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Dietary Olive Oil and Menhaden Oil Mitigate Induction of Lipogenesis in Hyperinsulinemic Corpulent JCR:LA-cp Rats: Microarray Analysis of Lipid-Related Gene Expression

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In the corpulent James C. Russell corpulent (JCR:LA-cp) rat, hyperinsulinemia leads to induction of lipogenic enzymes via enhanced expression of sterol-regulatory-binding protein (SREBP)-1c. This results in increased hepatic lipid production and hypertriglyceridemia. Information regarding down-regulation of SREBP-1c and lipogenic enzymes by dietary fatty acids in this model is limited. We therefore assessed *de novo* hepatic lipogenesis and hepatic and plasma lipids in corpulent JCR rats fed diets enriched in olive oil or menhaden oil. Using microarray and Northern analysis, we determined the effect of these diets on expression of mRNA for lipogenic enzymes and other proteins related to lipid metabolism. In corpulent JCR:LA-cp rats, both the olive oil and menhaden oil diets reduced expression of SREBP-1c, with concomitant reductions in hepatic triglyceride content, lipogenesis, and expression of enzymes related to lipid synthesis. Unexpectedly, expression of many peroxisomal proliferator-activated receptor-dependent enzymes mediating fatty acid oxidation was increased in livers of corpulent JCR rats. The menhaden oil diet further increased expression of these enzymes. Induction of SREBP-1c by insulin is dependent on liver × receptor (LXR)α. Although hepatic expression of mRNA for LXR itself was not increased in corpulent rats, expression of Cyp7a1, an LXR-responsive gene, was increased, suggesting increased LXR activity. Expression of mRNA encoding fatty acid translocase and ATP-binding cassette subfamily DALD member 3 was also increased in livers of corpulent JCR rats, indicating a potential role for these fatty acid transporters in the pathogenesis of disordered lipid metabolism in obesity. This study clearly demonstrates that substitution of dietary polyunsaturated fatty acid for carbohydrate in the corpulent JCR:LA-cp rat reduces *de novo* lipogenesis, at least in part, by reducing hepatic expression of SREBP-1c and that strategies directed toward reducing SREBP-1c expression in the liver may mitigate the adverse effects of hyperinsulinemia on hepatic lipid production. (*Endocrinology* 145: 5847–5861, 2004)

OBESITY AND INSULIN resistance result in increased plasma levels of triglyceride-rich lipoproteins and increased accumulation of triglyceride in the liver. In such individuals, this is accompanied by increased small low-density-lipoprotein particles and reduced levels of high-density lipoproteins or atherogenic dyslipidemia (1). Although current dietary recommendations advocate replacing dietary fat (saturated) with carbohydrate (Dietary Guidelines for Americans 2000; www.health.gov/dietary guidelines), such an approach may not be optimal for individuals with obesity and insulin resistance. High-carbohydrate diets can increase plasma triglyceride, whereas substitution of monounsaturated fat for carbohydrate reduces plasma triglyceride (1). Conversely, diets enriched in polyunsaturated fatty acids (PUFAs), including N-3 fatty acids, also reduce plasma triglyceride and reduce cardiovascular disease (2). In addition to their role as an energy source, fatty acids are also ligands for nuclear receptors [peroxisomal proliferator-activated receptors (PPARs), liver X receptor (LXR)], which in turn control metabolic pathways (3). In this context, a greater understanding of the effect of dietary fat on metabolic pathways in general and lipogenesis in particular is useful in designing optimal dietary fat intake for individuals with obesity and hyperinsulinemia.

The James C. Russell corpulent (JCR:LA-cp) rat, which lacks a functioning leptin receptor, is a useful model for study of the metabolic consequences of obesity and hyperinsulinemia (4, 5). When homozygous for the corpulent (cp) gene, these rats exhibit hyperphagia, obesity, hyperlipidemia, and hyperinsulinemia (6–8). Hypersecretion of very

Abbreviations: ABCD3, ATP-binding cassette subfamily DALD member 3; ACC-1, acetyl-coenzyme A carboxylase-1; acyl-CoA, acyltransferase-coenzyme A; apo, apolipoprotein; CD36, fatty acid translocase; C/EBP, CAAT/enhancer-binding protein; CoA, coenzyme A; cp, corpulent gene; Cyp7a1, cholesterol 7α-hydroxylase; EPA, eicosapentaenoic acid; FAS, fatty acid synthase; FXR, farnesoid X receptor; HDL, high-density lipoprotein; IGFBP, IGF binding protein; JCR:LA-cp, James C. Russell corpulent rat; Kid-1, zinc finger protein 354A; LCAT, lecithin/cholesterol acyltransferase; LXR, liver X receptor; NF-Y, nuclear factor-Y; NrOb2, nuclear receptor OB2; PL, phospholipid; PPAR, peroxisomal proliferator-activated receptor; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA-desaturase; SDS, sodium dodecyl sulfate; Sp1, specificity protein 1; SR-B1, scavenger receptor class B, type 1; SREBP, sterol-regulatory-binding protein; TG, triglyceride; UCP-2, uncoupling protein 2; VLDL, very low-density lipoprotein.

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low-density lipoprotein (VLDL) and resultant hyperlipidemia in the corpulent JCR:LA-cp rat and other models of obesity and hyperinsulinemia (*e.g.* fatty Zucker rat) results in part from increased *de novo* lipogenesis and increased use of fatty acid for triglyceride synthesis (9–11). Sterol-regulatory-binding protein (SREBP)-1c is a pivotal regulator of lipogenic enzyme expression (12, 13), Expression of SREBP-1c as well as the lipogenic enzymes fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase (ACC-1) is increased in the hyperinsulinemic corpulent JCR:LA-cp rat (9). Induction of SREBP-1c by insulin is the result, at least in part, of increased SREBP-1c gene transcription (14). Conversely, PUFAs have been shown to reduce expression of lipogenic enzymes by reducing levels of SREBP-1c (15). Proposed mechanisms for reduced SREBP-1c expression after exposure to PUFAs include accelerated degradation of SREBP-1 mRNA (16), a block in LXR-dependent activation of the SREBP-1c promoter (17), and inhibition of proteolytic processing (18).

We observed that fatty acids prevent activation of the SREBP-1c promoter by insulin (14). The N-3 PUFA, eicosapentaenoic acid (EPA, 20:5 N-3) was most effective in this regard, whereas the monounsaturated fatty acid, oleic acid (18:1, N-9) was ineffective (14). Although dietary PUFAs have been shown to suppress hepatic lipogenesis by reducing expression of lipogenic enzymes (19, 20), limited studies conducted to date in animal models of obesity and hyperinsulinemia suggest that such animals may be resistant to down-regulation of lipogenic enzymes by dietary fat (21–23). In addition, much of the information on effects of fatty acids on hepatic lipogenesis is derived from *in vitro* studies using individual fatty acids. It is important to extend this information by study of administration of real-life sources of dietary fatty acid *in vivo*. We therefore investigated whether diets enriched in two common sources of dietary fat, olive oil and fish oil (menhaden oil), one enriched in monounsaturated fatty acids, and another enriched in N-3 polyunsaturated fatty acids, could mitigate induction of SREBP-1c expression by hyperinsulinemia and inhibit both hepatic lipogenesis and expression of lipogenic enzymes in the corpulent JCR:LA-cp rat. We also examined the effect of obesity and dietary fatty acid on global lipid/lipoprotein-related gene expression in the livers of JCR:LA/cp rats using microarray analysis. We demonstrate coordinate regulation of a wide range of genes related to lipid and lipoprotein metabolism by both obesity and dietary fat in the corpulent JCR rat.

Materials and Methods

Animal procedures and dietary composition

The experimental protocol was approved by the University of Tennessee Animal Use Committee. All animal experimentation was conducted in accord with accepted standards of humane animal care. Male JCR:LA lean and corpulent rats were used in this study. JCR:LA-cp rats (obese) are homozygous for the defective leptin receptor gene (cp/cp), whereas lean animals are heterozygous for the cp gene (cp/+) or homozygous for the normal leptin receptor gene (+/+). Lean animals are phenotypically indistinguishable and are therefore designated as +/?. Nine corpulent (cp/cp) and nine lean (+/?) rats were obtained from the University of Alberta, Edmonton, Canada. The lean and obese rats were 6 wk of age and weighed, on average, 115 and 145 g, respectively, on arrival. They were individually caged and maintained on a standard rat chow diet (Harlan Teklad, Rodent Diet 8640, Madison, WI) (22% protein, 5% fat, 68.5% carbohydrate, and 4.5% fiber by weight) in the University of Tennessee Vivarium for 6 wk before being placed on one of three special diets for an additional 2 wk.

Three groups of rats consisting of three obese and three lean rats each were assigned to one of three diets: control diet (10% of calories from olive oil), olive oil-enriched diet (40% of calories from olive oil), or fish oil-enriched diet (40% of calories from menhaden oil), formulated by Research Diets, Inc. (New Brunswick, NJ) (Table 1). The carbohydrate content of the diets was composed of starch and sucrose in the ratio of 2.1:1 (wt/wt). All three diets also contained 2% soybean oil to ease compounding and contained 20% of calories as protein. The fatty acid composition of each diet is shown in Table 1. Although the predominant fatty acid in the olive oil diet (68% of fat calories) was the monounsaturated fatty acid oleic acid (18:1, N-9), this diet also included linoleic acid (18:2 n-6; 15% of fat calories) and palmitic acid (16:0; 17% of fat calories). The menhaden oil diet was enriched in N-3 PUFA (EPA, 20:5; docosahexaenoic acid, 22:6) (26% of fat calories) but also contained monounsaturated fatty acids (oleic acid and palmitoleic acid) (31% of fat calories) and saturated fatty acids (myristic acid, 14:0 and palmitic acid), 10 and 21% of fat calories, respectively (Table 1). The protein (casein) content was 20%. The energy content of the low fat diet was 3.85 calories/g, whereas that of the high-fat diets was 4.58 calories/g. Both corpulent and lean rats on the three diets were pair fed, respectively, to the lowest calorie intake (the high carbohydrate control diet) so that, within each dietary group, each rat consumed equal numbers of calories. Food consumption and animal weights were recorded at 2- to 3-d intervals at which time an appropriate amount of fresh food was provided. At the end of the experiment, rats were killed by exsanguination under deep Na pentobarbital anesthesia.

TABLE 1. Composition of test diets

Diet/nutrient	Control diet (C)	Olive oil diet (O)	Menhaden oil diet (F)
Fat (% calories)	10	40	40
Carbohydrate (% calories)	70	40	40
Protein (% calories)	20	20	20
Source of fat calories (% of total fat calories)			
Monounsaturated fatty acids (%)[a]	63	68	31[b]
n-6 Polyunsaturated fatty acids (%)[c]	20	15	9
n-3 Polyunsaturated fatty acids (%)[d]	1.3	0.4	26
Saturated fatty acids (%)[e]	16	17	29

Lean and corpulent rats were fed high-carbohydrate control diet (10% olive oil) and diets enriched in monounsaturated fatty acids (40% of calories from olive oil) and n-3 polyunsaturated fatty acids (40% of calories from menhaden oil).

[a] Predominantly oleic acid (18:1, N-9).

[b] Menhaden oil also contains approximately 9% palmitoleic acid (16:1, n-7).

[c] Predominantly linoleic acid (18:2, n-6), but menhaden oil also contains approximately 2% arachidonic acid (20:4, n-6).

[d] Menhaden oil contains the following n-3 polyunsaturated fatty acids: 13% eicosapentaenoic acid (20:5, n-3), 11% docosohexaenoic acid (22:6, n-3), and 2% α-linolenic (18:3, n-3).

[e] Predominantly palmitic acid (16:0); however, menhaden oil also contains significant quantities of myristic acid (14:0, 9%) and stearic acid (18:0, 6%).

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Determination of hepatic lipogenesis and fatty acid esterification

After 2 wk on the special diets, nonfasting rats were injected ip with 1 ml tritiated water (4.54 mCi, ICN Biochemials, Irvine, CA) at 0800–0900 h and allowed continued free access to food and water for 2 h. They were then anesthetized by ip injection of pentobarbital (60 mg/kg). A blood sample, anticoagulated with EDTA, was taken from the abdominal aorta after laparotomy. The liver was quickly excised, weighed, and a small piece transferred to RNA LATER (Ambion Inc., Austin, TX). A 1-g piece of liver was placed in methanol for lipid extraction and the remainder was stored at −80 C for further analysis. The epididymal fat pads were also excised, weighed, and stored at −80 C. An aliquot of whole blood was immediately transferred to liquid scintillation counting fluid (Econofluor, PerkinElmer, Wellesley, MA) to estimate the specific radioactivity of the body water (LS 5000, Beckman, Palo Alto, CA). Specific activity of the body water was used to calculate micromoles of tritiated water incorporated into lipid products. Extraction of liver lipids was accomplished by dispersing into methanol with a Polytron homogenizer after which two volumes of chloroform were added and was kept at 4 C overnight. To ensure the removal of unincorporated tritiated water from the lipid extract, the chloroform layer was washed several times with methanol/water (1/1.5, vol/vol) until the radioactivity associated with the methanol/aqueous layer was constant or near background. An aliquot of the chloroform phase was used for determination of the amount of radioactivity incorporated into total saponifiable fatty acids and cholesterol (24). Liver triglycerides (TGs), free cholesterol, cholesteryl ester, and phospholipids (PLs) were resolved by thin-layer chromatography for mass determinations (24). Radioactivity was determined in the TG and PL fractions.

Plasma was collected after brief centrifugation, and lipids were extracted for determination of individual lipid classes, after separation by thin-layer chromatography on silica gel G (25). Plasma samples from rats within each group were pooled and high-density lipoprotein (HDL) (density = 1.063–1.21) was isolated ultracentrifugally (26). Another aliquot of plasma was stored at −80 C for further analysis. The major lipid classes were separated from chloroform/methanol extracts of plasma and mass measurements were carried out as with liver lipid extracts.

Plasma glucose was determined using a glucose oxidase assay kit (Sigma Chemical, Inc., St. Louis, MO). Plasma insulin levels were determined with the Micromedic RIA kit (ICN Biochemicals Inc., Costa Mesa, CA). Protein analysis on HDL fractions was carried out using the Markwell modification of the Lowry procedure (27).

Measurement of steady-state levels of mRNA

To determine the abundance of mRNA for selected genes related to lipogenesis or lipogenic regulators, Northern analysis was performed on RNA from liver samples. Tissue slices in RNA LATER (Ambion) were dispersed into RNA Stat-60 (Tel-Test, Inc., Friendswood, TX), and total RNA was quantified by absorbance at 260 nm. Twenty micrograms of total RNA was loaded per lane of a formaldehyde/0.8% agarose gel, electrophoresed in 1× 3[*N*-morpholino]propanesulfonic acid buffer, blotted onto Nytran membranes (Schleicher and Schuell, Keene, NH), and UV cross-linked. Ribosomal RNA bands were visualized by staining with ethidium bromide before transfer. Blots were prehybridized for 3 h at 42 C in 50% formamide, 5× sodium chloride/sodium phosphate/EDTA, 5× Denhardt's solution (5Prime-3Prime, Boulder, CO), 7.5% dextran sulfate, 1.5% sodium dodecyl sulfate (SDS), and 100 μg/ml sheared salmon sperm DNA (Ambion).

cDNA probes for measurement of SREBP-1, FAS, LXRα, ACC-1, and specificity protein 1 (Sp1) mRNAs were prepared using plasmids provided by Bruce M. Spiegelman (Dana Farber Cancer Institute. Boston, MA), Stuart Smith (Children's Hospital Research Institute, Oakland, CA), David J. Mangelsdorf (Howard Hughes Medical Institute, Dallas, TX), Ki-Hankim (Purdue University, West Lafayette, IN), and Guntrum Suske (Institut fur Molekularbiologie und Tumor Forschung, Marburg, Germany), respectively. β-Actin mRNA was measured using mouse β-actin DECAprobe (Ambion). The cDNA probes were labeled with α-^{32}P-dCTP using a random primer labeling kit (Invitrogen, Carlsbad, CA). After hybridization, the membranes were washed twice with 2× saline sodium citrate + 0.1% SDS at room temperature and twice with 0.1× saline sodium citrate + 0.1% SDS at 65 C for 30 min each. Membranes were exposed to Bio-Max MS film (Eastman Kodak, Rochester, NY); a digital image of the developed film was created and RNA bands quantitated by densitometry (Alpha Innotech Corp., San Leandro, CA).

Analysis of hepatic expression of lipid-related genes by microarray

To confirm the findings of Northern analysis and survey the effect of obesity and fat-enriched diets on expression of a wider range of lipoprotein-related genes, mRNA from livers of lean and corpulent rats fed the control diet and corpulent rats fed olive oil and menhaden oil diets was used to synthesize cDNA for microarray analysis using the Affymetrix GeneChip (Rat Genome U34 Set, Affymetrix, Santa Clara, CA).

cRNA synthesis and labeling

First- and second-strand cDNA was synthesized from 5–15 μg of total RNA using the SuperScript double-stranded cDNA synthesis kit (Life Technologies, Inc., Grand Island, NY) and oligo-dT$_{24}$-T7 (5′-GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG-3′) primer according to the manufacturer's instructions. cRNA was synthesized labeled with biotinylated UTP and CTP by *in vitro* transcription using the T7 promoter coupled double-stranded cDNA as template and the T7 RNA transcript labeling kit (ENZO Diagnostics Inc., Farmingdale, NY). Briefly, double-stranded cDNA synthesized from the previous steps were washed twice with 70% ethanol and resuspended in 22 μl Rnase-free H_2O. The cDNA was incubated with 4 μl each of 10× reaction buffer, biotin-labeled ribonucleotides, dithiothreitol, RNase inhibitor mix, and 2 μl 20× T7 RNA polymerase for 5 h at 37 C. The labeled cRNA was separated from unincorporated ribonucleotides by passing through a CHROMA SPIN-100 column (Clontech, Palo Alto, CA) and precipitated at −20 C for 1 h to overnight.

Oligonucleotide array hybridization and analysis

The cRNA pellet was resuspended in 10 μl Rnase-free H_2O, and 10.0 μg were fragmented by heat and ion-mediated hydrolysis at 95 C for 35 min in 200 mM Tris-acetate (pH 8.1), 500 mM KOAc, 150 mM MgOAc. The fragmented cRNA was hybridized for 16 h at 45 C to oligonucleotide arrays (Affymetrix) containing approximately 12,500 full-length annotated genes together with additional probe sets designed to represent expressed sequence tag sequences. Arrays were washed at 25 C with 6× sodium chloride/sodium phosphate/EDTA (0.9 M NaCl, 60 mM NaH_2PO_4, 6 mM EDTA + 0.01% Tween 20) followed by a stringent wash at 50 C with 100 mM 2-(N-morpholine) ethane sulfonic acid, 0.1 M (NaCl), 0.01% Tween 20. The arrays were then stained with phycoerythrin-conjugated streptavidin (Molecular Probes, Eugene, OR), and the fluorescence intensities were determined using a laser confocal scanner (Hewlett-Packard, Portland OR). The scanned images were analyzed using Microarray software (Affymetrix). Sample loading and variations in staining were standardized by scaling the average of the fluorescent intensities of all genes on an array to constant target intensity (250) for all arrays used. Data analysis was conducted using Microarray Suite 5.0 (Affymetrix) following user guidelines. The signal intensity for each gene was calculated as the average intensity difference, represented by [Σ(PM-MM)/(number of probe pairs)], where PM and MM denote perfect-match and mismatch probes.

The resultant database was screened for mRNA species meeting the arbitrary detection, $P < 0.05$. This list was then searched for genes related to lipogenesis, cholesterol synthesis, apoproteins, lipoprotein-related genes, and transcription factors. To identify obesity-related changes in gene expression, mRNA in corpulent JCR/LA-cp rats fed the control diet was compared with that in lean JCR:LA rats fed the same diet. Similarly, to identify diet-related changes in lipid/lipoprotein gene expression, mRNA in livers of corpulent JCR:LA-cp rats fed the olive oil and menhaden oil diets was compared with that observed in corpulent rats fed the control diet. A pictorial display of metabolic genes whose expression was increased or decreased by 50% or more by obesity and dietary fatty acid was generated using Genesifter software (Visx Labs, Seattle, WA). Relative expression of the full panel of metabolic genes is presented in tabular form.

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Statistical analysis of lipid parameters

All analyses were conducted in SAS (version 9.0, SAS Institute, Cary, NC). Proc GLM was used to conduct a two way factorial ANOVA with interaction. The independent variables used were genotype, diet, and the interaction of genotype and diet. Pairwise comparisons of means were made through the least significant means procedure and associated *P* values were produced using the pdiff option. Diet effects were tested within each genotype (corpulent and lean) by comparing olive oil and menhaden oil diet to control diet within each genotype. The ability of the olive oil and menhaden oil diet to reverse obesity-related changes in lipid parameters was also tested by comparing corpulent rats consuming all three diets to lean animals consuming the control diet.

Results

To determine the effect of dietary fat on hepatic lipogenesis and lipogenic gene expression, rats were fed diets in which olive oil and menhaden oil were substituted for carbohydrate in eucaloric (within genotype) amounts. To accomplish this, caloric intake in lean and corpulent rats receiving both olive oil and menhaden oil diets was matched to that of the corresponding animals receiving the control diet. As expected, total caloric intake in corpulent rats was almost twice that of the lean controls, but food intake did not differ by diet within the corpulent and lean genotypes (Table 2). Body weight, liver weight, and epididymal fat pad weight were all significantly higher in corpulent rats. Increased liver weight in corpulent rats consuming the control diet corresponded to a marked accumulation of triglyceride (see Table 4). Reduced hepatic TG content of livers of corpulent rats fed the olive oil and menhaden oil diets resulted in reduced liver weight, compared with livers of corpulent rats consuming the control diet; however, liver weight remained increased, compared with that of lean rats (Table 2). There were no differences in liver weight by diet within the control group (Table 2). Nonfasting plasma insulin, which was significantly higher in the corpulent rats, was not significantly affected by diet. Plasma glucose did not differ by genotype and was also not significantly altered by diet (Table 2).

Diets enriched in olive oil and menhaden oil effectively reduce hepatic lipogenesis in the corpulent JCR rat

The ability of diets enriched in olive oil and menhaden oil to suppress hepatic lipogenesis was assessed in both lean and corpulent JCR:LA rats. *De novo* fatty acid synthesis was assessed by measuring incorporation of tritiated water into total fatty acids after saponification. *De novo* fatty acid synthesis in livers of corpulent rats fed the control diet was 6-fold higher than that observed in lean rats fed the same diet (Fig. 1). In the corpulent rats, both the olive oil and menhaden oil diets reduced hepatic lipogenesis; however, the menhaden oil diet was more effective ($P < 0.05$, olive oil *vs.* menhaden oil) (Fig. 1). In contrast, neither the olive oil nor menhaden oil diets significantly reduced hepatic lipogenesis in lean rats. In corpulent JCR rats, the menhaden oil diet reduced hepatic lipogenesis to levels comparable with those observed in the lean rats fed the control diet ($P = 0.71$), whereas lipogenesis, although reduced, remained significantly higher in corpulent rats fed the olive oil diet ($P < 0.01$) (Fig. 1). We also examined the rates of incorporation of tritiated water into specific liver lipids

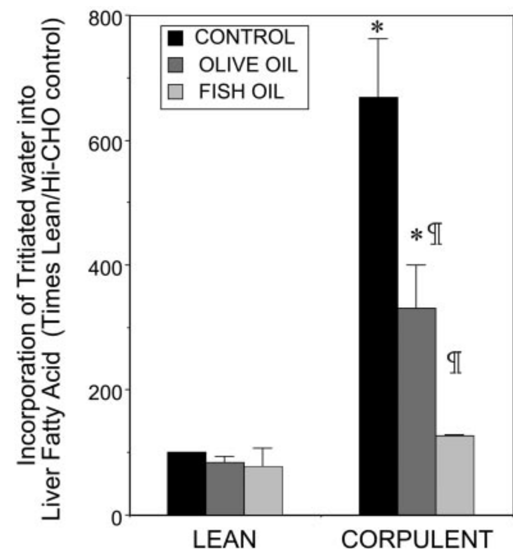

FIG. 1. Diets enriched in olive oil or menhaden oil effectively reduce hepatic lipogenesis in the corpulent JCR:LA/cp rat. Lean and corpulent rats were fed a low-fat control diet (10% calories from olive oil) and diets supplemented with olive oil (40% of calories) or menhaden (fish) oil (40% of calories). Rats were injected with tritiated water 2 h before being killed, and incorporation of label into hepatic total fatty acids was determined. Data are mean ± SEM of percent change, compared with lean rats consuming the control diet (n = 3 rats per group). Data were examined for differences between corpulent rats consuming all three diets *vs.* control rats consuming control diet (*, $P < 0.01$) and for effects of diet within each genotype (¶, $P < 0.01$). The distribution of tritiated water into specific lipid classes is shown in the accompanying table.

TABLE 2. Food intake, body weight, liver weight, epididymal fat pad weight, plasma insulin, and glucose in lean (Ln) and corpulent (Cp) JCR:LA-cp rats fed the control diet (C) containing 10% olive oil, or a fat-enriched (40% of calories) diet containing either olive oil (O) or menhaden (fish) oil (F)

Rat/diet	Food intake (cal/d)[a]	Body weight (g)	Epid. fat weight (g)	Liver weight (g)	Plasma insulin (μIU/ml)	Plasma glucose (mg/dl)
Ln/C	50.3 ± 4.9	328.7 ± 12.1	3.3 ± 0.2	10.2 ± 0.5	188 ± 43	218 ± 8
Ln/O	50.0 ± 9.7	333.7 ± 10.4	3.8 ± 0.2	10.3 ± 0.4	109 ± 18	220 ± 4
Ln/F	56.0 ± 4.2	320.7 ± 2.9	3.1 ± 0.3	11.3 ± 0.7	116 ± 6	201 ± 4
Cp/C	93.0 ± 5.6[b]	445.7 ± 10.2[b]	9.5 ± 0.2[b]	18.9 ± 1.1[c]	459 ± 85[c]	265 ± 35
Cp/O	101.0 ± 5.0[b]	448.3 ± 2.3[b]	9.4 ± 0.6[b]	14.9 ± 0.5[c,d]	528 ± 83[c]	237 ± 37
Cp/F	92.3 ± 4.2[b]	434.7 ± 16.0[b]	9.4 ± 0.8[b]	15.6 ± 0.2[c,d]	412 ± 43[b]	223 ± 21

[a] Within each rat phenotype (lean and corpulent), rats consuming the olive oil and menhaden oil diets were pair-fed with the corresponding control diet group to maintain equal caloric intake within phenotypes, n = 3 rats per group. Data are mean ± SEM for each group of three rats. Blood samples were taken between 0900 and 1000 h, and animals were allowed access to food up until the time of death.

[b] $P < 0.05$ or [c] $P < 0.001$, corpulent rats consuming each diet *vs.* lean rats consuming control diet (genotype effect).

[d] $P < 0.05$, olive oil or menhaden oil diet *vs.* control diet within each genotype (diet effect).

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(Table 3). TG synthesis was increased markedly (8-fold) in corpulent rats consuming the control diet, and, although reduced by the olive oil diet, remained greater than that observed in lean animals consuming the control diet (Table 3). The menhaden oil diet also reduced rates of TG synthesis from *de novo* fatty acid to levels comparable with those observed in lean rats consuming the control diet (Table 3). Thus menhaden oil diet completely reversed the increased levels of *de novo* lipogenesis and TG synthesis observed in the corpulent JCR rat. Although a similar trend toward reduced TG synthesis was observed with the olive oil and fish oil diet in lean JCR:LA rats, this did not achieve statistical significance. Although cholesterol synthesis tended to be higher in livers of obese rats and reduced by the menhaden oil diet, neither was statistically significant (Table 3). Incorporation of *de novo* synthesized fatty acids into PL was increased in the corpulent JCR rats and reduced by the menhaden oil diet but not by the olive oil diet (Table 3). Although the menhaden oil diet (and to a lesser extent the olive oil diet) reduced incorporation of tritiated water into all lipid products, there was a greater reduction in incorporation into TG than into PL with the menhaden oil diet.

Effect of olive oil and menhaden oil diet on hepatic lipid content

Hepatic TG content was markedly (20-fold) greater in livers of corpulent rats fed the control diet *vs.* lean rats fed the control diet (Table 4). In corpulent rats, hepatic content of TGs was significantly reduced by both the olive oil and menhaden oil diets, although the latter was slightly more effective (Table 4). In contrast, in lean rats, neither the olive oil nor menhaden oil diets altered hepatic TG content (Table 4). Hepatic PL content was unaffected by either obesity or fat feeding. Hepatic cholesteryl ester content was significantly increased in corpulent rats consuming the menhaden oil diet and in lean rats by the olive oil diet (Table 4). Free cholesterol content of livers was, however, not affected by either diet.

Effect of olive oil and menhaden oil diets on plasma lipids in lean and corpulent JCR rats

We also assessed the effect of diets enriched in olive oil and menhaden oil on plasma lipids in both lean and corpulent JCR rats. Plasma TG levels were significantly higher in the corpulent rats than lean animals (Table 5). Plasma PL, cholesterol, and cholesteryl-ester were increased in corpulent rats consuming the control and olive oil diets but not menhaden oil diet (Table 5). The menhaden oil diet reduced plasma PL, cholesterol, and cholesteryl-ester in both lean and corpulent rats (Table 5). Plasma TG was also reduced by the menhaden oil diet in lean rats. Unexpectedly, plasma TG was not reduced by the menhaden oil diet in corpulent rats and was higher in corpulent rats fed the olive oil diet (Table 5). Plasma content of cholesterol and cholesteryl-ester was significantly higher in corpulent JCR:LA-cp rats and was reduced by both the olive oil and menhaden oil diet in lean (cholesterol) and corpulent (cholesterol and cholesterol-ester) rats (Table 5). Similarly, plasma PL was increased in corpulent JCR:LA-cp rats and was reduced by the menhaden oil diet but not by the olive oil diet. Significantly, nonfasting plasma levels of nonesterified fatty acids were not increased by either obesity or the olive oil or menhaden oil diet (Table 5). Analysis of the individual plasma lipoproteins was not carried out; however, estimates of the protein level in the pooled plasma HDL fraction (density = 1.063–1.21) indicated the presence of higher levels of plasma HDL protein in the corpulent rats, compared with lean rats. The HDL protein level was 2-fold greater in corpulent rats (160 *vs.* 72 mg/dl) and was substantially decreased by the menhaden oil diet in the corpulent rats (84 mg/dl) but only slightly in lean rats (67

TABLE 3. Incorporation of tritiated water into hepatic lipids

Group/diet	Phospholipid	Triglyceride	Cholesterol
Lean/control (C)	9.68 ± 1.48	8.08 ± 1.43	1.43 ± 0.80
Lean/olive oil (O)	7.35 ± 0.60	5.33 ± 0.65	1.50 ± 0.88
Lean/menhaden oil (F)	7.18 ± 0.23	2.58 ± 0.30	0.58 ± 0.08
Corpulent/control (C)	22.58 ± 2.83[a]	62.95 ± 19.30[b]	3.75 ± 1.55
Corpulent/olive oil (O)	18.4 ± 4.73[a]	39.68 ± 11.80[a]	2.18 ± 0.70
Corpulent/menhaden oil (O)	13.18 ± 2.98[c]	11.25 ± 2.70[c]	1.33 ± 0.70

Liver samples were extracted and phospholipid and triglyceride separated by thin-layer chromatography. Total cholesterol was obtained from saponified extracts of liver. Data are micromoles ^3H_2O incorporated into lipid products per gram liver ± SEM.

[a] $P < 0.05$ and [b] $P < 0.01$ corpulent rats *vs.* lean rats fed control diet.

[c] $P < 0.05$ olive oil diet and menhaden oil diet *vs.* control diet within each genotype, n = 3 per group.

TABLE 4. Effect of feeding diets enriched in olive oil and menhaden oil on hepatic lipid content in lean and corpulent JCR:LA/cp rats

Group/diet	Triglyceride	Phospholipid	Cholesterol	Cholesteryl ester
Lean/control (C)	6.4 ± 1.0	22.6 ± 1.4	3.8 ± 0.2	0.8 ± 0.1
Lean/olive oil (O)	12.1 ± 3.0	23.7 ± 0.7	4.3 ± 0.3	2.7 ± 0.7[a]
Lean/menhaden oil (F)	6.0 ± 0.6	26.2 ± 2.2	4.3 ± 0.1	1.9 ± 0.3
Corpulent/control (O)	129 ± 34[b]	18.0 ± 1.9	3.9 ± 0.3	1.8 ± 0.6
Corpulent/olive oil (O)	47.7 ± 13.1[a,c]	25.4 ± 1.7	4.0 ± 0.2	1.5 ± 0.5
Corpulent/menhaden oil (F)	30.3 ± 2.8[a]	22.6 ± 2.7	3.9 ± 0.4	3.1 ± 0.5[b]

Hepatic lipid content was determined by chemical analysis. Data are mean ± SEM of hepatic lipids micromole/gram liver.

[a] $P \leq 0.05$ menhaden oil or olive oil diet *vs.* control diet within each genotype.

[b] $P < 0.05$ or [c] $P < 0.001$ corpulent rats *vs.* lean rats fed the control diet, n = 3 per group.

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mg/dl). Plasma HDL protein was unaffected by the olive oil diet.

Northern analysis of genes related to lipogenesis

We next examined the effect of obesity and dietary fat on hepatic expression of the key lipogenic enzymes, FAS and ACC-1, and the lipogenic regulators SREBP-1c, LXR, and Sp1 (Fig. 2). Expression of FAS and ACC-1 was increased in livers of corpulent JCR:LA-cp rats as was SREBP-1c (Fig. 2). Conversely, both the olive oil and menhaden oil diets reduced FAS, ACC-1, and SREBP-1c expression in both lean and corpulent JCR:LA-cp rats (Fig. 2). The menhaden oil diet was marginally more effective in this regard. We also determined the effect of obesity and dietary fat on expression of the metabolic regulator LXR. LXR induces SREBP-1c promoter

TABLE 5. Effect of feeding diets enriched in olive oil (O) and menhaden oil (F) on plasma lipids in lean and corpulent (Corp) JCR:LA/cp rats

Group/diet	Triglyceride	Phospholipid	Cholesterol	Chol-Ester	FFA
Lean/control (C)	57 ± 14	172 ± 7	44 ± 3	74 ± 11	46 ± 9
Lean/olive oil (O)	46 ± 13	161 ± 3	39 ± 2[a]	71 ± 3	40 ± 3
Lean/menhaden oil (F)	25 ± 11[a]	118 ± 14[a]	31 ± 4[a]	63 ± 5	38 ± 5
Corp/control (C)	107 ± 1[b]	361 ± 10[c]	94 ± 6[c]	196 ± 19[c]	45 ± 12
Corp/olive oil (O)	148 ± 9[a,c]	341 ± 14[c]	78 ± 4[a,c]	164 ± 10[a,c]	52 ± 6
Corp/menhaden oil (F)	103 ± 6[b]	204 ± 22[a]	49 ± 7[a]	110 ± 5[a]	38 ± 11

Data are mean ± SEM plasma lipid (micromoles per deciliter).
[a] $P < 0.05$ menhaden oil or olive oil *vs.* control diet within genotype (diet effect), n = 3 per group.
[b] $P < 0.05$ or [c] $P < 0.001$ corpulent rats *vs.* lean rats consuming control diet (obesity/diet effect).

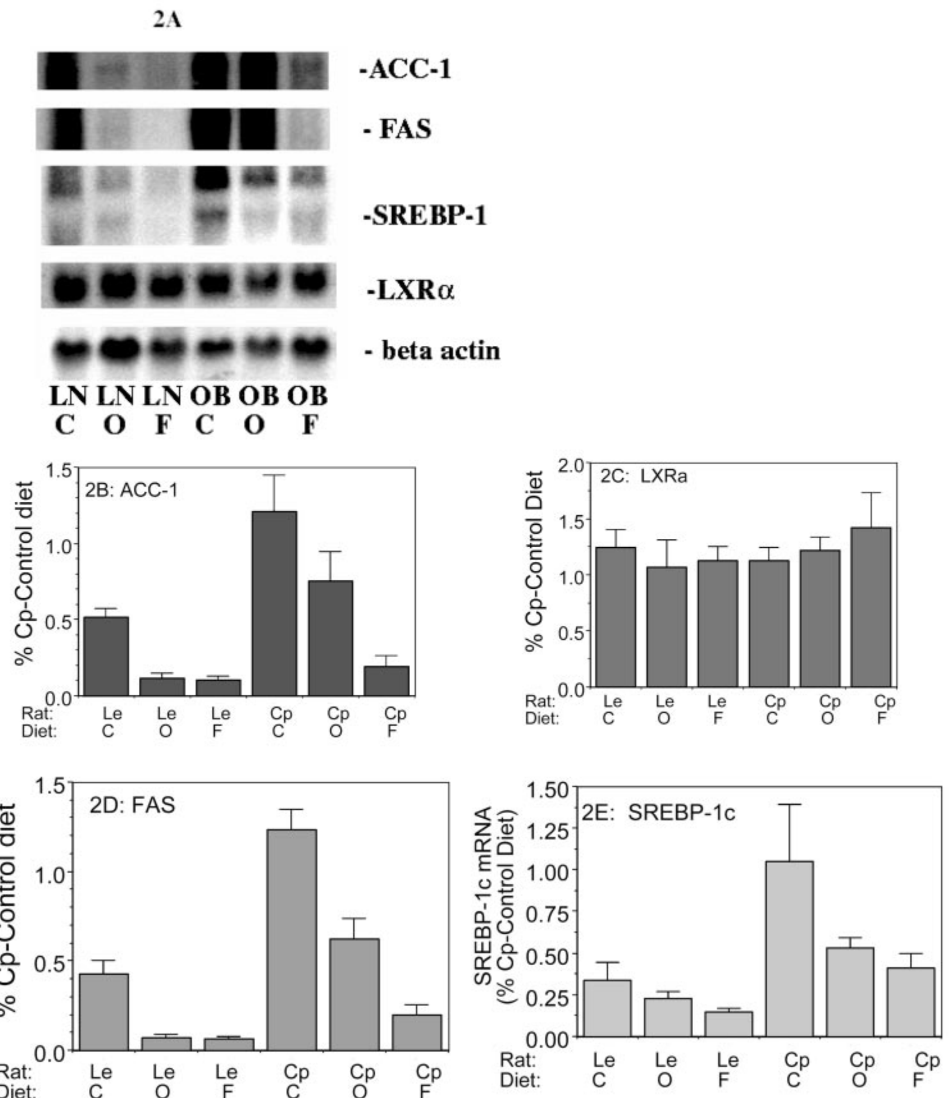

FIG. 2. Hepatic expression of lipogenic enzyme and lipogenic regulator mRNA in lean (Le) *vs.* corpulent (Cp) JCR:LA-cp rats after 2 wk of control (C), olive oil (O), or menhaden oil (F) diet. A, Representative Northern blot probed for ACC-1, FAS, SREBP-1, and LXRα. β-Actin was used as a loading control. mRNA expression relative to an index rat (corpulent rat, control diet), quantitated by densitometry, is depicted in B—E (n = 3 rats per group).

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activity (28), and induction of LXR gene expression by insulin has been proposed as one mechanism by which insulin increases SREBP-1c expression (29). Conversely, inhibition of SREBP-1c gene transcription by PUFAs may result from reduced LXR binding (17). Therefore, the effect of obesity and dietary fat on LXR expression was of interest. In these experiments, however, Northern analysis failed to show increased LXR expression in the corpulent JCR:LA-cp rat, and there was no LXR expression decreased by the fat-enriched diets (Fig. 2). The SREBP-1c promoter also contains several binding sites for Sp1 (14), and activity of Sp1 is regulated by insulin (30). To determine whether obesity/hyperinsulinemia and fatty acid feeding altered hepatic Sp1 mRNA levels in the corpulent JCR rat, we performed Northern analysis of mRNA from lean and corpulent rats fed control, olive oil, and menhaden oil diets. Neither obesity nor fatty acid feeding altered hepatic Sp1 mRNA expression (data not shown).

Microarray analysis of the effect of obesity and dietary fat on hepatic lipogenic enzyme and lipid-related gene expression

To confirm the findings of altered lipogenic enzyme gene expression by Northern analysis and further examine the effect of obesity and dietary fat on expression of a wide range of lipid-related genes, we determined expression of mRNA for genes related to lipid and lipoprotein metabolism in livers of lean and corpulent JCR rats fed the control diet using Gene-Chip microarray (Affymetrix). Similarly, to identify potential mechanisms for altered lipid synthesis in response to the olive oil and menhaden oil diets in the corpulent JCR rat, we compared lipid/lipoprotein gene expression in livers of corpulent rats fed the olive oil and menhaden oil diets with that observed in corpulent rats consuming the control diet. Qualitative expression of metabolic genes whose expression was altered 50% or more by obesity or dietary fatty acid are presented in Fig. 3. Quantitative results of microarray analysis of the complete panel of metabolic genes are presented in Table 6.

A total of 65 genes related to lipid and lipoprotein metabolism were identified from the microarray (Table 6). These include genes related to lipid synthesis, fatty acid oxidation and transport, apoproteins, genes related to transcriptional regulation, and insulin-related genes. Forty-six genes whose expression was maximally altered by either obesity or dietary fat are presented in Fig. 3. Expression of a cluster of genes related to fatty acid oxidation and fatty acid transport was increased in the corpulent JCR:LA-cp rats consuming the control diet, relative to lean rats consuming control diet, and was either maintained or further increased in corpulent rats consuming fat-enriched diets (Fig. 3). Expression of several genes related to lipid synthesis was increased in the corpulent JCR:LA rats consuming the control diet and decreased by the fat-enriched diets (Fig. 3). Quantitative expression of the entire panel of 65 metabolic genes is presented by functional group in Table 6 as detailed below.

Effect of obesity and dietary fat on hepatic expression of mRNA for enzymes of lipid synthesis and metabolism. Rates of lipogenesis were increased in livers of corpulent rats and were effectively suppressed by both fat-enriched diets (Fig. 1). Consistent with this, there was coordinate up-regulation of hepatic expression of the lipogenic regulator SREBP-1c and a wide range of SREBP-responsive enzymes catalyzing *de novo* synthesis of fatty acid (malic enzyme, FAS, and pyruvate kinase.) in corpulent livers and corresponding down-regulation of these lipogenic enzymes by both olive oil and menhaden oil diets (Table 6). Gene expression of other enzymes involved in lipid synthesis and metabolism, including stearoyl coenzyme A (CoA) desaturase (SCD), ATP-citrate lyase, and hepatic lipase was unaffected by obesity but was decreased by the menhaden oil diet (Table 6). Expression of phosphatidate phosphohydrolase (type 2) and lecithin-cholesterol acyltransferase was unaffected by either obesity or fat-enriched diets (Table 6). Because down-regulation of SCD by leptin has been proposed as a potential mechanism for weight loss (31), our data suggest that failure of leptin to down-regulate SCD in the leptin-receptor-deficient corpulent JCR:LA-cp rat may contribute to the development of obesity.

As stated earlier, our pulse-chase study indicated that TG synthesis is increased in corpulent rats fed the high-carbohydrate control diet and decreased with fat-enriched diet. This is consistent with previous reports of increased TG synthesis in livers of both corpulent JCR and fatty Zucker rats (9, 11). We therefore examined the microarray results for an effect of either obesity or fat-enriched diet on hepatic expression of genes related to TG synthesis (Table 6). Consistent with the changes in TG synthesis observed in the pulse-chase experiment, expression of diacylglycerol acyltransferase was modestly increased in corpulent JCR rats consuming the control diet and was correspondingly reduced by both fat-enriched diets.

Expression of two isoforms of carboxylesterase 1 family, ES-3 (32) and ES-10 (33), was reduced in livers of corpulent rats and tended to be increased by both the olive oil and menhaden oil diets (Table 6). These enzymes catalyze the hydrolysis of short- and long-chain acyl-glycerols, long-chain acylcarnitine, and acyltransferase-CoA (acyl-CoA) esters as well as a variety of ester- and amide-containing chemicals and drugs (34).

Effect of obesity and dietary fat on enzymes related to mitochondrial and peroxisomal fatty acid oxidation and fatty acid transport. Reduced fatty acid oxidation contributes to increased lipid synthesis in other animal models of obesity and hyperinsulinemia such as the Zucker fatty rat (11). The nuclear receptor PPARα is a pivotal regulator of β-oxidation of long-chain fatty acids (35). Long-chain PUFAs such as arachidonic acid (20:4, n-6) and docosahexaenoic acid (22:5, n-3) as well as long-chain saturated fatty acids, are PPARα ligands (36). Dietary intake of long-chain fatty acids in the olive oil and menhaden oil diets may have reduced triglyceride synthesis in part by diverting fatty acids into oxidative pathways. In addition, the effect of obesity and hyperinsulinemia on hepatic expression of enzymes involved in fatty acid oxidation is not known. Therefore, we examined the microarray results for changes in expression of PPAR-responsive enzymes involving fatty acid oxidation. Unexpectedly, expression of several enzymes related to fatty acid oxidation (carnitine *O*-octanoyltransferase, peroxisomal membrane protein Pmp26p, carnitine palmitoyl transferase 2,

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FIG. 3. Microarray analysis of metabolic genes regulated by obesity and dietary fatty acid. Data represent average expression of genes that are up-regulated (*red*) and down-regulated (*green*) by 50% or more in corpulent (obese) JCR:LA-cp rats consuming control (OBC), olive oil (OBO) and menhaden (fish) oil (OBF) diets, compared with lean JCR:LA rats consuming control diet (LC).

mitochondrial multienzyme complex, enoyl coenzyme A hydratase1, and carnitine palmitoyltransferase 1α) was increased in livers of corpulent JCR rats (Table 6). The menhaden oil diet further increased expression of many PPARα-dependent enzymes (carnitine *O*-octanoyltransferase, carnitine palmitoyl transferase 2, enoyl coenzyme A hydratase 1, acetyl-CoA acyltransferase 1, very long-chain acyl-CoA dehydrogenase, and acyl-CoA thioesterase), consistent with the property of long-chain fatty acids to act as PPAR agonists (37). Other PPARα-responsive enzymes [acyl-CoA oxidase and uncoupling protein (UCP)-2] (38, 39) were not induced by either fat-enriched diet (Table 6).

The fatty acid transport proteins fatty acid translocase (CD36) and ATP-binding cassette sub-family DALD member 3 (ABCD3) were markedly up-regulated in the corpulent rat, and conversely CD36 was down-regulated by both the olive oil and menhaden oil diets (Table 6). The striking changes in expression of these fatty acid transport proteins may reflect an adaptive response to increased intracellular fatty acid flux that accompanies increased lipogenesis in the corpulent rat

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TABLE 6. Summary of ratios of expression of selected metabolic mRNA species in livers of lean and obese rats measured by microarray analysis

Functional Group/Name	OBC/LC	OBF/OBC	OBO/OBC	Regulated by	Unigene ID
Lipid synthesis and metabolism					
Malic enzyme	5.24[a]	0.08[a]	0.27[a]	SREBP-1/2	Rn.64900
Fatty acid synthase	3.15[a]	0.14[a]	0.45[a]	SREBP-1	Rn.9486
Diacylglycerol acyltransferase	1.33[a]	0.76[a]	0.78[a]		Rn.252
Pyruvate kinase	1.43	0.26	0.51		Rn.48821
Glycerol-3-phosphate acyltransferase mitochondrial	1.27	0.47	0.59	SREBP-1	Rn.44456
Acetyl CoA synthetase long chain	1.14	1.06	1.12	SREBP-1	Rn.6215
Lipoprotein lipase	1.10	0.63	0.84	LXR	Rn.3834
Stearoyl CoA desaturase	1.06	0.49	0.96	LXR	Rn.1023
Lecithin-cholesterol acyltransferase	0.99	1.08	1.26		Rn.10481
Hepatic lipase	0.98	0.66[a]	1.07		Rn.1195
ATP citrate lyase	0.92	0.38	0.64	SREBP-1/2	Rn.29771
Carboxylesterase 1 (ES-3)	0.42[a]	1.74	1.50	PPAR	Rn.82692
Carboxylesterase 1 (ES-10)	0.32[a]	2.44[a]	1.62	PPAR	Rn.34885
Fatty acid oxidation					
Carnitine O-octanoyltransferase	2.85[a]	1.95[a]	1.32	PPAR	Rn.4896
Peroxisomal membrane protein Pmp26p (Peroxin-11)	2.03[a]	1.07	0.84	PPAR	Rn.14519
Carnitine palmitoyl transferase 2	1.74[a]	1.34[a]	0.92	PPAR	Rn.11389
Mitochondrial multienzyme complex (B subunit)	1.58[a]	1.26[a]	0.99		Rn.11253
Enoyl coenzyme A hydratase 1	1.87	1.84[a]	1.14	PPAR	Rn.6148
Carnitine palmitoyltransferase 1 α	1.86	0.50	0.80	PPAR	Rn.2856
Acetyl-CoA acyltransferase 1	1.23	2.05[a]	1.11	PPAR	Rn.8913
Acyl-coA oxidase	1.14	1.10	1.01	PPAR	Rn.31796
Very long chain Acyl-Coa dehydrogenase	1.09	1.47[a]	1.18	PPAR	Rn.33319
Medium chain Acyl-Coa dehydrogenase	1.07	1.21	1.05	PPAR	Rn.6302
Peroxisomal multifunctional enzyme type II	0.98	1.07	1.03	PPAR	Rn.2082
Uncoupling protein 2 mitochondrial	0.93	1.26	1.21	PPAR	Rn.13333
Acyl-CoA thioesterase 1 (cytosolic)	0.79	2.76[a]	0.83	PPAR	Rn.11326
Fatty acid transport					
CD36 (fatty acid translocase)	6.86[a]	0.61[a]	0.56[a]		Rn.3790
ATP-binding cassette ABCD3	1.96[a]	0.72	0.66		Rn.7024
Apolipoproteins					
Apolipoprotein A-I	1.54	0.22[a]	1.09		Rn.10308
Apolipoprotein C-III	1.35	1.02	0.99		Rn.36813
Apolipoprotein C1	1.32	0.74	0.88		Rn.8887
Apolipoprotein A-II	1.30	0.70	0.90		Rn.10309
Apolipoprotein E	1.24	0.77	0.95	LXR	Rn.32351
Apolipoprotein B	1.18	0.81	0.95		Rn.33815
Apolipoprotein AIV	1.06	0.59[a]	1.40[a]		Rn.15739
Apolipoprotein M	0.79	1.27	1.17		Rn.262
Transcriptional regulation					
SREBP-1	2.16	0.43	0.54		L16995
Farnesoid X activated receptor	1.44[a]	0.74[a]	0.72[a]		Rn.42943
NFY-C	1.33[a]	0.78[a]	0.82		Rn.1457
HNF-4a	1.30	0.81	0.80		Rn.44442
USF2	1.22	0.75	1.09		Rn.44637
CREM	1.15	0.68	0.86		Rn.10251
PPAR-a	1.12	0.82	0.85		Rn.9753
USF1	0.87	1.04	0.89		Rn.37514
NFY-B	0.85	1.17	0.94		Rn.1131
NrOb2	0.74	1.81[a]	1.74[a]	PPAR	Rn.10712
PPAR-γ	0.70	0.97	1.12		Rn.23443
Cbp/p300-interacting transactivator 2	0.65[a]	1.07	0.66		Rn.31765
Kid-1 (zinc finger protein 354A)	0.64	2.42[a]	1.96[a]		Rn.11049
ESTs highly similar to I55595 splicing factor (*H. sapiens*)	0.50[a]	1.45	1.33		Rn.8555
C/EBP-B	0.48	1.98	1.65		Rn.6479
Cholesterol related					
Cholesterol-7α hydroxylase (Cyp7a1)	3.43[a]	0.91	0.51	LXR	Rn.10737
Scavenger receptor class B, member 1	1.71[a]	0.75	1.12		Rn.3142
Lecithin:cholesterol acyltransferase (LCAT)	0.99	1.08	1.26		Rn.10481
HMG-CoA reductase	1.83	0.65	0.83	SREBP-2	Rn.10469
HMG-CoA synthase	1.33	0.68	0.73	SREBP-2	Rn.5106
ATP-binding cassette subfamily ABCB11	0.92	0.86	0.93		Rn.14539
ATP-binding cassette subfamily ABCC9	0.71	0.95	1.04		Rn.10528
ATP-binding cassette subfamily ABCC2	0.53	1.40	1.40		Rn.10265
Cyp7b1	0.66	2.05[a]	0.85		Rn.53969
Insulin-related genes					
Prolactin receptor	2.95[a]	0.61	0.88		Rn.9757
Insulin receptor-related receptor	1.64[a]	0.66[a]	0.68[a]		Rn.44446
IRS-1	0.85	0.73	0.86		Rn.10476
IGF-1	0.69	1.21	1.34		Rn.6282
IGF binding protein 1	0.29[a]	0.89	0.88		Rn.34026

Ratios of mRNA expression in livers of corpulent and lean JCR:LA rats consuming the control diet (OBC/LC) are presented as are the expression of mRNA species in corpulent rats fed either olive oil or menhaden oil compared to that of corpulent rats fed the control diet (OBO/OBC and OBF/OBC), n = 3 rats per group. Genes known to be regulated by SREBP, LXR, and PPAR are noted, as is the Unigene Identification number for each gene.

[a] $P < 0.05$ *vs.* obese rat consuming control diet (OBC).

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because CD36 and ABCD3 transport fatty acids across cell membranes and into peroxisomes, respectively (40, 41). Alternatively, increased expression of CD36 may contribute to increased TG production in the corpulent livers by increasing uptake of fatty acid by the hepatocyte.

Effect of obesity and dietary fat on apoprotein expression. Hyperlipidemia in the corpulent JCR:LA/cp rat results, in part, from increased hepatic secretion of VLDL lipid and apoprotein (9). Therefore, altered expression of hepatic apolipoprotein genes may contribute to hyperlipidemia in the corpulent rat by changes in production of apoproteins involved in VLDL synthesis [apolipoprotein (apo) B] or removal (apoE, apoCII, apoCIII). We therefore analyzed the microarray data for differences in VLDL apoprotein gene expression between lean and corpulent rats consuming the control diet, and in corpulent rats fed the olive oil and menhaden oil diets. We did not detect significant alterations in VLDL apoprotein expression between lean and corpulent JCR:LA-cp rats, nor was there any detectable effect of diet on expression of VLDL apoproteins (apoB, apoE, apoCII, or apoCIII). Therefore, altered VLDL apoprotein expression does not appear to be a major mechanism for increased VLDL secretion in the corpulent JCR rat, nor do high fat diets exert significant effects on VLDL apoprotein expression. On the other hand, the menhaden oil diet reduced expression of both apoAI and apoAIV mRNA in livers of corpulent rats (Table 6). Conversely, the olive oil diet had no effect on ApoAI expression but increased apoAIV mRNA in livers of corpulent rats (Table 6). The ability of the menhaden oil diet to suppress apoAI expression corresponds with reduced plasma HDL protein. Changes in apoAIV expression in response to dietary fat may be of importance insofar as food intake may be affected. ApoAIV is synthesized by the intestine and liver, is increased by fat absorption, and serves as a satiety signal after a high fat meal (42).

Effect of obesity and dietary fat on expression of mRNA for transcription factors. Both microarray and Northern analysis indicated increased SREBP-1 expression in livers of hyperinsulinemic corpulent rats and suppression of SREBP-1 by both the olive oil and menhaden oil diets (Table 6 and Fig. 2). We previously demonstrated that insulin increases SREBP-1c transcription and that PUFAs prevent this effect (14). The SREBP-1 promoter contains response elements for the transcription factors Sp1, nuclear factor-Y (NF-Y), and LXR (14, 43). We therefore examined the microarray data for changes in expression of transcription factors to gain insight into potential mechanisms by which obesity/hyperinsulinemia and dietary fat might regulate SREBP-1c transcription. Sp1 mRNA expression was too low to detect a reproducible signal from the microarray probe, and LXRα was not present in the microarray panel. These mRNAs were therefore assessed by Northern analysis. As reported earlier in this section, neither Sp1 nor LXR expression was altered in corpulent rats, nor were they affected by either high-fat diet (data not shown, Fig. 2). NF-Y is another candidate for regulation of SREBP-1c transcription by PUFAs. Not only is an NF-Y binding site present on the SREBP-1c promoter, but also mutation of the NF-Y binding site in the insulin-response unit of the FAS promoter has been shown to attenuate the inhibitory effect of PUFAs on that promoter (44). Expression of the C-subunit of NF-Y was modestly increased in corpulent rats and decreased with the menhaden oil diet (Table 6). This suggests that NF-Y may be involved in regulation of SREBP-1c by both insulin and PUFAs.

The abundance of mRNA for the farnesoid X activated receptor (FXR), which acts as a bile acid sensor in the liver (45), was modestly increased in the corpulent rat and decreased by both high-fat diets (Table 6). Expression of mRNA for the nuclear receptor OB2 (NrOb2), which has an FXR response element, was increased by both high-fat diets (Table 6). NrOb2 functions as a repressor of the rate-limiting enzyme of bile acid synthesis, cholesterol 7α-hydroxylase (Cyp7a1), by inactivating the transcription factor liver receptor homolog-1/Cyp7a promoter binding factor (NR5A2) (45). Significantly, expression of Cyp7a1 was robustly increased in the corpulent rat and decreased with the olive oil diet (Table 6, cholesterol-related genes). Expression of Kid-1 (also known as zinc finger protein 354A), a member of the C2H2 gene family (46), tended to be lower in corpulent rats and was significantly increased by both fat-enriched diets (Table 6). Kid-1 is highly expressed in the kidney in which it is thought to mediate differentiation and proliferative response and functions as a transcriptional repressor (47). The significance of the observed regulation of Kid-1 expression by obesity and dietary fat is intriguing and may be significant in view of the presence of significant microalbuminuria and glomerular sclerosis in young cp/cp rats (Russell, J. C., unpublished observations). Expression of mRNA for CAAT/enhancer-binding protein (C/EBP)β also tended to be negatively regulated by obesity and positively by dietary fat in a manner similar to Kid-1 (Table 6). C/EBPs are important for regulation of gene expression in insulin-responsive tissues (48). Insulin suppresses C/EBP-β-mediated transactivation of the IGF binding protein (IGFBP)-1 gene by disrupting its interaction with p300/cAMP response element-binding protein (48). Significantly, expression of IGFBP-1 was markedly reduced in the corpulent JCR:LA-cp rat (Table 6, insulin-related genes). Expression of other transcription factors of interest, including hepatocyte nuclear factor-4a, upstream stimulatory factor-1 and -2 (USF1, USF2), cAMP response element modulator, and NF-Y B was unaffected by either obesity or high-fat diet (Table 6).

Effect of obesity and dietary fat on hepatic expression of cholesterol and bile-acid-related genes. Expression of the scavenger receptor class B, type 1 (SR-B1) was increased in the corpulent rat. SR-B1 is highly expressed in liver and has been shown to bind to HDL and mediate internalization of HDL cholesterol (49). Expression of the enzyme lecithin/cholesterol acyltransferase (LCAT), which also plays a major role in reverse cholesterol transport, was unchanged by either obesity or fat-enriched diet (Table 6). Expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase tended to be higher in corpulent rats, suggesting coordinate up-regulation of this SREBP-2 target gene by hyperinsulinemia (Table 6). Cyp7a1, the rate-limiting enzyme of bile acid synthesis, was strongly up-regulated in corpulent rats (Table 6). Cyp7a1 is induced by dietary cholesterol via LXR (50). Oxysterol 7α-hydroxy-

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lase, which is part of an alternative pathway of bile acid synthesis (51), was up-regulated by the fish oil but not olive oil diet. In addition to the fatty acid transporter ABCD3 whose expression was increased in corpulent rats and decreased by both fat-enriched diets (Table 6), three additional members of the ATP-binding Cassette family of genes (ABCC2, ABCC9, ABCB 11) were identified on the microarray. Unlike ABCD3, their expression was not altered by either obesity or either fat-enriched diet.

Effect of obesity and dietary fat on expression of insulin and hormone-receptor-related genes. To gain insight into potential mechanisms underlying the insulin resistance in the corpulent JCR:LA/N rat, we surveyed the microarray for changes in expression of genes related to insulin action and related hormone receptors. Expression of mRNA for the prolactin receptor was increased 3-fold in livers of corpulent JCR rats (Table 6). Secretion of prolactin by the pituitary is increased by leptin, and prolactin levels are increased in leptin-receptor-deficient obesity (52). Conversely, inhibition of prolactin secretion in obese female spontaneously hypertensive rats reverses insulin resistance (53). Prolactin may therefore play a role in the development of insulin resistance in leptin-receptor-deficient obesity. Expression of the insulin receptor-related receptor was also significantly increased in the corpulent rat and was decreased by high-fat diet (Table 6). The insulin-related receptor is a heterotetrameric transmembrane receptor with intrinsic tyrosine kinase activity and homology with the insulin and the IGF-I receptor (54). IGFBP-1 is strongly repressed in the corpulent JCR rat (Table 6), most likely in response to hyperinsulinemia (55).

Discussion

Dietary fatty acids suppress hepatic lipogenesis by reducing transcription of genes coding for participating enzymes, including FAS and ACC-1 (19) (20). Few studies have, however, examined regulation of lipogenesis by dietary fat in animal models of obesity and hyperinsulinemia. The previous limited reports suggested that obese, insulin-resistant animals may be resistant to down-regulation of lipogenic enzymes by dietary fat (21–23). In contrast, we found that diets enriched in monounsaturated fatty acids (olive oil) or N-3 polyunsaturated fatty acid (menhaden oil) effectively suppressed hepatic lipogenesis and lipogenic gene expression in the corpulent JCR:LA-cp rat. Reduced lipogenic gene expression by the fat-enriched diets was most likely the result of decreased expression of the lipogenic regulator SREBP-1c. The difference in response of the corpulent rats in the present study with that of previous studies may reflect methodological differences between the present study and prior studies. Differences in prior studies include use of diets enriched in saturated fat, short feeding duration (3 d), use of control diets enriched in PUFAs, and differences in mechanisms of regulation of lipogenesis among these models of obesity and hyperinsulinemia. Our results do not support the hypothesis that the corpulent JCR:LA rat is resistant to down-regulation of hepatic lipogenesis by dietary fat.

Several lines of evidence point to reduced expression of SREBP-1c as a primary mechanism by which dietary fatty acids reduce hepatic lipogenesis. SREBP-1c stimulates transcription of the enzymes that participate in synthesis of fatty acids and TGs (56). We observed parallel changes in expression of lipogenic enzymes and SREBP-1c in corpulent *vs.* lean rats and with the various diets. Several mechanisms have been identified by which fatty acids may suppress SREBP-1c expression. Fatty acids decrease the abundance of both SREBP-1c mRNA and the transcriptionally active nuclear SREBP-1c fragment in livers of normal mice (15, 18, 57). Fatty acids reduce nuclear content of transcriptionally active SREBP-1c by decreasing cleavage of full-length SREBP-1c (18, 58) and accelerate degradation of SREBP-1c mRNA (16). In addition, fatty acids prevent activation of the SREBP-1c promoter *in vitro* by insulin (14) and LXR agonists (59). These latter observations suggest that at least part of the effect of fatty acids to suppress SREBP-1c expression is exerted at the transcriptional level. Recently the mechanism by which dietary fats reduce SREBP-1c expression has been shown to be dependent on the percentage of calories derived from fat. Supplementation with fish oil at low levels (10% of calories) in mice decreased nuclear content of transcriptionally active SREBP-1c fragment by reducing proteolytic processing, whereas dietary supplementation with fish oil at 30% of calories or greater also lowered SREBP-1c mRNA abundance (60), consistent with the present study.

In the present study the olive oil diet, although not as effective as menhaden oil, also suppressed hepatic expression of SREBP-1 and lipogenic enzymes. This observation is in contrast to the inability of oleic acid (18:1, N-9) to suppress SREBP-1c promoter activity (14) and the failure of dietary triolein to reduce SREBP-1 and FAS mRNA in livers of normal rats (15). In the present study, we used olive oil that, unlike triolein, also contains significant quantities of linoleic acid (18:2, N-6). It is possible that the reduced expression of SREBP-1 and lipogenic enzymes observed in corpulent rats fed the olive oil diet was attributable at least in part to the PUFA content of that diet.

The rat SREBP-1c promoter has binding sites for the transcription factor LXRα (14), an important regulator of SREBP-1c transcription (28, 61, 62). The effect of insulin to induce SREBP-1c expression has been postulated to involve increased expression of LXRα (29). Because we did not observe increased LXRα mRNA levels in the livers of hyperinsulinemic JCR:LA-cp rats, nor were LXRα mRNA levels reduced by dietary fat, neither insulin nor PUFAs appear to regulate LXRα expression at the transcriptional level in the corpulent JCR rat. These findings do not, however, exclude posttranscriptional effects of either hyperinsulinemia or dietary fat on LXR protein levels or on its transcriptional activity. Fatty acids have, in fact, been shown to inhibit agonist-dependent activation of LXR (59). Although we did not examine this question directly, the activity of endogenous LXRα can be assessed indirectly by examining changes in expression of LXRα-responsive genes. Of the LXR-responsive genes on the microarray panel, only one, Cyp7a1, was increased in the corpulent JCR rat. Other LXR-responsive genes (apoE, lipoprotein lipase, and stearoyl CoA desaturase) (63, 64) were unchanged in corpulent rats. Expression of some, but not all, of these LXR-responsive genes, was reduced by the menhaden oil and olive oil diets (SCD and Cyp7a1, respectively). These findings suggest a differential

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response of LXR-regulated genes to obesity, hyperinsulinemia, and fatty acid feeding. A recent report (65) suggests that, in contrast to previous findings in human embryonic kidney-293 cells, suppression of SREBP-1 and lipogenic enzymes by fatty acids in hepatocytes and liver may be independent of LXRα

PPARα is a pivotal regulator of lipid metabolism, stimulating β-oxidation of PUFAs in peroxisomes and saturated fatty acids in mitochondria (35). A number of PPARα-regulated genes (acyl-CoA thioesterase, NrOb2, carnitine palmitoyl transferase 2, enoyl coenzyme A hydratase 1, acetyl-CoA acyltransferase 1, and very long-chain acyl-CoA dehydrogenase) were increased by the menhaden oil diet. Other PPARα-regulated enzymes (acyl-CoA oxidase, peroxisomal multifunctional enzyme, and UCP2) were not increased by dietary fatty acid. Failure of dietary fatty acid to induce certain PPAR-regulated genes has been observed previously (66). An unexpected finding was that expression of mRNA for several enzymes involved in fatty acid oxidation were also increased in corpulent rats consuming the control diet, compared with lean rats. This increase may reflect an adaptive response to increased total intake of dietary fatty acid in the corpulent rats. The recent finding that expression of the peroxisomal enzyme L-specific multifunctional β-oxidation protein (GenBank no. AJ011864) is increased in the SREBP-1a overexpressing transgenic mouse (67) indicates that some enzymes associated with fatty acid oxidation may also be up-regulated by SREBP-1.

In addition to the proposed direct effects of PUFAs on hepatic lipid-related gene expression, we cannot exclude the possibility that dietary PUFAs may have also acted indirectly by affecting insulin sensitivity. Although postprandial plasma insulin and glucose were not significantly changed by either fat-enriched diet, we did not perform direct analyses of insulin sensitivity or insulin secretion. Dietary intake of saturated fat and selected monounsaturated fats has been shown to induce insulin resistance; however, dietary PUFAs, particularly fish oil, are considered either neutral or protective (68). Fish oil may, in fact, protect against fat-induced insulin resistance by serving as a PPARα ligand, thereby increasing β-oxidation of fatty acids (69). The potential importance of changes in hepatic insulin signaling pathways in the pathogenesis of increased SREBP-1c expression and fatty acid synthesis in obesity and diabetes is underscored by the recent demonstration that overexpression of suppressors of cytokine signaling-1 and -3 (which are increased in livers of obese diabetic *db/db* mice) causes insulin resistance and increases hepatic SREBP-1c expression (70).

In addition to observing coordinate regulation of enzyme systems involved in *de novo* lipogenesis, fatty acid oxidation, and cholesterol synthesis, the microarray analyses also identified other genes that may be important in the pathogenesis of the metabolic changes that accompany obesity and dietary fat intake. The significance of changes in hepatic expression of these mRNA species, which include the class B scavenger receptor, Cyp7a1, oxysterol 7α-hydroxylase, ABCD3, prolactin receptor, insulin-related receptor, IGFBP-1, FXR, C/EBP-B, and Kid-1 requires further study. One of the most striking findings from the microarray data was the marked increase in expression of mRNA for the fatty acid transport protein CD36 in the corpulent JCR rat (control diet). CD36 encodes the enzyme fatty acid translocase that mediates transport of fatty acids into the cell. Increased expression of CD36 has been observed in heart, muscle, and adipose of the Zucker fatty rat, and translocation of CD36 to the plasma membrane is increased by insulin (71, 72). A deficiency of CD36 results in insulin resistance in the spontaneously hypertensive rat (73). Increased expression of CD36 in the liver of the corpulent JCR:LA-cp rat could result in increased transport of fatty acids into the liver and contribute to the overproduction of triglyceride. Conversely, reduced CD36 expression after fat feeding might be expected to reduce entry of fatty acid into the liver and reduce triglyceride production.

An unexpected finding of the present study was that, despite markedly reduced *de novo* hepatic lipogenesis, plasma TG was not reduced in corpulent JCR:LA-cp rats consuming the menhaden oil diet. Other studies have shown reduced plasma TG with fish oil feeding in both rat (74) and human (75). Possibly at this level of supplementation (40% of calories from menhaden oil), reduced endogenous fatty acid synthesis might have been counterbalanced by use of the dietary fatty acid for triglyceride (and VLDL) synthesis. Similarly, increased plasma TG in JCR:LA-cp rats fed olive oil may have been the result of the use of dietary fatty acid for TG synthesis insofar as oleic acid is readily incorporated into TG. Others have, in fact, observed increased plasma TG in rats fed olive oil (74). These data indicate that the type and amount of dietary fat ingested may determine the net effect on plasma TG.

In the present study, consumption of diets enriched in olive oil and menhaden oil reduced plasma cholesterol and cholesteryl esters in both lean and corpulent rats. In addition, plasma PL was reduced by menhaden oil diet in both lean and corpulent JCR:LA rats. This latter finding most likely reflects reduced plasma HDL because this fraction is the major carrier of PL, and free and esterified cholesterol, in the rat. Consistent with this, we observed decreased HDL protein in plasma of corpulent rats fed menhaden oil. This has been observed by others in both rat (76) and human (77). Despite this, diets enriched in PUFAs, including the n-3 PUFA found in fish oil, have been shown to reduce cardiovascular disease (2). The mechanism for reduced HDL with dietary PUFAs is poorly understood. In the human, decreased HDL cholesterol in response to dietary PUFAs has been linked to a polymorphism in the apoA1 promoter (78). In the present experiments, expression of mRNA for apoAI was, in fact, significantly reduced by the menhaden oil diet in corpulent JCR:LA-cp rats.

In the present studies, not only was TG synthesis increased in the JCR:LA-cp rat, but also PL synthesis was increased as well. Cholesterol synthesis, although not significantly increased in the corpulent rats, trended higher, in keeping with the observation of increased hepatic expression of mRNA for 3-hydroxy-3-methylglutaryl coenzyme A reductase. This indicates coordinate up-regulation of enzymes related to the synthesis of these components of VLDL. This did not result in increased accumulation of hepatic PL or cholesterol, however, indicating that the excess lipid may have been primarily used for VLDL production. Conversely, synthesis of PL and

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cholesterol tended to be lower in corpulent rats fed menhaden oil (Table 3). Although we did not directly assess cholesterol ester synthesis in these experiments, hepatic cholesterol esters were increased by dietary fat in lean (olive oil) and corpulent (menhaden oil) rats. Increased hepatic cholesterol ester after administration of dietary fat is an interesting finding and may reflect up-regulation of enzymes related to cholesterol ester formation by fatty acids, as observed for acyl-CoA-acyltransferase (ACAT) in human hepatoma (HepG2) cells (79). Alternatively, down-regulation of the rate-limiting enzyme of bile acid synthesis (7α-hydroxylase) by the olive oil diet may have also resulted in increased cholesterol ester formation.

In conclusion, the present study demonstrates that substitution of dietary fat, particularly n-3 long-chain PUFAs, for carbohydrate can effectively mitigate up-regulation of SREBP-1c and lipogenic enzymes in the hyperinsulinemic corpulent JCR:LA-cp rat. Although the menhaden oil diet, enriched in marine PUFAs, EPA (20:5 n-3) and docosahexanoic acid (22:6, n-3), was most effective in this regard, the olive oil diet that contained the monounsaturated fatty acid oleic acid (18:1, N-9) as its predominant fatty acid was also effective, perhaps due to its content of PUFA (linoleic acid, 18:2, n-6). In the corpulent JCR:LA-cp rat, down-regulation of SREBP-1c by dietary fat is accompanied by a coordinate reduction in hepatic expression of the panel of enzymes participating in lipid synthesis and variable up-regulation of PPARα-responsive genes responsible for fatty acid oxidation. This results in reductions in both *de novo* fatty acid and TG synthesis in corpulent rats consuming fat-enriched diets. Contrary to limited previous reports in this and other animal models of obesity and hyperinsulinemia, corpulent JCR:LA-cp rats were not resistant to down-regulation of lipogenic enzymes by dietary fat and were in fact more responsive to dietary fat than were lean animals. This study clearly demonstrates that substitution of dietary PUFAs for carbohydrate in the corpulent JCR:LA-cp rat reduces *de novo* lipogenesis, at least in part, by reducing hepatic expression of SREBP-1c and that a strategy directed toward reducing SREBP-1c expression in the liver can mitigate the adverse effects of hyperinsulinemia on hepatic lipid production. Whether a similar mechanism is operative in obese humans requires further study.

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