours after the pulse than did those from the donors fed the FO diet: 54±6% versus 40±6% of peak value, respectively \((n=7, P<0.05)\). There were no significant differences in the rate of degradation of apoB-100. By contrast, with each type of hepatocyte preparation, when oleate was replaced by EPA in the medium, there was an increase in the rate of apoB degradation between 0.25 hour (the time of peak incorporation) and 2 hours. In the hepatocytes from the LF-fed donors, the addition of EPA gave rise to a degradation of 17±4% of apoB-48 between 0.25 and 2 hours \((P<0.05)\) compared with 7±17% (NS) in the presence of oleate. EPA addition to hepatocytes from the FO-fed rats gave rise to a degradation of 28±8% of apoB-48 between 0.25 and 2 hours \((P<0.05)\) compared with 1±6% (NS) over this period in the presence of oleate. At 24 hours there was no difference in the amount of

Figure 5. Newly synthesized apoB contained in the cells immediately after the pulse and during the chase in the presence of either oleate or EPA. Hepatocytes were pulse-labeled for 1 hour with \(^{[35}S\)methionine (100 μCi/dish) and then chased for various times up to 24 hours. The value marked * at zero time was significantly less than that in the LF in the presence of oleate at \(P<0.02, n=7\).

Figure 6. The effect of the in vitro addition of oleate or EPA on the proportion of apoB-100 and apoB-48 degraded by hepatocytes from rats fed either the LF diet or the FO diet. Hepatocytes were pulse-labeled for 1 hour with \(^{[35}S\)methionine and then chased with cold methionine (10 mmol/L) for various times up to 24 hours. Results are expressed as percentage of maximum values obtained from the summation of apoB values for the cells and the total medium at each time point. The value marked * was significantly different from that for the FO at \(P<0.05, n=7\).
**TABLE 1.** Response of VLDL TAG Secretion and Cellular TAG Synthesis to EPA Feeding (FO) or Direct EPA Addition to Hepatocyte Cultures

<table>
<thead>
<tr>
<th>Diet</th>
<th>VLDL TAG Output, nmol TAG/mg Cell Protein</th>
<th>Total TAG Synthesis, nmol Oleate/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>130±14</td>
<td>471±108</td>
</tr>
<tr>
<td>Olive oil</td>
<td>146±10</td>
<td>375±111</td>
</tr>
<tr>
<td>FO (EPA)</td>
<td>93±9†</td>
<td>237±66‡</td>
</tr>
</tbody>
</table>

In experiment 1, hepatocytes were cultured in the presence of [3H]oleate (0.75 mmol/L, 978 dpm/nmol) for 24 hours, after which time the mass and 3H lable of cellular and VLDL TAG were determined. Total TAG synthesis was the sum of the cell and VLDL TAG label divided by the specific radioactivity of the exogenous oleate. The results are expressed as mean±SEM (n=5). Values marked † and ‡ are significantly different (P<0.05 and P<0.01, respectively) from the corresponding values for the olive oil diet. The values marked * are significantly different (P<0.05) from the corresponding values for the LF diet.

**TABLE 2.** De Novo Fatty Acid Synthesis in Hepatocytes From Rats Fed LF and FO-Enriched Diets: Effects of Fatty Acids In Vitro

<table>
<thead>
<tr>
<th>Diet</th>
<th>Incubation Conditions</th>
<th>Fatty Acid Synthesis, nmol 3H2O/mg Cell Protein per 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>Albumin</td>
<td>341.3±76.1</td>
</tr>
<tr>
<td>LF</td>
<td>Oleate</td>
<td>279.0±70.9</td>
</tr>
<tr>
<td>LF</td>
<td>EPA</td>
<td>223.1±44.7</td>
</tr>
<tr>
<td>FO</td>
<td>Albumin</td>
<td>67.5±13.4*</td>
</tr>
<tr>
<td>FO</td>
<td>Oleate</td>
<td>58.8±10.4*</td>
</tr>
<tr>
<td>FO</td>
<td>EPA</td>
<td>64.0±16.8*</td>
</tr>
</tbody>
</table>

Hepatocytes were incubated for 3 hours in the presence of oleate (0.75 mmol/L) or EPA (0.75 mmol/L) bound to albumin or with albumin alone (0.5% wt/vol). Each value is the mean±SEM for 3 hepatocyte preparations. Values marked * are significantly different (P<0.05) from the corresponding incubations with the hepatocytes from the LF-fed rats.

apoB-48 degraded in the LF hepatocytes whether incubated with oleate or EPA. In the FO hepatocytes, however, 65±5% of apoB-48 had been degraded at 24 hours in the presence of EPA, but only 44±8% in the presence of oleate (P<0.05).

**Synthesis and VLDL Secretion of TAG**

Administration of n-3 PUFAs via the dietary route gave rise to a decreased secretion of VLDL TAG in the derived hepatocyte cultures. This effect was not a nonspecific result of feeding dietary fat, since dietary supplementation with an identical concentration of olive oil did not inhibit VLDL TAG (Table 1). Direct addition of EPA to hepatocytes from donors fed the LF diet also gave rise to a decreased output of VLDL TAG compared with that observed when extracellular oleate was present (Table 1). However, determination of the rate of cellular TAG synthesis in each experiment showed that this was decreased only in the hepatocytes from donors fed the high n-3 PUFA diet. Direct administration of n-3 PUFAs as EPA to hepatocytes from animals fed the LF diet, despite decreasing VLDL TAG output, had no significant effect on the rate of TAG synthesis compared with that observed in the presence of oleate (Table 1).

**De Novo Fatty Acid Synthesis**

Table 2 shows the response of fatty acid synthesis de novo to either FO feeding on the 1 hand or addition of EPA in vitro on the other. After feeding with the FO diet, the rate of fatty acid synthesis was reduced to only 20% of that which occurred in the hepatocytes derived from the LF-fed animals (P<0.05). On the other hand, addition of EPA directly to hepatocytes from animals fed the LF diet had relatively little effect; in this case fatty acid synthesis was decreased to ~70% of the controls incubated in the presence of albumin alone. Addition of oleate had an effect that was similar to the 1 observed with EPA. The low rates of fatty acid synthesis observed in the hepatocytes from the FO-fed animals were not further decreased by the addition of either EPA or oleate to the incubation medium.

**Discussion**

The lack of unanimity concerning the mechanism(s) by which n-3 fatty acids suppress VLDL secretion probably arises from differences in the experimental approach used to investigate this problem. For instance, some studies have used liver preparations from animals fed diets supplemented with FOs containing n-3 fatty acids, whereas others have used liver preparations from animals fed an LF diet to which n-3 fatty acids have been added directly in vitro. The major novel findings of the present work are that dietary supplementation with marine FOs, containing n-3 fatty acids, induces long-term changes in hepatic lipid metabolism that suppress apoB-containing lipoprotein assembly via different mechanisms to those that occur after short-term treatment of hepatocytes with the n-3 fatty acid (EPA). These differences have been unequivocally demonstrated by comparing, under identical culture conditions, the behavior of hepatocytes from donor animals fed an FO-supplemented diet on the 1 hand and after exposure of hepatocytes from donor animals fed an LF diet to n-3 fatty acids on the other. In addition, at any point in time in vivo, the livers of rats fed the FO-supplemented diet were also exposed to an increased concentration of n-3 fatty acids in the plasma. So far as we are aware, there have been no previous studies in which this complex, but more physiologically realistic, situation has been modeled. We have tried to simulate these conditions in vitro by culturing hepatocytes from FO-fed rats with EPA. Comparison of the results obtained in the 2 experimental designs clearly showed that there are major differences in the metabolism of lipids and of apoB, depending on the approach adopted. For example, addition of EPA directly gave rise to a more pronounced inhibition of VLDL apoB (Figure 2) and TAG
Dietary administration of n-3 fatty acids suppressed post-translational apoB-48 degradation, whereas compared with oleate, direct addition of EPA enhanced this process, particularly in hepatocytes from the n-3 fatty acid–fed animals (Figure 6). The present work documents, for the first time, this important difference between dietary n-3 fatty acid feeding on the 1 hand and on the other, n-3 addition to hepatocytes in vitro, on apoB metabolism.

Finally, dietary consumption of n-3 fatty acids resulted in profound changes in lipid metabolism, eg, in TAG synthesis (Table 1) and de novo lipogenesis (Table 2) that could not be reproduced in vitro by addition of EPA to the medium of hepatocytes from animals fed the LF diet. It is possible, therefore, that the potent effects of EPA addition in vitro on apoB metabolism may not have occurred as a result of the relatively small changes in TAG or fatty acid synthesis. The causes of the inhibitory effects on lipid metabolism in vivo are unknown but may be linked to the widespread changes that occur in the metabolic profile of the liver after the induction of peroxisome proliferator–activated receptor-α. In particular, the increase in fatty acid oxidation mediated by peroxisome proliferator–activated receptor-α probably contributed to the decreased rate of TAG synthesis.

Interestingly, relationships between apoB-48 net accumulation and degradation; between apoB-48 net accumulation and apoB-48 VLDL secretion, and between apoB-48 degradation at 2 hours of chase and the secretion of apoB-48 into the VLDL 22 hours later (Figure 7). Small but significant correlations also existed for apoB-48 degraded at 2 hours post chase and the secretion of apoB-48 into the d<1.006 fraction of the culture medium after 24 hours (r²=0.54, P<0.01; n=11). Hepatocytes from rats fed the FO or LF diet were cultured with either albumin alone or with complexes of albumin and oleate (0.75 mmol/L). Each point represents the value obtained in hepatocytes from a single rat.

Figure 7. The comparative effect of feeding either an FO diet or an LF diet for 2 weeks on the interrelationship between net accumulation, posttranslational degradation, and VLDL output. a, The correlation between net apoB-48 accumulation during the pulse and the percentage of newly synthesised apoB-48 that is degraded at 2 hours post chase (r²=0.56, P<0.0001; n=23). b, The correlation between net apoB-48 accumulation during the pulse and the secretion of apoB-48 into the d<1.006 fraction of the culture medium after 24 hours (r²=0.63, P<0.001; n=14). c, The correlation between the percentage of newly synthesized apoB-48 degraded at 2 hours post chase and the secretion of apoB-48 into the d<1.006 fraction of the culture medium after 24 hours (r²=0.54, P<0.01; n=11).
term manipulations of the culture medium in vitro rather than from long-term changes in metabolic patterns imprinted on the liver over a period of time, as was the case in the present work. Thus, the present work suggests that, while rapid changes in the rate of apoB degradation may be the primary response to metabolic changes in the short term, longer-term changes are reflected, at least in part, by changes in the rate of apoB synthesis. This may contribute to the amounts of labeled apoB that accumulate during the 1-hour pulse.

In hepatocytes from the LF-fed animals cultured with oleate, most of the newly synthesized apoB-48 and apoB-100 were secreted as dense, lipid-depleted particles during the first hour of the chase. Little was secreted as VLDL (Figures 2 and 3). However, after 1 hour, there was very little further secretion of labeled apoB as dense particles. By contrast, most of the secreted, newly synthesized apoB appeared in the medium as VLDL between 1 and 24 hours of chase (Figures 2 and 3). It appears therefore, that under these conditions, a lag phase of ~1 hour is required for most of the newly synthesized apoB to acquire sufficient lipid for secretion mainly as VLDL. The pattern of apoB secretion in hepatocytes from animals fed the FO diet is somewhat different. In this case, when oleate was present, cells continued to secrete a considerable amount of apoB, particularly apoB-48, as smaller, dense particles between 1 and 2 hours’ chase. Consumption of an FO diet therefore appears to retard the ability of hepatocytes to complete the intracellular maturation of large, TAG-rich particles, even when an easily utilizable TAG precursor such as oleate was available extracellularly. This defect may be related to the low rates of TAG (Table 1) and fatty acid (Table 2) synthesis in hepatocytes from FO-fed rats.

Finally, it should of course be recognized that FO is a complex mixture of TAGs and contains large quantities of fatty acids other than n-3 fatty acids. The exact fatty acid composition of the FO used (MaxEPA) is given in the Methods section, and it shows, for instance, that palmitic acid (16:0) and oleic acid (18:1) are major components in addition to the n-3 fatty acids EPA and DHA. It could therefore be argued that the effects observed after FO feeding result primarily from fatty acids other than n-3 or from a precise combination of n-3 with other types of fatty acid. Nevertheless, it is generally considered that the hypotriglyceridemic effects of FO, which derive from a reduced hepatic secretion of VLDL, are the specific result of the n-3 component of the diet, and this concept is supported by recent studies using dietary concentrates containing as much as 85% combined EPA and DHA. Our previous studies have also shown that in hepatocytes from rats fed a diet supplemented with olive oil (rich in 18:1), there was no significant difference in VLDL TAG and apoB secretion compared with that observed in hepatocytes from rats fed the LF diet.

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References

Dietary vs In Vitro Effects of Fatty Acids on Hepatic ApoB

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Results in Different Effects on Hepatocellular ApoB Metabolism and Secretion
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