Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids

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Abstract Using a combination of in vivo and in vitro studies, we have investigated the impact of polyunsaturated fatty acids (PUFA) on the expression of several genes encoding proteins involved in hepatic glycolysis and lipogenesis. Meal-training rats to a high glucose diet containing 10% triolein led to a significant induction of hepatic mRNAs encoding glucokinase (GK), pyruvate kinase (PK), fatty acid synthase (FAS), malic enzyme (ME), and the S14 protein (S14), but had no effect on thyroid hormone receptor-β1 (TRβ1) and c/EBPα gene expression. Replacing triolein with menhaden oil attenuated (by 50–90%) the induction of mRNA encoding GK, ME, PK, FAS, and S14. This effect was rapid (within hours) and for FAS and S14, directed at the transcriptional level. The mRNAs encoding TRβ1, c/EBPα and β-actin were unaffected by menhaden oil. Studies with cultured primary hepatocytes showed that C18:3,ω3 (n-3), C18:3,ω6 (n-6), C20:4, ω6 (n-6), and C20:5,ω3 (n-3) were all equally effective at suppressing the level of mRNAs encoding FAS, S14, and PK. This effect was specific for glycolytic and lipogenic enzymes, as expression of β-actin was not affected by these fatty acids. Moreover, the fatty acids had only marginal effects on cell viability as judged by lactate dehydrogenase release. These data indicate that polyunsaturated fatty acids coordinately regulate the expression of several enzymes involved in carbohydrate and lipid metabolism. The mechanism of control does not require extrahepatic factors or fatty acid metabolism.—**Jump, D. B., S. D. Clarke, A. Thelen, and M. Liimatta.** Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J. Lipid Res.* 1994. **35:** 1076–1084.

Supplementary key words dietary polyunsaturated fat • primary hepatocytes • glycolytic and lipogenic gene expression • transcription

In contrast to saturated and monounsaturated fatty acids, dietary polyunsaturated fatty acids (PUFA) inhibit hepatic de novo fatty acid synthesis (1–3). Based on studies of fatty acid synthase (FAS) and the S14 protein (S14), the mechanism for this suppression is due to an inhibition of gene transcription accompanied by a decline in the corresponding mRNAs and subsequent reduction in protein and/or enzymatic activity (2, 4–7).

Most studies investigating the physiological processes affected by dietary PUFA, i.e., the regulation of serum triglycerides or the regulation of gene expression, have involved feeding rats or humans diets supplemented with ω3 and/or ω6 fatty acids for extended periods of time (1–10). Recent studies with the S14 protein have shown that S14 gene transcription is rapidly inhibited (within hours) after a single meal enriched in ω3 fatty acid (7). Transfection analysis using S14–chloramphenicol acetyl transferase (CAT) fusion genes further indicated that PUFA-regulated factors targeted *cis*-linked elements within the S14 gene proximal promoter.

Because the regulation of expression of the S14 protein is similar to many glycolytic and lipogenic enzymes (4, 5, 7), we hypothesized that the rapid action of PUFA on S14 gene expression may extend to other genes encoding glycolytic and lipogenic enzymes. Several hepatic proteins involved in glycolysis and lipogenesis have been reported to be regulated by dietary PUFA, including glucokinase (10), malic enzyme, (11), glucose-6-phosphate dehydrogenase (11, 12), citrate lyase (10), acetyl-CoA carboxylase (2, 11) and Δ^9-desaturase (13). We selected the glycolytic enzymes, glucokinase (GK) and pyruvate kinase (PK), the lipogenic enzymes, FAS and malic enzyme (ME) and two transcription factors involved in hepatic lipid metabolism, i.e., cEBP/α and thyroid hormone receptor β1 (TRβ1) (14, 15). The response of these genes was compared to the PUFA control of S14 in vivo and in cultured hepatocytes. Our studies suggest that PUFA coordinately regulate several enzymes involved in hepatic glycolysis and lipogenesis.

Abbreviations: PUFA, polyunsaturated fatty acids; FAS, fatty acid synthase; GK, glucokinase; PK, pyruvate kinase; ME, malic enzyme; TO, triolein; DHA, docosahexaenoic acid; LDH, lactic dehydrogenase; TBARS, thiobarbituric acid-reacting substances.

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METHODS AND MATERIALS

Animals and diets

Male Sprague-Dawley rats (125–150 g) were obtained from Charles River Breeding Laboratories (Kalamazoo, MI). A high carbohydrate–fat-free diet (58% glucose) was obtained from ICN (Cleveland, OH). Rats (1/cage) were trained to a meal feeding regimen, i.e., access to food from 0900 to 1200 h (7). Body weights and food intakes were measured daily. Rats were first trained to a meal feeding protocol by being fed a high glucose diet supplemented with either 10% olive oil or 10% triolein (TO: 1,2,3-tri-[(*cis*)-9-octadecenoyl]-glycerol; > 95% by gas chromatography; Sigma Chemical Co., St. Louis, MO) plus 0.1% butylated hydroxytoluene. After 10 days of adaptation, the rats were switched to a diet containing 10% fish oil or its fatty acid constituents. The fish oils tested include: *a)* menhaden oil ("MaxEPA", Scherer, Fl); *b)* concentrated ethyl esters of menhaden oil containing ω3 fatty acids; *c)* eicosapentaenoic acid (EPA, 20:5,ω3), and *d)* docosahexaenoic acid (DHA, 22:6,ω3) (Southeast Fisheries Science Center, Charleston, SC). Diets containing the various oils were supplemented with 0.1% butylated hydroxytoluene as an antioxidant and were prepared fresh daily. An analysis of the fatty acid composition of these various fats is shown in **Table 1.** Rats were maintained on the meal feeding protocol through all studies.

Preparation of hepatocytes

Male Sprague-Dawley rats (150–350 g) maintained on rat chow (Teklad) were fasted for 24 h prior to hepatocyte preparation. Primary hepatocytes were prepared using the collagenase perfusion method of Berry and Friend (16) as modified by Jacoby, Zilz, and Towle (17). Viable hepatocytes were separated through percol and seeded at a density of 10^7 cells/100-mm primary culture dish (Falcon) in Williams E medium containing 25 mM glucose, 10 nM dexamethasone, 1 μM insulin, 10% fetal calf serum. After a 4-h attachment period, cells were switched to Williams E medium containing no serum for an overnight treatment. Cells were switched to media containing hormones, i.e., insulin (1 μM), dexamethasone (10 nM), triiodothyronine (T_3, 1 μM) and albumin (50 μM) plus a specific fatty acid, at 300 μM. The fatty acids examined in these studies included: oleic acid (18:1, ω9), α-linolenic acid (18:3, ω3), γ-linolenic acid (18:3, ω6), arachidonic acid (20:4, ω6), and eicosapentaenoic acid (20:5, ω3). The media also contained 1 μM vitamin E and 2.7 μM butylated hydroxytoluene to prevent oxidation of the fatty acids. All fatty acids were obtained from Nu-Chek Prep (Elysian, MN) and were > 95% pure by gas chromatography.

TABLE 1. Fatty acid composition of dietary fats

Fatty Acids	Olive Oil	Menhaden Oil "MaxEPA"	Menhaden Oil ω3 Esters	C20:5 Ester	C22:6 Ester
			%		
Sat.	13.5	26.0			
Mono.	73.7	21.4			
Poly.	8.4	43.4	88.7		
16:0	11.0	15.9			
18:0	0.2	2.9			
18:1	72.5	7.4			
18:2	7.9	1.1			
18:3	0.6	0.7			
20:4		0.8	1.7		
20:5		16.1	41.3	90.5	1.1
22:6		11.2	23.4	0.2	85.7
Total ω3	0.6	34.7	76.6	91.2	88.7
Total ω6	7.9	2.7	3.3		

RNA extraction and transcription analysis

Liver and hepatocyte total RNA was isolated using the 4 M guanidinium thiocyanate procedure and was used for measurement of specific mRNAs (7, 18). Specific mRNAs were examined by Northern analysis and quantified by dot blot analysis. The corresponding cDNAs were labeled with [^{32}P]dCTP by random priming. The cDNAs include: S14 (S14 exoPEII6), fatty acid synthase (FAS17, provided by M. Magnuson, Vanderbilt University), pyruvate kinase (pLPK, provided by A. Kahn, INSERM, France), glucokinase (pBGK2, provided by P. Iynedjian, University of Geneva, Switzerland), malic enzyme (provided by V. Nikodem, NIH), β-actin (provided by L. Kedes, Stanford University), c/EBPα (provided by S. Mcknight, Carnegie Institute of Washington, and thyroid hormone receptor β1 (rc-erbAβ1, provided by H. Towle, University of Minnesota).

Hepatic nuclei were isolated for run-on transcription analysis of the FAS gene using the FASg57-1.8B genomic clone as previously described for the analysis of the S14 gene (7). The FASg57-1.8B genomic clone contains sequences extending from +704 to +2504 relative to the 5' end of the rat FAS gene.

Determination of cell viability

Viability of hepatocytes after treatment with fatty acids was determined by measuring the leakage of lactate dehydrogenase (LDH) from hepatocytes. Hepatocytes were treated with fatty acids as described above. After the 48-h treatment, media were collected and cell debris was removed (12,000 *g;* 5 min). Buffer (500 μl; 250 mM Tris-Cl, pH 7.5) was added to the plates which were then frozen at −80°C. Cells were thawed and scraped, and then subjected to three freeze/thaw cycles. The cell homogenate and the cell culture media were assayed for LDH activity using an LDH kit (Sigma; LDH-10). Total protein within the homogenate was determined using the Bradford assay, Bio-Rad, Inc. (19). Total barbituric acid reactive substance (TBARS) was assayed in the medium (20, 21).

RESULTS

Effects of dietary fatty acid on hepatic glycolytic and lipogenic gene expression

The meal feeding protocol was used to induce both glycolytic and lipogenic enzymes. **Fig. 1** illustrates the effects of meal feeding on mRNAs encoding S14, FAS, ME, GK, PK, c/EBPα, TRβ1, and β-actin. The mRNAs encoding S14, FAS, ME, GK, and PK were significantly induced (3- to 43-fold) by the high glucose diet containing triolein. While $mRNA_{\beta actin}$ was marginally induced (2-fold); mRNAs encoding c/EBPα and TRβ1 were not affected by this treatment. These observations are consistent with previous reports on these genes (4, 5, 7, 22–28) and illustrate that we are able to coordinately regulate several glycolytic and lipogenic enzymes by this dietary manipulation.

The large induction of mRNA abundance of the various transcripts was dependent on the fatty acid composition of the diet. **Fig. 2A** illustrates the effect of menhaden oil on S14 and FAS gene expression, while Fig. 2B illustrates the effect of triolein and menhaden oil on the levels of hepatic mRNAs encoding ME, PK, GK, c/EBPα, TRβ1, and β-actin in both premeal (PRE) and postmeal (POST) animals. When menhaden oil replaced triolein in the diet, mRNAs encoding FAS, ME, GK, PK, and S14 were attenuated by ≥ 70% (Fig. 2; **Table 2**). Menhaden oil did not significantly attenuate hepatic levels of $mRNA_{\beta actin}$, $mRNA_{TR\beta1}$, or $mRNA_{c/EBP\alpha}$. The decline in mRNAs encoding FAS, S14, ME, PK, and GK was due to both a reduced basal level of expression in the premeal

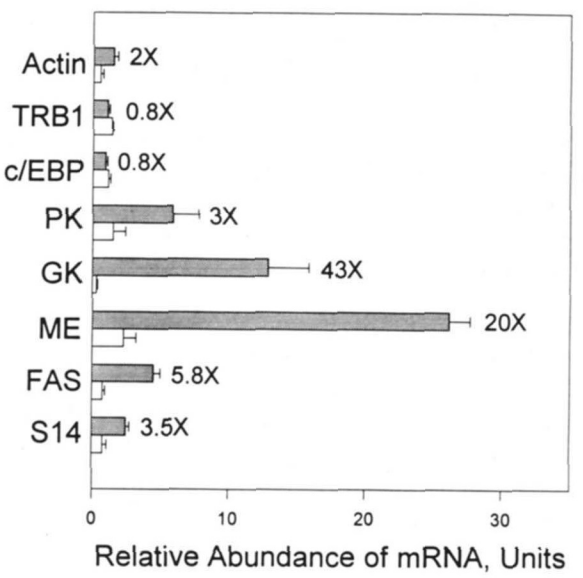

Fig. 1. Effect of meal feeding on hepatic gene expression. Rats were meal-fed a high glucose diet supplemented with 10% triolein. Rats received food from 0900 until 1200 daily. After 10 days on this regimen, rats were killed prior to the meal (premeal, open bars) and 2 h after completion of the meal (postmeal, cross-hatched bars) for extraction of hepatic RNA. The numerical values to the right of cross-hatched bars represent the fold induction of the specific mRNAs. mRNAs encoding various proteins were measured by dot and Northern blot analysis. Results of the dot blots were quantified and expressed as mean ± SE units, n ≥ 7.

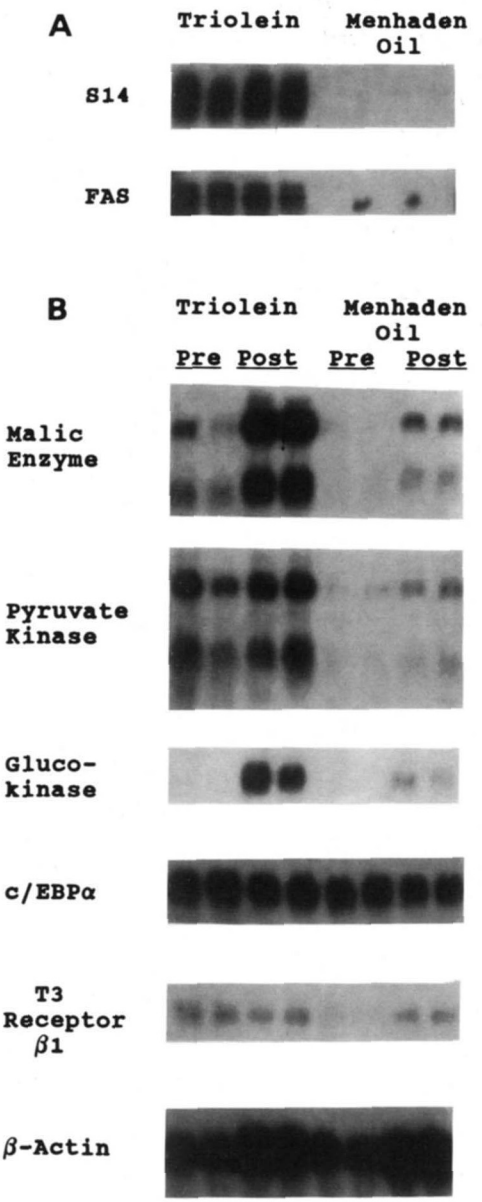

Fig. 2. Effect of triolein and menhaden oil on hepatic gene expression. A: Rats were meal-fed a high glucose diet supplemented with triolein or menhaden oil for 5 days. Hepatic RNA was isolated and examined by Northern analysis for the effect of feeding on $mRNA_{S14}$ (1.3 and 1.5 kb) and $mRNA_{FAS}$ (8.0 and 8.5 kb) levels, n = 4. B: Rats were meal-fed a high glucose diet supplemented with triolein oil or menhaden oil for 5 days. Hepatic RNA was isolated and examined by Northern analysis for the level of mRNAs encoding malic enzyme (2.25 and 3.1 kb), pyruvate kinase (4.2 and 3.2 kb), glucokinase (2.4 kb), c/EBPα (2.7 kb), T3 receptor β1 (6.5 kb), and β-actin. The apparent effect of menhaden oil on TRβ1 expression (see premeal-menhaden oil) was not seen consistently. Animals were killed prior to (PRE) and 90 min after completion of the meal (POST), n = 2.

TABLE 2. Effect of triolein and menhaden oil on hepatic gene expression

mRNA	Triolein	Menhaden Oil	% Change
		units	
Fatty acid synthase	10.5 ± 1.3	0.3 ± 0.1	−97
Malic enzyme	24.6 ± 7.1	3.0 ± 0.2	−99
Glucokinase	7.9 ± 2.1	2.2 ± 0.6	−72
Pyruvate kinase	9.6 ± 1.7	1.1 ± 0.1	−89
S14	5.3 ± 0.9	0.3 ± 0.1	−94
c/EBPα	0.9 ± 0.1	0.9 ± 0.3	− 4
Thyroid hormone receptor β1	1.1 ± 0.1	1.0 ± 0.1	+ 1
β-Actin	2.0 ± 0.3	3.1 ± 0.2	+55

Rats were meal-fed a high glucose diet supplemented with triolein oil or menhaden oil for 5 days. Hepatic RNA was isolated and examined for the levels of mRNAs encoding the proteins listed above by dot analysis. Results are expressed as units (7), mean ± SE, n ≥ 7.

state and an attenuation of the glucose-mediated induction the specific mRNAs in the postmeal state (Fig. 2). Thus, menhaden oil affects the apparent steady-state level of the mRNA in the premeal state, as well as the inducibility of the mRNA after meal ingestion.

Eicosapentaenoic acid (20:5,ω3) and docosahexaenoic acid (22:6,ω3) can substitute for menhaden oil in controlling hepatic gene expression

While the inhibitory component of menhaden oil has been ascribed to the presence of ω3 fatty acids (8, 9), direct analysis of either eicosapentaenoic acid (20:5,ω3) or docosahexaenoic acid (22:6,ω3) on hepatic gene expression has not been reported. Accordingly, rats were fed diets containing menhaden oil ethyl esters, ethyl esters of eicosapentaenoic acid (20:5,ω3), and docosahexaenoic acid (22:6,ω3). Controls for these studies included triolein and olive oil (**Fig. 3**).

Both menhaden oil and its concentrated ω3 fatty acids were strongly inhibitory to S14, FAS, ME, GK, and PK gene expression. More importantly, both 20:5,ω3 and 22:6,ω3 were as effective as menhaden oil at suppressing the mRNAs encoding these various proteins. Based on these findings, the inhibitory component of menhaden oil would appear to be the ω3 fatty acid component of menhaden oil. Both 20:5,ω3 and 22:6,ω were equally effective at the dose and duration of treatment used in this study. When compared to rats receiving triolein, olive oil and olive oil ethyl esters (not shown) were mildly inhibitory to S14, FAS, ME, GK, and PK gene expression. This inhibitory effect may be due to the presence of linoleic acid in olive oil (Table 1).

Kinetics of PUFA action on hepatic gene expression

A recent study showed that menhaden oil rapidly (within hours) inhibited S14 gene transcription (7). We hypothesized that the rapid effect of dietary ω3 fatty acids extends to other glycolytic and lipogenic enzymes. Accordingly, the kinetics of inhibition of FAS, GK, PK, and ME gene expression were examined.

We first examined the kinetics of menhaden oil action on FAS gene expression at both the mRNA and transcriptional levels. Rats were meal-fed a high glucose diet containing 10% triolein for 10 days. Half of the rats were maintained on this diet, and the other half were switched to a 10% menhaden oil diet. Both triolein- and menhaden oil-fed rats were killed 90 min after the completion of the meal. The effects of menhaden oil were examined after 1, 2, or 5 meals (**Fig. 4**). In contrast to the rats maintained on the triolein diet, rats switched to the menhaden oil diet showed a rapid and progressive decline in both FAS mRNA and gene transcription. A single meal of menhaden oil was sufficient to suppress FAS gene transcription by 70%. After 2 and 5 days on the menhaden oil diet, FAS gene transcription was suppressed by 80 and 90%, respectively, when compared to triolein-fed rats. FAS gene transcription rates paralleled the decline in $mRNA_{FAS}$, indicating that principal site of menhaden oil action was at the level of FAS gene transcription. This rapid effect of menhaden oil on FAS gene expression is essentially identical to the kinetics of menhaden oil inhibition of S14 gene

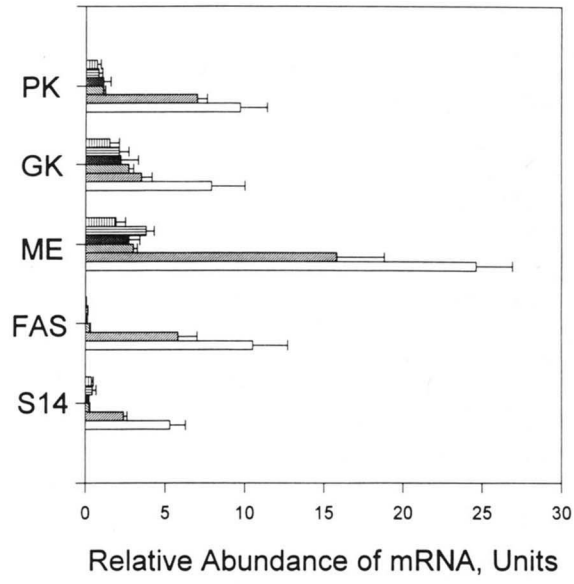

Fig. 3. Effects of different oils and fatty acid esters on hepatic gene expression. Rats were meal-fed high glucose diets supplemented with either triolein (open bar), olive oil (right cross-hatched bar), olive oil ethyl esters (not shown), menhaden oil (left cross-hatched bar), ethyl esters of ω3 fatty acids concentrated from menhaden oil (crisscrossed bar), ethyl esters of 20:5,ω3 (bar with horizontal lines), and ethyl esters of 22:6,ω3 (bar with vertical lines). As the effects of olive oil and olive oil ethyl esters on hepatic gene expression were not significantly different, only the olive oil data are shown in the figure. The composition of the oils used in this study is shown in Table 1. Oils were present at 10% w/w and rats were meal-fed for 5 days. RNA was extracted and specific mRNAs were quantified by RNA dot blot. The results are represented as mean ± SE units, n ≥ 4.

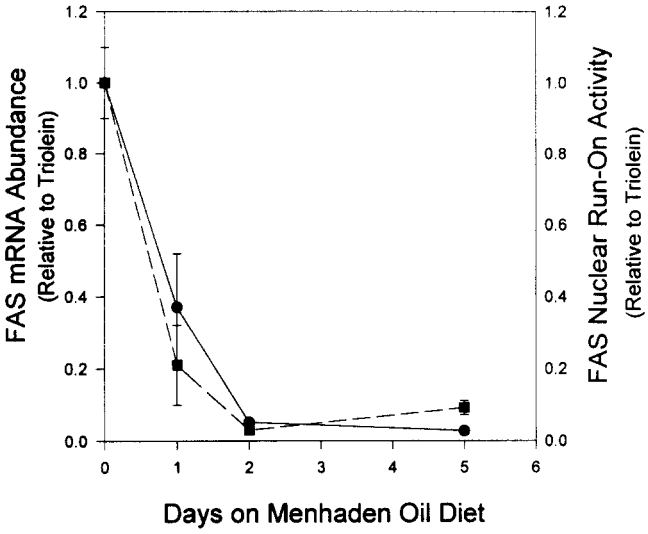

Fig. 4. Kinetics of menhaden oil inhibition of FAS gene expression. Rats were meal-fed a triolein diet as described in Fig. 1. Rats were either maintained on the triolein diet or switched to a menhaden oil diet for 1, 2, or 5 meals (days). Triolein- and menhaden oil-fed rats were killed 90 min after completion of the meal. Hepatic RNA and nuclei were isolated for $mRNA_{FAS}$ (closed circles, solid line) and FAS run-on transcription (closed squares, dashed line) analysis. Results are expressed as mean ± SE relative to the triolein control. Hepatic $mRNA_{FAS}$ (19.1 ± 3.1 units) and transcription rates (58.9 ± 14.5 ppm) in triolein-fed rats did not vary significantly over the 5-day experiment. Three animals were in each group. Similar results were obtained when rats were killed 5 h after food removal. Hepatic run-on transcription rates for phosphoenolpyruvate carboxykinase (62 ± 18 ppm), β-actin (4.1 ± 1.3 ppm), and tyrosine aminotransferase (12 ± 4 ppm) and the corresponding mRNAs (0.2 ± 0.05, 2.5 ± 0.7 and 0.65 ± 0.3 units, respectively) were not consistently affected by menhaden oil treatment.

transcription (7). Transcriptional run-on activity and mRNA levels for phosphoenolpyruvate carboxykinase, tyrosine aminotransferase, and β-actin were not consistently affected by menhaden oil treatment, indicating the specificity of menhaden oil effects on hepatic gene expression.

The second study compared the kinetics of menhaden oil inhibition of mRNAs encoding ME, GK, and PK to FAS and S14. In this study, olive oil was used as the control diet. All of the mRNAs examined showed essentially the same rapid blockade (by ≥ 50%) of the glucose-mediated induction after a single meal as FAS and S14 (**Fig. 5**). In contrast, mRNAs encoding c/EBPα, β-actin, and TRβ1 were not affected by menhaden oil at any time during the 5-day kinetic study (not shown). These findings provide the first evidence for a ω3 fatty acid-mediated coordinate regulatory mechanism that attenuates both hepatic glycolytic and lipogenic enzyme gene expression.

Effects of PUFA on primary hepatocyte gene expression

To determine the molecular basis for this rapid effect of ω3 fatty acids on hepatic gene expression, we needed to establish whether these fatty acids acted directly on hepatocytes. Previous studies on hepatocytes have shown that arachidonic acid, 20:4,ω6 and eicosapentaenoic acid, 20:5,ω3 suppress $mRNA_{FAS}$ and $mRNA_{S14}$ (7, 29).

The first study examined the effects of 18:1,ω9 and 20:5,ω3 on hepatocyte gene expression under defined culture conditions. Hepatocytes were treated with T_3 and insulin to induce the mRNAs encoding the various hepatic proteins. Hepatocytes were also treated for 48 h with albumin in the absence or presence of fatty acids. The levels of mRNAs encoding S14, FAS, ME, PK, GK, and β-actin are shown in **Fig. 6.** With the exception of β-actin, the level of mRNA for the various proteins in the albumin-treated cells was similar to that found in livers of chow-fed rats. $mRNA_{\beta actin}$ increased 6-fold upon hepatocyte culture and the fatty acid treatment had no effect on the accumulation of $mRNA_{\beta actin}$ in these cells.

When compared to the albumin (fatty acid-free)-treated cells, the mRNAs encoding the various proteins were not significantly affected by the oleic acid treatment. In contrast, the level of mRNAs encoding S14, FAS, and PK were attenuated by ≥ 70% after the 48-h exposure to 300 μM 20:5,ω3. $mRNA_{ME}$ was inhibited by 45%, while $mRNA_{GK}$ was not significantly affected by this treatment. With the exception of GK, all of the mRNAs affected by ω3 fatty acids in vivo were also inhibited by 20:5,ω3 treatment of hepatocytes. The most pronounced effect of 20:5,ω3 was on FAS, PK, and S14.

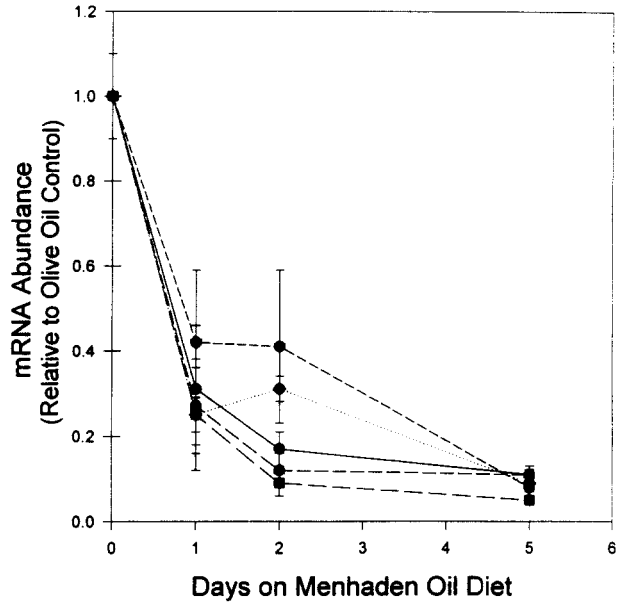

Fig. 5. Kinetics of menhaden oil inhibition of hepatic gene expression. Rats were treated and RNAs were examined as described in Fig. 3, except control animals were fed olive oil. The results are expressed as relative to the olive oil control; mean ± SE units, n = 4. FAS (long dashed line, bottom curve); S14 (medium dashed line, second from bottom curve); ME (solid line), PK (stippled line), and GK (short dashed line, top curve). Menhaden oil feeding had no effect on hepatic levels of mRNAs encoding c/EBPα, TRβ1, or β-actin mRNA.

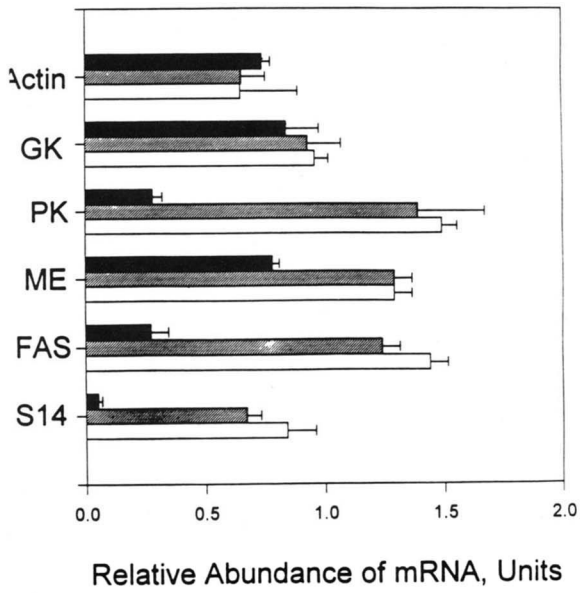

Fig. 6. Effects of fatty acids on hepatocyte gene expression. Primary hepatocytes were treated with media containing 1 μM T_3, 1 μM insulin, and 50 μM albumin. Cells received either no fatty acid treatment (open bar) or were treated with 300 μM 18:1,ω9 (right cross-hatched bar) or 20:5,ω3 (solid bar) for 48 hr. RNA was extracted and analyzed for S14, FAS, PK, ME, GK, and β-actin. Results are expressed as mean ± SE units, n ≥ 4. The values for βactin and malic enzyme (ME) were multiplied by (0.1) to fit on the graph.

We next examined the effects of various fatty acids on S14, FAS, and PK gene expression (**Fig. 7**). S14, FAS, and PK mRNAs were strongly suppressed by α-linolenic (18:3,ω3), γ-linolenic (18:3,ω6), arachidonic (20:4,ω6), and eicosapentaenoic acids (20:5,ω3). No major difference in sensitivity was detected as the fatty acid chain length increased from 18 to 20 or the degree of unsaturation increased from 3 to 5 double bonds. Previous dose–response analysis of 20:4,ω6 and 20:5,ω3 on S14 showed an ED_{50} ≤ 100 μM (7) indicating that ω3 and ω6 were equipotent.

Toxicity of PUFA to cultured hepatocytes

PUFA rapidly undergo peroxidation and these oxidation products can be toxic to cells. In fact, it has been suggested that the principal inhibitory action of PUFA on hepatocyte lipogenesis is due to cell death (30). We examined the effects of the mono- and polyunsaturated fatty acids on hepatocyte viability by measuring levels of LDH activity (**Fig. 8**) and lipid peroxides released from hepatocytes (see below). The level of total hepatocyte protein per tissue culture plate (2.45 ± 0.18 mg, n = 21) was not affected by addition of 18:1, 18:3 (α or γ) or 20:4,ω6 fatty acids to the media. However, treatment of cells with 20:5,ω3 led to a 19% reduction of total protein. The %LDH activity in media of hepatocytes treated with 18:1,ω9 and 18:3, (both ω3 and ω6) was 2.0 ± 0.2. In 20:4- and 20:5-treated cells, the percentage of total LDH released into the media increased to 5.2 ± 0.6 and 8.8 ± 0.4, respectively. The level of peroxides released into the media of 18:1- and 20:5-treated cells, as measured by TBARS, was 2.7 ± 0.9 (n = 10) and 6.0 ± 0.6 μM (n = 10), respectively. When the LDH activity was expressed as a function of the LDH retained within cells, i.e., LDH units/mg protein (1.1 ± 0.1 LDH units/mg protein), no fatty acid cytotoxicity was apparent.

These results suggest that treatment of cells with 20:4,ω6 and 20:5,ω3 promoted a loss of cells from the culture. Presumably, these cells undergo lysis and release LDH activity to the media. Cells remaining attached to the plate show no signs of PUFA cytotoxicity. These cells were intact and functional. Moreover, the lack of any significant effect of PUFA on the levels of mRNAs encoding β-actin or glucokinase would indicate that these cells retain the capacity to express the mRNAs coding for these proteins. This is particularly significant because β-actin mRNA levels increased 6-fold during the 48–72 h primary culture period. These observations suggest that cells used in our assay, i.e., those attached to the plate, are intact and that cytotoxicity cannot account for the specific effects of PUFA on the expression of these glycolytic and lipogenic genes.

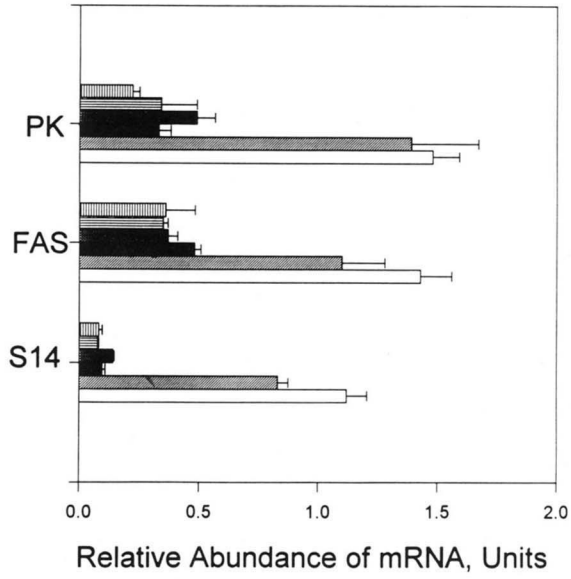

Fig. 7. Effects of ω3, ω6, and ω9 fatty acids on hepatocyte gene expression. Primary hepatocytes were treated with 1 μM T_3, 1 μM insulin, and 50 μM albumin in the absence (open bar) and presence of various fatty acids at 300 μM for 48 h. The fatty acids examined include oleic acid (18:1,ω9) right cross-hatched bar; α-linolenic acid (18:3,ω3) solid bar; γ-linolenic acid (18:3,ω6) criss-crossed bar; arachidonic acid (20:4,ω6) bar with horizontal lines; and eicosapentaenoic acid (20:5,ω3) bar with vertical lines. RNAs were extracted and analyzed for expression of S14, FAS, and PK as described in Fig. 6. The results are expressed as mean ± SE units, n = 4.

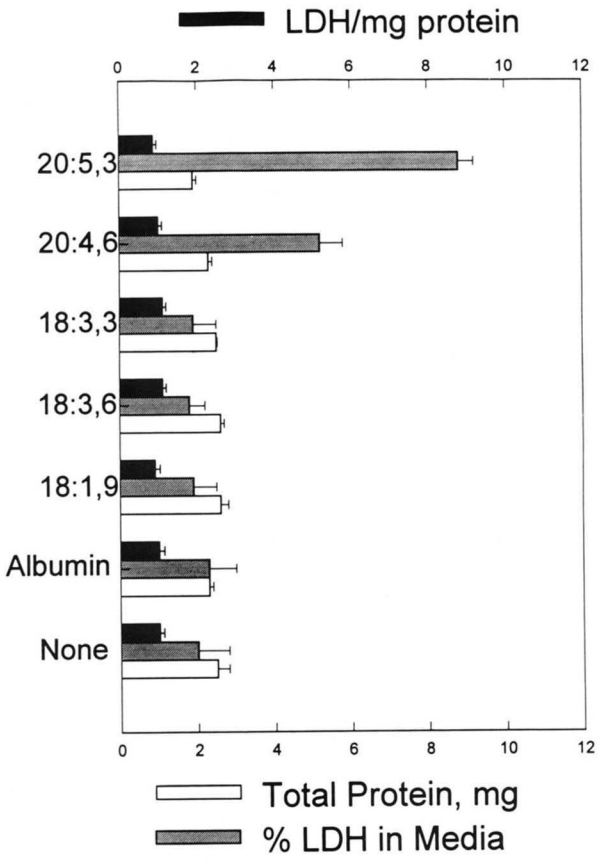

Fig. 8. Effect of fatty acids on release of lactate dehydrogenase from primary hepatocytes. Primary hepatocytes were treated without and with fatty acids (300 μM) as described in Fig. 6. At the completion of the 48-h treatment, hepatocytes and media were harvested as described in Materials and Methods. Results are represented as mean ± SD, n = 3. The following determinations were made: *1)* total cell protein/plate, expressed as total protein, mg (open bar); *2)* LDH activity in hepatocytes, expressed as LDH/mg protein (solid bar); and *3)* LDH activity in media; the fraction of the total LDH activity found in the media was expressed as % LDH in media (cross-hatched bar).

DISCUSSION

We have examined the effects of dietary fish oil and its constituents on the expression of several hepatic genes encoding proteins involved in glycolysis and lipogenesis. The expression of genes encoding both glycolytic (GK and PK) and lipogenic (FAS, ME, and S14) enzymes was inhibited when rats were fed diets containing ω3 PUFA. However, two transcription factors implicated in lipogenic gene expression, i.e., c/EBPα and TRβ1, were not affected by PUFA (Table 2, Figs. 2–5). The effects of PUFA on GK, PK, FAS, ME, and S14 gene expression were rapid (within hours) and significant (> 70% suppression) for each gene examined. This analysis used the meal feeding protocol which orchestrated a coordinate activation of gene transcription for GK, PK, FAS, and S14 (4, 5, 22–27) and enhanced $mRNA_{ME}$ stability (28). Both S14 (7) and FAS (Fig. 4) were inhibited at the transcriptional level. Based on the role transcription plays in the accumulation of $mRNA_{GK}$ and $mRNA_{PK}$ in a fast-refeeding regimen, we speculate that PUFA also attenuated the transcription of these genes. However, additional studies will be required to support this notion.

The lack of PUFA attenuation of mRNAs encoding c/EBPα, TRβ1, β-actin (Table 2, Fig. 2), Pep-carboxykinase (6, 7), or tyrosine aminotransferase (7) indicates that the action of PUFA is gene-specific. Moreover, we have found no evidence for a similar rapid PUFA-mediated control of S14 or FAS in white adipose tissue (7). Thus, the rapid effects of PUFA on gene expression are tissue-specific. This report extends previous in vivo studies (4–7) by showing that the effects of menhaden oil feeding leads to a rapid and coordinate inhibition of several genes encoding proteins involved in hepatic glycolysis as well as lipogenesis. This rapid inhibitory action of PUFA on both glycolytic and lipogenic gene expression may account, at least in part, for the hypertriglyceridemic effect of ω3 fatty acids.

Previous studies on the effects of dietary fat on hepatic glycolytic and lipogenic enzymes showed that the activity of FAS, GK, and PK were suppressed after rats were fed diets containing linoleic acid, C18:2 (10). However, subsequent studies using a similar feeding protocol found no PUFA effect on PK activity (2). The disparity between these previous studies and the present studies can be explained by the following observations. *1)* PUFA suppression of PK enzyme activity correlates with the decline in $mRNA_{PK}$ (preliminary studies); *2)* menhaden oil is 2–4 times more effective at suppressing hepatic FAS activity (and mRNA) than corn oil (3); *3)* products of Δ^6 desaturase activity, e.g., 20:4,ω6 or 20:5,ω3, are more potent suppressors of FAS activity than the respective fatty acid precursors (see below and ref. 3). Thus, the lack of a 18:2 effect on hepatic PK activity (10) is consistent with our view that long chain PUFA (> 20 carbons) are potent suppressors of hepatic lipogenic and glycolytic gene activity.

The in vitro studies have shown that of the mRNAs affected by PUFA in vivo, FAS, PK, and S14, were highly sensitive to ω3 and ω6 fatty acid inhibition, while ME was moderately sensitive. Thus, the PUFA-mediated suppression of mRNAs encoding S14, FAS, PK, and ME did not require extrahepatic factors or fatty acid metabolism. However, the lack of significant attenuation of GK by PUFA in hepatocytes suggests that either extrahepatic factors are required for the PUFA suppression in vivo or that the hepatocyte model is not adequate to examine the PUFA control of GK. The hepatocyte model was initially optimized to study S14 gene expression by T_3, insulin, and PUFA. While GK is regulated by T3 and insulin (31), culture conditions may need to be modified to reveal PUFA control of this gene.

No evidence was found for a differential action of ω3 and ω6 fatty acids on S14, FAS, and GK gene expression. This confirms and extends previous reports on FAS and S14 by showing that not only is 20:4,ω6 inhibitory, but so are 18:3 (both α and γ) and 20:5,ω3 (7, 29). The lack of evidence for a differential action of ω3 or ω6 fatty acids on hepatic gene expression might suggest that PUFA do not utilize a prostanoid pathway to generate reactive intermediates affecting gene expression. Inhibiting cyclooxygenase and the δ5 and δ15-lipoxygenases did not prevent the inhibition of fatty acid synthase by 18:3,ω6, 20:4,ω6, or fish oils (3). However, there does appear to be a requirement for Δ^6-desaturation. Inhibition of this activity by eicosatetraynoic acid (ETYA) blocked the inhibitory effects of 18:3,ω6 on FAS gene expression. Our studies show that both 18:3,ω3 and 18:3,ω6 were as effective as 20:4,ω6 and 20:5,ω3 indicating that the Δ^6-desaturase activity was active in rat hepatocytes. The identity of the cellular intermediates generated from dietary PUFA that affect gene transcription remain to be determined.

Finally, the analysis of fatty acid toxicology in cultured hepatocytes showed that while 20:4,ω6 and 20:5,ω3 promoted some loss of cells from the culture, the remaining cells (> 80%) presented no evidence of toxicity. The PUFA-treated cells retained LDH activity, increased $mRNA_{\beta actin}$ 6-fold, expressed high levels of the transfected RSV-chloramphenicol acetyltransferase activity (7), and expressed $mRNA_{GK}$ as well as cells receiving no fatty acid. In addition, 18:3,ω3 and 18:3,ω6 were as effective as 20:4,ω6 and 20:5,ω3 at inhibiting S14, PK, and FAS gene expression. However, neither 18:3,ω3 or 18:3,ω6 promoted any loss of cells or increased LDH release over control treated cells. Based on these results, the inhibitory action of PUFA on hepatocyte lipogenic and glycolytic gene expression does not appear to be due to generalized toxic effects caused by fatty acid micelle formation (detergent effects) or lipid peroxidation.

In conclusion, the rapid effects of PUFA first reported for the S14 gene (7) now extend to several hepatic genes encoding proteins involved in both glycolysis and lipogenesis. The apparent coordinate and rapid effect on these genes may suggest a common mechanism of repression. The finding that S14, FAS, and PK are similarly regulated by insulin, dietary carbohydrate, and PUFA suggests that these genes might share common response elements which are targeted by the same *trans*-acting factor(s). The PUFA-regulated *cis*-acting elements have been localized for the S14 gene (7). Similar studies are currently underway for the PK and FAS genes.

The authors wish to thank Drs. A. Kahn, P. Iynedjian, M. Magnuson, V. Nikodem, L. Kedes, S. Mcknight, and H. Towle for kindly providing the plasmids used in these studies. The authors also wish to thank Dr. Pat Fair and her colleagues at the Southeast Fisheries Science Center-Charleston Laboratory (Charleston, SC) for the fatty acid analysis of MaxEPA and for the various fractions of menhaden oil used in this study. This work was supported by the National Institutes of Health (DK43220).

Manuscript received 18 October 1993 and in revised form 6 January 1994.

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