Site-specific regulation of gene expression by n–3 polyunsaturated fatty acids in rat white adipose tissues

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Abstract Dietary n–3 polyunsaturated fatty acids (n–3 PUFAs) limit abdominal fat depot hypertrophy. This could be due to regulation of the expression of proteins involved in adipose tissue metabolism. We investigated in vivo whether fatty acid synthase (FAS), hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), phosphoenolpyruvate carboxykinase (PEPCK), CCAAT/enhancer binding protein α (C/EBPα), and leptin mRNA levels are affected in retroperitoneal (RP) and subcutaneous adipose tissues (SC) of rats fed n–3 PUFAs. For 4 weeks rats were fed high fat diets (20% fat) containing n–3 PUFAs given as eicosapentaenoic acid (EPA group), docosahexaenoic acid (DHA group), a mixture of these two fatty acids (MIX group), or native fish oil (FO group). A control group was fed with lard plus olive oil (LOO group). Final mean fat cell weight in RP ranged according to: LOO ≥ EPA ≥ DHA = FO = MIX. There was no difference in fat cell size of SC when comparing the LOO and MIX groups. The fatty acid compositions of RP and SC were similar and resemble that of dietary fat within each experimental group. In RP and compared to the LOO group, FAS, HSL, PEPCK, LPL, C/EBPα, and leptin mRNA levels decreased although not significantly in the EPA group, and were 40–75% lower in the DHA and MIX groups. mRNA levels were positively correlated to fat cell size in RP. In contrast, n–3 PUFAs had no effect on gene expression in SC. We conclude that n–3 PUFAs and mainly 22:6n–3 affect gene expression in a site-dependent manner in white adipose tissues via possible antiadipogenic effects.—**Raclot, T., R. Groscolas, D. Langin, and P. Ferré.** Site-specific regulation of gene expression by n–3 polyunsaturated fatty acids in rat white adipose tissues. *J. Lipid Res.* 1997. **38:** 1963–1972.

Supplementary key words fat cell hypertrophy • gene regulation • adipocyte • fish oil • eicosapentaenoic acid • docosahexaenoic acid

N–3 polyunsaturated fatty acids (n–3 PUFAs) present in fish oil are known to have numerous beneficial effects on health (1–3). This includes amelioration of pathological conditions such as inflammatory diseases (1, 2), atherosclerosis (1, 3), hypertension (1, 4), non-insulin-dependent diabetes (5), as well as blood lipid-lowering actions in rodents (6, 7) and humans (3, 8). The amount and the type of dietary fats have also been shown to affect the development of fat depots in a site-dependent manner in rats fed high fat diets (9). During high fat feeding and for a similar energy intake, hypertrophy of perirenal and epididymal adipose tissues is less important with diets containing fish oil than with diets containing lard or beef tallow (10–13). Conversely, subcutaneous and mesenteric adipose tissues were not affected after the same feeding protocol (11–13). Differences in the lipid gain in adipose tissues were explained by differences in fat cell hypertrophy (10, 11).

Part of these effects are related to changes in plasma membrane fatty acid composition (14) and/or eicosanoid biosynthesis (2, 4, 15). In the liver, fish oil fatty acids could also influence lipid metabolism independently of changes in phospholipid composition and prostanoid production (16, 17). N–3 PUFAs modulate the activity of several hepatic enzymes (6, 7, 18, 19). For instance, it has been reported that fish oil fatty acids suppressed fatty acid synthesis (19), increased oxidation of fatty acids (6, 7, 18, 19), and reduced triacylglycerol synthesis (6, 7). Recently, n–3 PUFAs were shown to

Abbreviations: PUFAs, polyunsaturated fatty acids; RP, retroperitoneal adipose tissue; SC, subcutaneous adipose tissue; LOO, lard and olive oil; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MIX, docosahexaenoic acid and eicosapentaenoic acid; FO, fish oil; FAS, fatty acid synthase; HSL, hormone-sensitive lipase; PEPCK, phosphoenolpyruvate carboxykinase; LPL, lipoprotein lipase; C/EBPα, CCAAT/enhancer binding protein α.

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regulate hepatic gene expression in vitro (20–24) and in vivo (22, 25). Based on previous studies showing that fish oil fatty acids can limit the hypertrophy of abdominal fat depots in rats fed high fat diets (10–13), it is tempting to hypothesize regulation of the expression of adipose tissue proteins by n−3 PUFAs. Indeed, the regulation of the expression of adipose tissue proteins involved in lipid metabolism could contribute to explain the partition between lipid storage and oxidation. Fatty acids of various chain lengths and degrees of unsaturation have been reported to affect gene expression in adipocyte cell lines (26, 27). However, whether n−3 PUFAs affect gene expression in adipose tissue in vivo and whether this is in any way similar to that in the liver is largely unknown.

The two major n−3 PUFAs found in fish oil are eicosapentaenoic acid (20:5n−3) and docosahexaenoic acid (22:6n−3) (28, 29). The level at which each fatty acid contributes to the regulation of gene expression, and whether they are equipotent or not, remains to be documented. The first aim of this study was to determine whether the regulation of key proteins involved in adipocyte metabolism is affected by 20:5n−3 and 22:6n−3 fed alone or in combination. Specifically, the gene expression of fatty acid synthase (FAS), lipoprotein lipase (LPL), phosphoenolpyruvate carboxykinase (PEPCK), hormone-sensitive lipase (HSL), leptin, and CCAAT/enhancer binding protein α (C/EBPα) were studied.

Fat depots are characterized by marked metabolic differences according to their anatomical location (30, 31). These site-specific differences also hold for the gene expression that encodes various proteins in adipose tissue (32–34.) Hence, the second aim of the present study was to examine whether the regulation of adipose tissue gene expression by n−3 PUFAs is site-dependent. For this purpose, an internal depot, retroperitoneal adipose tissue (RP), and a subcutaneous depot (SC), inguinal adipose tissue, were compared.

MATERIALS AND METHODS

Animals and diets

Thirty 50-day-old male Wistar rats (200–220 g; IFFA CREDO, L'Arbresle, France) were housed in individual plastic cages at 25°C with a 12 h/12 h light–dark cycle. They were fed a standard laboratory diet (A04, UAR, Villemoison, France) for 1 week until they reached 240–250 g. They were then randomly shifted for 4 weeks (6 animals per group) to one of five experimental high-fat diets containing (in g/kg): 450 sucrose, 250 casein, 200 fat, 45 alphacel, 45 mineral mix (205B, UAR) and 10 vitamin mix (200, UAR), as previously described (11). The fat part of the diet was a mixture of two to four different fats or oils so that the content in saturated fatty acids and n−6 PUFAs was similar, as determined by gas–liquid chromatography (**Table 1**). The four diets high in fatty acids of marine origin contained the same amounts of n−3 PUFAs and monounsaturated fatty acids (Table 1). N−3 PUFAs were given as the purified ethyl ester of eicosapentaenoic acid (EPA diet) or docosahexaenoic acid (DHA diet), or as a mixture of ethyl esters of these two fatty acids (MIX diet), or as native fish oil (FO diet). The LOO diet contained no n−3 PUFAs (replaced by monounsaturated fatty acids,

TABLE 1. Fatty acid composition of dietary lipids

Fatty Acids	LOO	EPA	DHA	MIX	FO
14:0	0.8	1.0	1.0	1.0	5.9
16:0	18.9	18.7	18.6	18.0	18.9
18:0	12.3	12.6	12.9	12.8	6.0
16:1	1.4	1.2	1.3	1.8	6.8
18:1	58.2	31.1	30.6	30.5	26.9
20:1	0.6	0.6	0.5	0.6	1.5
18:2n−6	7.2	6.8	6.6	6.8	6.1
18:3n−3	0.6	0.5	0.5	0.6	0.8
18:4n−3	—	—	—	1.2	1.7
20:5n−3	—	27.4	1.1	9.9	13.3
22:5n−3	—	—	—	1.4	2.4
22:6n−3	—	—	26.9	15.2	9.5
Sum SFA	32.0	32.4	32.5	31.9	30.8
Sum MUFA	60.2	32.9	32.4	32.9	35.3
Sum n−6 PUFA	7.2	6.8	6.6	6.8	6.1
Sum n−3 PUFA	0.6	27.9	28.5	28.4	27.8

Values (weight percentage) are means of three determinations. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Minor fatty acids (<0.5%) were not considered; —, not determinable.

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mainly 18:1n–9). Diets were prepared weekly, mixed with α-tocopherol (300 mg/kg) as an antioxidant, and stored as daily rations at −20°C. A new ration was given daily. The fatty acid composition of the diets was not affected by 1 week at −20°C nor by 1-day exposure at 25°C. Individual food intake was recorded daily and rat body weight was measured twice a week.

Measurements on adipose tissue and plasma

At the end of the feeding period, rats (355–385 g) were killed by cervical dislocation in the post-absorptive state (1000 h), and exsanguinated by cardiac puncture. Plasma was prepared by centrifugation and kept frozen at −80°C until analysis. Samples of RP and SC (inguinal) were rapidly dissected out, weighed, put into liquid nitrogen, and stored at −80°C until RNA extraction. An aliquot of RP and SC was dissected, minced with scissors, and total lipids were extracted according to Folch, Lees, and Sloane Stanley (35) to gravimetrically determine the lipid content of the two depots and for analysis of the fatty acid composition of stored triacylglycerols. Fat cells were isolated from the remaining RP (all groups) or SC (LOO and MIX groups) according to Rodbell (36). The diameter of 100 fat cells was determined to ± 1 μm using a reticular scale in the eyepiece of a microscope. Fat cell (lipid) mass was calculated from cell volume assuming a density of 0.915 g/ml. Plasma insulin was measured by radioimmunoassay using a commercial kit (ICN, Costa Mesa, CA). Plasma triacylglycerols and non-esterified fatty acids were measured enzymatically using commercials kits (Boehringer, Mannheim, Germany).

Extraction and Northern-blot analysis of total RNA

Adipose tissue total RNA was extracted using a single-step guanidinium thiocyanate–phenol–chloroform extraction (37). The extracted RNA samples (20 μg) were resolved by electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde. The RNA was then transferred onto a nylon membrane (Hybond-N, Amersham) and cross-linked by UV. Prehybridization and hybridization of the blots were performed as described previously (38) using specific cDNA probes labeled with [^{32}P]dCTP by random priming. The cDNA fragment (660 bp in length) for rat liver FAS and the full-length cDNA probe (2.6 kb in length) for rat liver cytosolic PEPCK were kindly provided by Dr. A. G. Goodridge (Iowa City, IA) and Dr. R. W. Hanson (Cleveland, OH), respectively. Full-length rat HSL, rat LPL, and rat C/EBPα cDNAs were generous gifts from Dr. C. Holm (Lund University, Lund, Sweden), Dr. J. Auwerx (INSERM, Lille, France), and Dr. S. L. McKnight (Tularik Inc., CA), respectively. The 236 bp of the rat β-actin gene corresponding to exon 3 was amplified from 500 ng of rat genomic DNA by the polymerase chain reaction using sense primer: 5′-GAGACCTTCAACACC CC-3′ and antisense primer: 5′-GTGGTGGTGAAGCTG TAGCC-3′. A rat leptin cDNA fragment (nt 86 to 436 of the coding sequence) was cloned from adipose tissue by reverse transcription and polymerase chain reaction amplification using sense primer: 5′-TGACACCAAAAC CCTCATCAAG-3′ and antisense primer: 5′-GCCACCA CCTCTGTGGAGTA-3′. Polymerase chain reaction fragment(s) of the predicted size were isolated and cloned into pBluescript. Sequence determination showed complete identity to the reported rat leptin and β-actin cDNA sequences. Autoradiograms were performed by exposure of radioactive membranes to Hyperfilm (Amersham) at −80°C with intensifying screens. Quantifications were performed by scanning densitometry of the autoradiograms. A β-actin cDNA fragment was used as a control probe.

Lipid analysis

To obtain triacylglycerols, samples (about 1 mg) of total lipid extracts from RP and SC were purified

TABLE 2. Body mass, food intake, fat depot cellularity and lipid content, and plasma insulin of rats fed the experimental diets

Parameters	LOO	EPA	DHA	MIX	FO
Final body weight (g)	373.8 ± 3.0	365.3 ± 5.2	371.6 ± 1.8	371.1 ± 3.5	382.5 ± 3.6
Food intake (g)	16.6 ± 0.3	16.2 ± 0.4	16.8 ± 0.2	16.3 ± 0.2	16.9 ± 0.3
Retroperitoneal fat					
Cell weight (μg)	0.69 ± 0.06^{a}	0.57 ± 0.03^{a,b}	0.48 ± 0.04^{b,c}	0.42 ± 0.02^{c}	0.47 ± 0.03^{b,c}
Lipid content (%)	93.2 ± 0.3^{a}	91.0 ± 0.4^{b,c}	88.4 ± 1.2^{c,d}	84.5 ± 1.2^{d}	87.9 ± 0.6^{c,d}
Subcutaneous fat					
Cell weight (μg)	0.31 ± 0.02	ND	ND	0.28 ± 0.03	ND
Lipid content (%)	71.7 ± 1.6	ND	ND	70.3 ± 1.2	ND
Insulin (μU/ml)	38.6 ± 8.9^{a}	22.4 ± 3.4^{a,b}	24.0 ± 2.4^{a,b}	22.0 ± 2.7^{a,b}	18.8 ± 1.0^{b}

Values are means ± SE (n = 6). Within a line, values that do not share the same superscript letter are significantly different ($P < 0.05$); ND, not determined.

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TABLE 3. Fatty acid composition in triacylglycerols of retroperitoneal adipose tissue from rats fed the experimental diets

Fatty Acids	LOO	EPA	DHA	MIX	FO
Sum SFA	26.2 ± 0.5^a	34.6 ± 0.4^b	33.8 ± 0.4^b	34.0 ± 0.5^b	36.4 ± 0.7^b
Sum MUFA	63.3 ± 0.3^a	42.2 ± 0.6^b	43.0 ± 0.6^b	40.9 ± 0.8^b	40.8 ± 0.6^b
Sum n–6 PUFA	10.0 ± 0.3	11.9 ± 0.6	11.8 ± 0.3	11.8 ± 0.3	10.4 ± 0.3
Sum n–3 PUFA	0.5 ± 0.0^a	11.4 ± 0.7^b	11.4 ± 0.4^b	13.3 ± 1.0^b	12.3 ± 0.4^b
20:5n–3	—	9.1 ± 0.5^a	0.5 ± 0.0^b	3.0 ± 0.4^c	3.9 ± 0.2^c
22:6n–3	—	—	10.2 ± 0.4^a	7.9 ± 0.5^b	5.1 ± 0.3^c

Values (weight percentage) are means ± SE (n = 6). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Within a line, values that do not share the same superscript letter are significantly different ($P < 0.05$). Minor fatty acids (<0.5%) were not considered; —, not determinable.

by thin-layer chromatography using hexane–diethyl ether–acetic acid 70:30:1 (v/v/v) as the developing solvent (29). The thin-layer chromatography plates were then dried under nitrogen, sprayed with primulin, and the triacylglycerol bands were scraped into vials. After saponification, fatty acid methyl esters were prepared using 14% boron trifluoride in methanol, as reported earlier (29). Fatty acid derivatives were analyzed by gas–liquid chromatography using a Chrompack CP9000 chromatograph (Chrompack, Les Ulis, France) equipped with a capillary column (AT-WAX, 60 m-long, 0.25 mm internal diameter, 0.25 μm thickness, Alltech, Templeuve, France) and quantified with an integrator (SP 4290, Spectra-Physics, Les Ulis, France). Butylated hydroxytoluene at the final concentration of 0.05% was added to all solvent mixtures as an antioxidant.

Statistics

Results are expressed as the means ± SE of 4 to 6 determinations. The statistical significance of differences between means was assessed using Peritz' F-test (39) for multiple comparisons and the criterion of significance was $P < 0.05$. Linear regression analyses with the F test were performed on individual values for statistical analysis of the correlations.

RESULTS

Animals and measurements on adipose tissue and plasma

Final body weight and daily food intake were not significantly different among groups (**Table 2**). The daily n–3 PUFA intake was similar (close to 0.9 g/d) in the four groups fed diets enriched with these fatty acids (EPA, DHA, FO, and MIX groups).

When comparing all experimental groups, fat cell weight in RP decreased in the following order: LOO ≥ EPA ≥ DHA = FO = MIX (Table 2), ranging from 0.7 μg (LOO group) to 0.4 μg (MIX group). The same trend applies also for lipid content, ranging from 93% (LOO group) to 85% (MIX group). The various experimental diets did not induce changes in the number of RP fat cells, as obtained by dividing the total lipid con-

TABLE 4. Fatty acid composition in triacylglycerols of subcutaneous adipose tissue from rats fed the experimental diets

Fatty Acids	EPA	DHA	MIX	FO
Sum SFA	33.6 ± 0.5	31.5 ± 0.5	32.5 ± 0.5	33.4 ± 0.8
Sum MUFA	41.2 ± 0.3	39.8 ± 0.7	39.4 ± 1.7	39.8 ± 1.1
Sum n–6 PUFA	15.7 ± 0.6	14.8 ± 0.3	14.9 ± 1.3	14.2 ± 0.4
Sum n–3 PUFA	9.5 ± 0.6^a	13.9 ± 0.8^b	13.1 ± 1.1^b	12.6 ± 0.6^b
20:5n–3	7.4 ± 0.5^a	0.6 ± 0.0^b	3.2 ± 0.3^c	4.0 ± 0.3^c
22:6n–3	—	12.0 ± 0.8^a	7.5 ± 0.7^b	5.2 ± 0.4^c

Values (weight percentage) are means ± SE (n = 6). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Within a line, values that do not share the same superscript letter are significantly different ($P < 0.05$). Minor fatty acids (<0.5%) were not considered; —, not determinable.

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tent of RP by the average mass of RP fat cells (data not shown). In contrast, fat cell size and lipid content of SC were similar in the LOO and MIX groups. Thus, n−3 PUFA intake influences fat cell hypertrophy and lipid content differently depending on the depot location.

Plasma concentration of insulin tended to be lower in the four experimental groups fed n−3 PUFAs than in the LOO group (Table 2). However, probably due to the wide dispersion of individual values in the LOO group, the difference was significant only when compared with the FO group. Plasma concentration of triacylglycerols were similar in the four groups fed n−3 PUFAs and significantly 70–80% lower than in the LOO group ($P < 0.001$) whereas plasma concentration of non-esterified fatty acids did not differ significantly between the five groups (data not shown).

Fatty acid composition of retroperitoneal and subcutaneous adipose tissues

The fatty acid composition of both adipose tissues is shown in **Table 3** (RP) and **Table 4** (SC). It resembles that of dietary fat (see Table 1). A high level of n−3 PUFAs was found in adipose tissue triacylglycerols of animals fed with EPA, DHA, FO, and MIX diets, the proportion of 20:5n−3 and 22:6n−3 depending on their respective proportions in the diets. In contrast, adipose tissue triacylglycerols of the LOO group contained higher proportions of monounsaturated fatty acids (mainly 18:1n−9) and almost no n−3 PUFAs of marine origin. In RP and SC, the content of triacylglycerols in saturated, monounsaturated, n−6 PUFAs, and n−3 PUFAs did not differ among the four groups of rats fed fatty acids of marine origin (Tables 3 and 4). Within each experimental group, the fatty acid composition of RP (Table 3) and SC (Table 4) was similar. Hence, both adipose tissues can serve as a reservoir for dietary n−3 PUFAs.

Effects of dietary n−3 polyunsaturated fatty acids on adipose tissue gene expression

Dietary manipulation was used to study the expression of several gene-encoding enzymes involved in lipid and glucose metabolism, a transcription factor (C/EBPα) and leptin in rat white adipose tissues. Northern blot hybridization of RP total RNA with cDNA probes for FAS, HSL, PEPCK, LPL, C/EBPα and leptin mRNA is shown in **Fig. 1.** The intensity of the specific signals for the adipocyte mRNAs tended to be lower in animals fed EPA, DHA, FO, and MIX diets than in animals fed the LOO diet. By contrast, the β-actin mRNA level was not affected by the dietary treatments, indicating the relative specificity of n−3 fatty acids on adipose tissue gene expression. Quantitative analyses were performed after correction for β-actin mRNA levels considering mRNA levels in FO as unity (**Fig. 2**). mRNA levels for all the adipocyte genes fell into the following order: LOO group (no n−3 PUFAs of marine origin) ≥ EPA group (20:5n−3 is the main n−3 PUFA) ≥ DHA group (22:6n−3 is the main n−3 PUFA) = MIX group (20:5n−3 and 22:6n−3 are both present). The difference between the LOO and EPA groups did not reach statistical significance. When compared to the LOO group, a maximal decrease of about 2-fold was observed in the DHA and MIX groups for FAS, HSL, PEPCK, LPL, and C/EBPα mRNA levels, and of about 4-fold for the leptin mRNA level (Fig. 2). The inhibitory effect of 22:6n−3 on adipose tissue gene expression was not significantly different when fed alone or in combination with 20:5n−3.

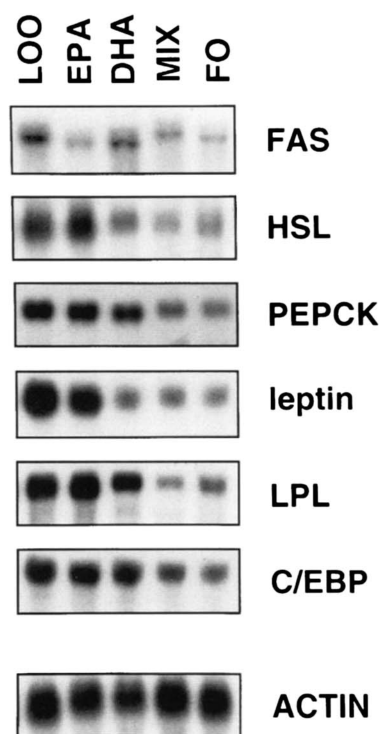

Fig. 1. Northern-blot analysis of retroperitoneal adipose tissue mRNA from rats fed high-fat diets differing in fatty acid composition. 20 μg of total RNA was electrophoresed in a 2.2 M formaldehyde, 1% agarose gel and blotted to nylon membrane. The blots were hybridized with ^{32}P-labeled cDNA probes. LOO (lard plus olive oil), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), MIX (docosahexaenoic acid plus eicosapentaenoic acid), and FO (fish oil). FAS, fatty acid synthase; HSL, hormone-sensitive lipase; PEPCK, phosphoenolpyruvate carboxykinase; LPL, lipoprotein lipase; C/EBP, CCAAT/enhancer binding protein α.

The data in Table 2 and Fig. 2 suggest that changes in RP fat cell weight parallel changes in adipose tissue gene expression among the experimental groups. Linear-regression analyses were performed between fat cell weight and relative mRNA levels and a significant

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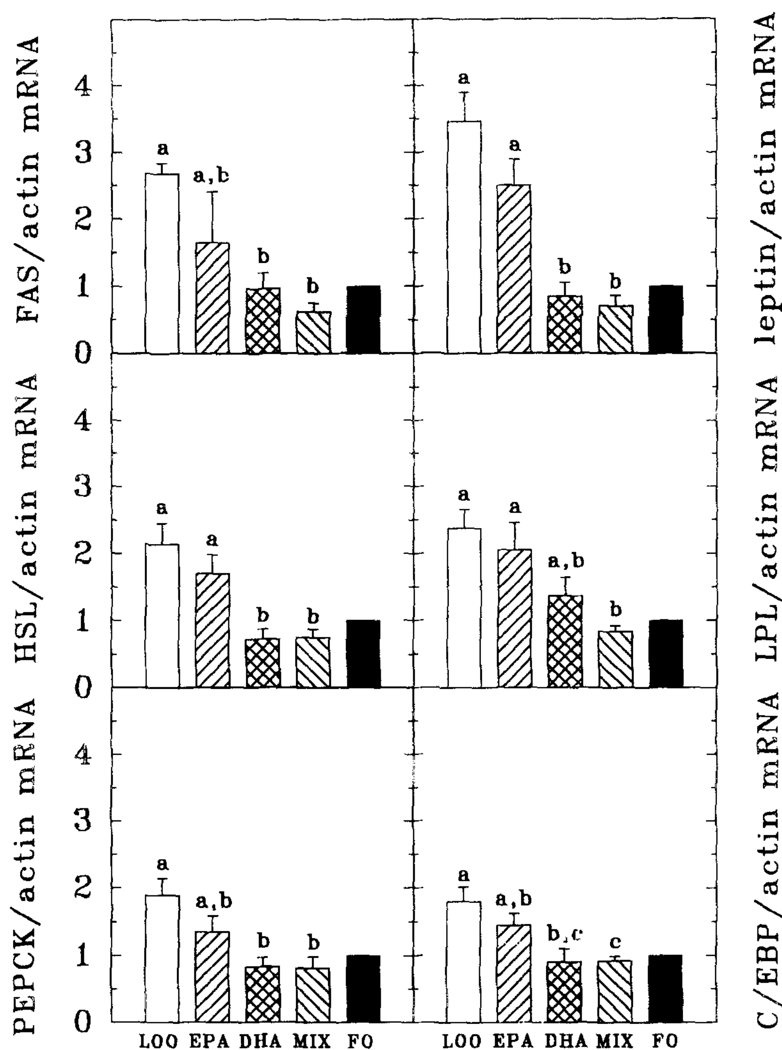

Fig. 2. Effect of dietary fatty acids on retroperitoneal adipose tissue gene expression. Results of densitometry scanning of autoradiograms are expressed relative to actin mRNA in arbitrary units normalized to the FO group as unity. Bars that do not share the same superscript letter are significantly different ($P < 0.05$). Values are means ± SE of 4–6 determinations. Abbreviations as in Fig. 1.

positive relationship was found for all the genes (**Fig. 3**). Slope values clustered around 3–4 for HSL, PEPCK, LPL and C/EBPα whereas the slope was above 7 for leptin, indicating that the leptin mRNA level is more sensitive to variation of fat cell size.

Expression of the same genes was studied in SC (**Fig. 4** and **Table 5**). In marked contrast with RP, n–3 PUFAs had no effect on gene expression. This holds also for the leptin mRNA level which was the most depressed in RP. Fed alone or in combination, 20:5n–3 and 22:6n–3 were similarly ineffective at repressing mRNA expression in SC.

DISCUSSION

There is now clear evidence that the nature of the dietary fat can influence the overall lipid metabolism such as plasma lipid profile and body fat deposition. In full agreement with previous studies (10, 11, 13), we found that n–3 PUFA intake limits fat cell hypertrophy and lipid content of adipose tissue according to the depot location. The hypothesis that n–3 PUFAs influence lipid accretion in adipose tissue by affecting the expression of enzymes involved in the control of lipid metabolism has been examined here by focusing attention on the regulation of genes encoding lipogenic (FAS and LPL), lipolytic (HSL), and glyceroneogenic enzymes (PEPCK). In order to evaluate fat cell differentiation and hypertrophy, mRNA levels of C/EBPα (40) and leptin (41–43) were measured.

Among metabolic pathways that could be influenced by n–3 PUFAs and contribute to explain the limitation of fat cell hypertrophy in RP, increase of lipolysis by induction of HSL expression, as already reported during fasting (44), and/or decrease of lipogenesis by repression of FAS expression, as previously shown in the liver (22, 25), can be hypothesized. A decrease of lipid storage in adipose tissue by decreased hydrolysis of circulat-

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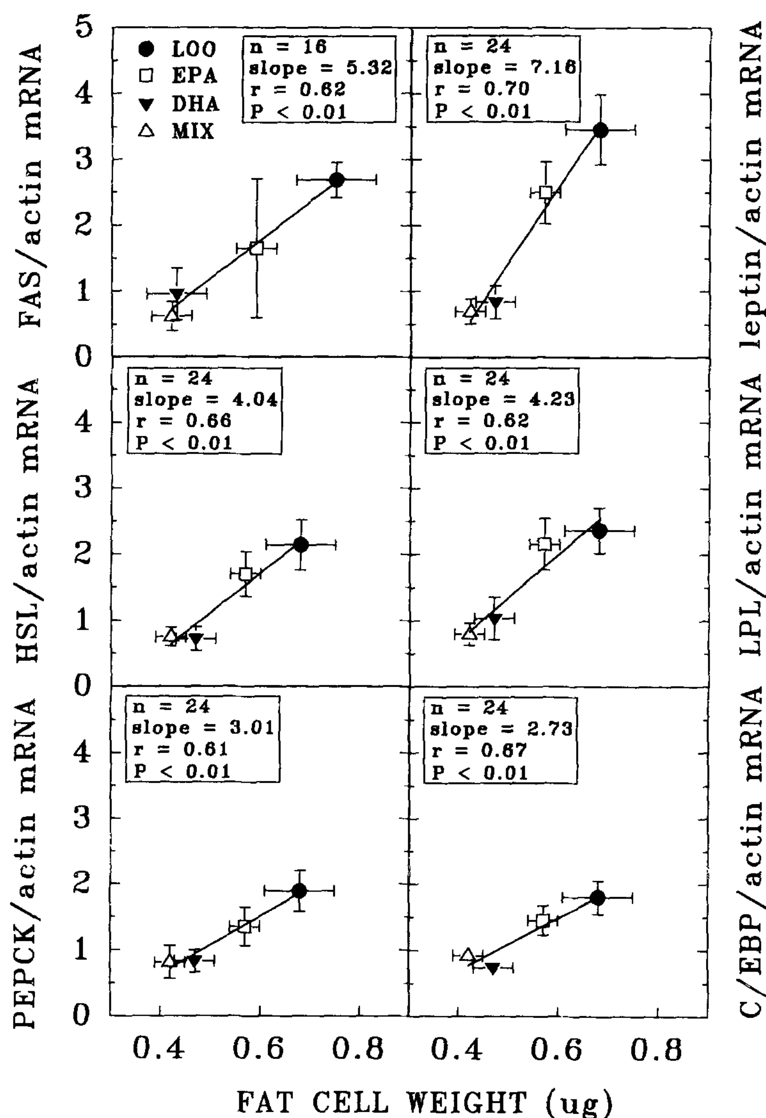

Fig. 3. Relationship between gene expression and fat cell weight in retroperitoneal adipose tissue. Data are from Fig. 2 and Table 2. Each point corresponds to a mean ± SE. Linear regression analyses were performed on individual values. Abbreviations as in Fig. 1.

ing triacylglycerols through diminished LPL expression can also be proposed. PEPCK catalyzes a critical step in adipose tissue glyceroneogenesis and thereby permits fatty acid reesterification (27). In this connection, a limitation of lipid accretion in adipose tissue by a depressed PEPCK expression could also be expected. A limitation of fat cell hypertrophy could result from a decrease in food intake and/or an increase in energy expenditure. Indeed, the ob gene product leptin is thought to be a fat-derived satiety factor acting on brain centers controlling energy homeostasis (45, 46). Leptin mRNA and/or plasma leptin levels are correlated with the percentage of body fat in rodents (47) and in humans (41). Therefore, an increase of leptin gene expression in rats fed n−3 PUFAs could be expected. In the present study, we report a 25–75% decrease of adipose tissue gene expression in RP but none in SC in response to n−3 PUFA intake. These data are in accordance with the previously reported decrease of S14 mRNA levels by 50% in rat white adipose tissue after menhaden oil feeding (20). The decrease of FAS, LPL, and PEPCK expression in RP would be in rather good agreement with the limitation of fat cell hypertrophy by n−3 PUFAs but not the low HSL expression. A major role for leptin gene expression is not supported by our data as leptin gene expression was decreased in RP and remained unchanged in SC after n−3 PUFA intake. Moreover, the daily food intake did not differ among the experimental groups. On the other hand, low plasma insulin levels may contribute to explain the up to 4-fold decrease of leptin gene expression in RP in the groups fed n−3 PUFAs (48). However, variations in plasma insulin concentrations do not explain the difference in leptin mRNA levels in RP between the EPA and

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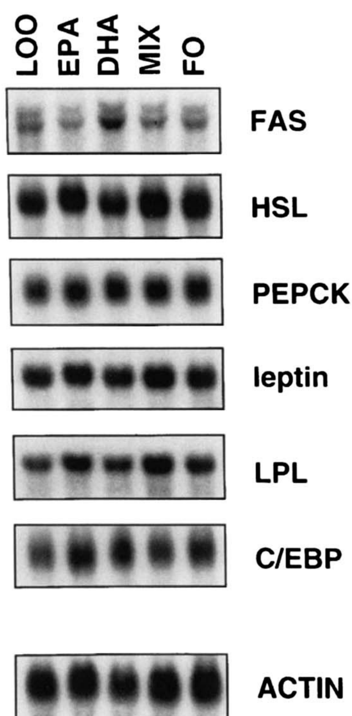

Fig. 4. Northern-blot analysis of subcutaneous adipose tissue mRNA from rats fed high-fat diets differing in fatty acid composition. Abbreviations and footnotes as in Fig. 1.

DHA groups (Table 2, Fig. 2) and between RP and SC (Table 5, Fig. 2). The site-specific regulation of adipose tissue gene expression may depend upon the time and duration of the dietary treatment. Hill and coworkers (12) showed that feeding rats fish oil for 6 months significantly limited the hypertrophy of the four major fat depots including SC. Therefore, with a long-term treatment, the effects of n−3 PUFAs on gene expression in SC could become comparable to those in RP. Hence it should not be definitively concluded that RP but not SC responds to n−3 PUFAs. This would be in line with earlier studies that found no evidence for rapid PUFA-mediated control of gene expression in white adipose tissue, contrary to what occurs in the liver (16, 17, 20). Altogether, because of similar gene repression of proteins involved in lipogenic and lipolytic metabolic pathways, these results provide no clear evidence for a coordinated modulation of gene expression in RP as an explanation of the limitation of fat cell hypertrophy by n−3 PUFAs.

The mRNAs studied herein, except β-actin mRNA, are expressed during the conversion of preadipocytes into adipocytes and can be considered as markers of the adipocyte phenotype (49, 50). The LPL gene is one of the first genes to be transcribed during adipocyte differentiation and is defined as an early marker (49). FAS, HSL, PEPCK, and leptin are late markers (49). The lower expression of early and late markers suggests a modulation of the adipocyte phenotype in RP according to the dietary treatment. In addition, the mRNA level of C/EBPα, a transcriptional activator of adipocyte genes during preadipocyte differentiation, was also lower (Fig. 2). Expression of the transcription factor C/EBPα is necessary for the induction of adipocyte conversion (40). When considering all the experimental groups, fat cell weights and adipocyte marker mRNA levels are directly related (Fig. 3). Our results suggest that n−3 PUFAs exert an antiadipogenic effect in RP. An alternative explanation could be that fat cell size and gene expression are closely related but independent of n−3 PUFA intake. Then other factors decreasing fat cell size could also down-regulate gene expression. Interestingly, the reduction of leptin gene expression was more pronounced than that of the other late markers. In agreement with previous studies (41–43), the leptin mRNA level was closely correlated with fat cell size (Fig. 3). These data confirm that leptin mRNA is a good marker of fat cell hypertrophy (43) but provide evidence that other adipocyte marker mRNA levels may vary according to fat cell size. This is further reinforced by the finding that there were neither changes in fat cell weight nor in gene expression in SC (Tables 2 and 5). Altogether, these findings provide evidence for a mechanism leading to the attenuation of adipogenesis by n−3 PUFAs in a site-specific manner.

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TABLE 5. Effect of dietary fatty acids on subcutaneous adipose tissue gene expression

	LOO	EPA	DHA	MIX	FO
FAS	1.09 ± 0.38	0.87 ± 0.31	1.31 ± 0.46	1.13 ± 0.48	1.00
HSL	1.10 ± 0.19	0.90 ± 0.06	1.13 ± 0.16	0.87 ± 0.07	1.00
PEPCK	0.88 ± 0.19	0.72 ± 0.08	1.07 ± 0.18	1.04 ± 0.10	1.00
Leptin	0.92 ± 0.04	0.92 ± 0.16	1.16 ± 0.10	0.82 ± 0.12	1.00
LPL	0.90 ± 0.07	0.92 ± 0.18	1.07 ± 0.10	0.90 ± 0.14	1.00
C/EBPα	0.85 ± 0.05	0.93 ± 0.10	1.05 ± 0.26	0.87 ± 0.08	1.00

Values are means ± SE of 4–6 determinations. Results of densitometry scanning of autoradiograms are expressed relative to β-actin mRNA in arbitrary units taking the FO group as unity. FAS, fatty acid synthase; HSL, hormone-sensitive lipase; PEPCK, phosphoenolpyruvate carboxykinase; LPL, lipoprotein lipase; C/EBPα, CCAAT/enhancer binding protein α.

Lastly, our data show a significant inhibitory effect on RP gene expression of 22:6n−3 alone (DHA group) or in combination with 20:5n−3 (MIX group) but not of 20:5n−3 alone (EPA group). This seems different from data previously reported for the liver where both 20:5n−3 and 22:6n−3 were equally effective at repressing gene expression (22). The mechanisms through which n−3 PUFAs, and mainly 22:6n−3, could affect adipose tissue gene expression are still unclear. The suppression of gene expression by fish oil fatty acids might suggest that a prostanoid pathway is involved through the production of reactive intermediates. In this connection, recent evidence indicates that fatty acid metabolites such as prostaglandins of the 2-series (e.g., PGI_2, PGJ_2) exert adipogenic effects (51, 52). By competing for cyclooxygenase with 20:4n−6, the precursor of the 2-series of prostaglandins (15), 20:5n−3 and/or 22:6n−3 could affect adipogenesis.

In conclusion, the expression of the genes encoding lipogenic (FAS, LPL), lipolytic (HSL), glyceroneogenic (PEPCK) enzymes, the transcription factor (C/EBPα) and leptin was decreased by 22:6n−3 fed alone or in combination with 20:5n−3 in RP but not in SC. The decrease of mRNA levels is closely related to the decrease of fat cell size. As RP and SC have similar fatty acid composition within each experimental group, the decrease of mRNA levels is not directly related to the fatty acid profile of the adipose tissues. The marked differences in adipose tissue gene expression according to its anatomic location suggest a role for autocrine, paracrine, and/or endocrine factors. The mechanisms underlying these site-specific changes induced by n−3 PUFAs remain to be elucidated. On the other hand, such a dietary model of obesity should be useful to study relationships between fat cell size and gene expression in vivo.

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