Genes Involved in Fatty Acid Partitioning and Binding, Lipolysis, Monocyte/Macrophage Recruitment, and Inflammation Are Overexpressed in the Human Fatty Liver of Insulin-Resistant Subjects

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OBJECTIVE—The objective of this study is to quantitate expression of genes possibly contributing to insulin resistance and fat deposition in the human liver.

RESEARCH DESIGN AND METHODS—A total of 24 subjects who had varying amounts of histologically determined fat in the liver ranging from normal ($n = 8$) to steatosis due to a nonalcoholic fatty liver (NAFL) ($n = 16$) were studied. The mRNA concentrations of 21 candidate genes associated with fatty acid metabolism, inflammation, and insulin sensitivity were quantitated in liver biopsies using real-time PCR. In addition, the subjects were characterized with respect to body composition and circulating markers of insulin sensitivity.

RESULTS—The following genes were significantly upregulated in NAFL: peroxisome proliferator–activated receptor (PPAR)γ2 (2.8-fold), the monocyte-attracting chemokine CCL2 (monocyte chemoattractant protein [MCP]-1, 1.8-fold), and four genes associated with fatty acid metabolism (acyl-CoA synthetase long-chain family member 4 [ACSL4] [2.8-fold], fatty acid binding protein [FABP]4 [3.9-fold], FABP5 [2.5-fold], and lipoprotein lipase [LPL] [3.6-fold]). PPARγ coactivator 1 (PGC1) was significantly lower in subjects with NAFL than in those without. Genes significantly associated with obesity included nine genes: plasminogen activator inhibitor 1, PPARγ, PPARδ, MCP-1, CCL3 (macrophage inflammatory protein [MIP]-1α), PPARγ2, carnitine palmitoyltransferase (CPT1A), FABP4, and FABP5. The following parameters were associated with liver fat independent of obesity: serum adiponectin, insulin, C-peptide, and HDL cholesterol concentrations and the mRNA concentrations of MCP-1, MIP-1α, ACSL4, FABP4, FABP5, and LPL.

CONCLUSIONS—Genes involved in fatty acid partitioning and binding, lipolysis, and monocyte/macrophage recruitment and inflammation are overexpressed in the human fatty liver. ***Diabetes*** **56:2759–2765, 2007**

Excess fat accumulation in the liver characterizes insulin-resistant subjects with both too much and too little subcutaneous fat (1). Obesity and insulin resistance are the most common associates of nonalcoholic fatty liver disease (NAFLD), a condition characterized by >10% fat (by histology) in the liver in the absence of other causes of steatosis (2). NAFLD covers a spectrum from simple steatosis (nonalcoholic fatty liver [NAFL]) to nonalcoholic steatohepatitis (NASH), including NASH with cirrhosis, and predicts type 2 diabetes, cardiovascular disease, and liver failure (1). The mechanisms underlying fat accumulation in the human liver and those linking fat to insulin resistance are poorly understood.

Insulin resistance is frequently accompanied by low-grade inflammation. Circulating markers of inflammation include elevated serum levels of C-reactive protein (3) and cytokines and chemokines attracting monocytes/macrophages, such as monocyte chemoattractant protein (MCP)-1 (4). An absence of the C-C motif chemokine receptor-2 (CCR2) regulating monocyte/macrophage recruitment protects the liver against fat accumulation in diet-induced obesity in mice (5). MCP-1 is overexpressed in insulin-resistant human adipose tissue (6,7). Adipose tissue of obese subjects is also characterized by increased accumulation of macrophages as determined by immunohistochemistry (6,8) and expression of macrophage-specific markers, such as CD68 (6–8). The fatty liver of obese mice overexpresses plasminogen activator inhibitor 1 (PAI-1) (9). We have previously demonstrated circulating PAI-1 concentrations to be closely correlated with liver fat content in patients with lipoatrophy (10), but it is unknown whether the human fatty liver of insulin-resistant subjects overexpresses MCP-1, macrophage inflammatory protein (MIP)-1α, CD68, or PAI-1.

In human NAFL, the main source of free fatty acid (FFA) in triglycerides in hepatocytes is peripheral lipolysis from subcutaneous sources (11). In addition, postprandially, de novo lipogenesis contributes significantly to hepatic fat accumulation (11). Fatty acid transport proteins (FATPs) and fatty acid binding proteins (FABPs) regulate fatty acid fluxes. FATP5 is exclusively expressed in the liver, and its overexpression in cell cultures increases FFA uptake (12). Conversely, FFA uptake is reduced in hepatocytes isolated

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ADIPOR, adiponectin receptor; CCR2, C-C motif chemokine receptor-2; FATP, fatty acid transport protein; FABP, fatty acid binding protein; FFA, free fatty acid; LPL, lipoprotein lipase; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; NAFL, nonalcoholic fatty liver; NAFLD, NAFL disease; NASH, nonalcoholic steatohepatitis; PAI-1, plasminogen activator inhibitor 1; PGC1, PPARγ coactivator 1; PPAR, peroxisome proliferator–activated receptor. RPLP0, ribosomal protein, large P0; TBP, TATA-binding protein.

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from FATP5 knockout animals (12). FABP4- and FABP5-deficient mice are protected against diet-induced obesity, insulin resistance, type 2 diabetes, and a fatty liver (13,14). There are no data on expression of FATPs or FABPs or other genes, such as acyl-CoA synthetase long-chain family member 4 (ACSL4) and FAS, in the human fatty liver. Peroxisome proliferator–activated receptor (PPAR)γ has two major isoforms, -γ1 and -γ2. Both isoforms activate several adipogenic and lipogenic genes regulating adipocyte maturation, lipid accumulation, and insulin sensitivity. In mice, PPARγ2 is overexpressed in the fatty liver (15), but data regarding PPARγ, -α, and -δ expression in the human fatty liver are lacking.

In the present study, we quantified, by real-time PCR, expression of genes related to inflammation (CD68, MCP-1, MIP-1α, and PAI-1), lipolysis (lipoprotein lipase [LPL]), FFA binding (FABP4 and FABP5), transport (FATP5 and CPT1), and synthesis (ACSL4, SCD1 [stearoyl-coenzyme A desaturase 1], and acetyl-coenzyme A carboxylase-α [ACACA]) in the liver of subjects with varying amounts of liver fat ranging from normal to NAFL (>10% fat as determined by histology [2]). In addition, expression of PPARα, -γ, -δ, PPARγ coactivator 1 (PGC1), and adiponectin receptors 1 and 2 were measured. Data were also analyzed after dividing the subjects into groups based on the presence and absence of NAFL.

RESEARCH DESIGN AND METHODS

A total of 30 Caucasian subjects were recruited from patients undergoing a laparoscopic gastric bypass operation or among those referred to the gastroenterologist because of elevated liver function tests. Inclusion criteria were as follows: *1*) age of 18–60 years; *2*) alcohol consumption less than two drinks, i.e., 20 g ethanol/day; and *3*) no histological evidence of NASH. The following causes of liver diseases were excluded: chronic hepatitis B or C, thyroid dysfunction, autoimmune hepatitis (smooth muscle and anti-nuclear antibodies), primary biliary cirrhosis (anti-mitochondrial antibodies), primary sclerosing cholangitis, use of hepatotoxic medications or herbal products, or use of medications known to be associated with steatohepatitis. None of the subjects used antidiabetic or lipid-lowering medications, including PPARα or -γ agonists. The liver biopsy was taken if considered clinically indicated. The subjects were divided into groups with (NAFL+) or without (NAFL−) NAFL (2). Of the 30 patients, 6 were excluded because of NASH ($n = 2$), undefined hepatitis ($n = 1$), cirrhosis ($n = 1$), or insufficient sample for RNA isolation ($n = 2$), resulting in the patient sample of 24 patients for final analysis.

The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki. The protocol was approved by the ethics committee of the Helsinki University Central Hospital.

Liver biopsy and total RNA cDNA preparation. Needle or wedge biopsies (10–400 mg) were taken after an overnight fast. Approximately one-half of the sample was sent to the pathologist for routine histopathological assessment, while the rest was immediately frozen and stored in liquid nitrogen. Frozen tissue samples (2–30 mg) were homogenized in 1 ml QIAzol Lysis Reagent (Qiagen, Valencia, CA), and total RNA was isolated as previously described (16). RNA was stored at −80°C until quantification of target mRNAs. A total of 0.1 μg RNA was transcribed into cDNA using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Paisley, U.K.) and oligo $(dT)_{12–18}$ primers (16).

PCR analyses. The mRNA concentrations were quantified by real-time PCR using the ABI 7000 Sequence Detection System instrument and software (Applied Biosystems). cDNA synthesized from 10 ng total RNA was mixed with TaqMan Universal PCR Master Mix (Applied Biosystems) and a gene-specific primer and probe mixture (predeveloped TaqMan Gene Expression Assays; Applied Biosystems) in a final volume of 25 μl. The assays used were as follows: Hs00167155_m1 for PAI-1, Hs00234592_m1 for PPARγ, Hs00602622_m1 for PPARδ, Hs00360422_m1 for adiponectin receptor (ADIPOR)1, Hs00154355_m1 for CD68, Hs00231882_m1 for PPARα, Hs00748952_s1 for SCD, Hs00234140_m1 for MCP-1, Hs00234142_m1 for MIP-1α, Hs00226105_m1 for ADIPOR2, Hs00173304_m1 for PGC1α, Hs01115510_m1 for PPARγ2, Hs00244871_m1 for ACSL4, Hs00157079_m1 for carnitine palmitoyltransferase 1A (CPT1A), Hs00236877_m1 and Hs00426285_m1 for IGFBP1 (insulin-like growth factor binding protein-1), Hs00167385_m1 for ACACA, Hs00202073_m1 for FATP5, Hs00609791_m1 for FABP4, Hs02339439_g1 for FABP5, Hs00173425_m1 for LPL, Hs00194145_m1 for HMGCS2, Hs99999910_m1 for TATA-binding protein (TBP), and Hs99999902_m1 for ribosomal protein, large P0 (RPLP0). All samples were run in duplicate. Relative expression levels were determined using a 7-point serially diluted standard curve, generated from cDNA of human liver. The mRNA concentrations of specific genes were expressed in arbitrary units and normalized to the mean of the mRNA concentrations of RPLP0 and TBP to correct for differences in cDNA loading.

Other measurements. Blood samples were taken after an overnight fast for measurement of plasma glucose, serum insulin, C-peptide, serum triglycerides, and total and HDL cholesterol concentrations as described previously (7). Serum adiponectin concentrations were measured using an enzyme-linked immunosorbent assay kit from B-Bridge International (San Jose, CA). Serum FFA were measured by an enzymatic colorimetric method using a kit from Wako Chemicals (Neuss, Germany) and PAI-1 antigen using an enzyme-linked immunosorbent assay kit from Trinity Biotech (Wicklow, Ireland).

Statistical analyses. Comparisons between NAFL+ and NAFL− groups were performed using nonparametric methods, and correlations coefficients were calculated using Spearman's rank correlation coefficient. Nonparametric statistics were used because of relatively small sample size, although all gene expression data passed the normality test. Adjustment of liver fat for BMI was performed using analysis of covariance after logarithmic transformation of the variables if necessary. A P value of <0.05 was considered statistically significant. The calculations were performed using SPSS 11.0 for Windows (SPSS, Chicago, IL). All data are shown as means ± SE.

TABLE 1
Characteristics of the study subjects

	All subjects	NAFL−	NAFL+
n	24	8	16
Histological liver fat (%)	38.5 ± 6.8	2.6 ± 1.2	56.4 ± 6.5*
Women/men	14/10	4/4	10/6
Age (years)	44 ± 2	47 ± 4	42 ± 3
Body weight (kg)	105 ± 6	85 ± 9	116 ± 8†
BMI (kg/m^2)	37.1 ± 2.4	29.9 ± 3.8	40.8 ± 2.6†
Components of the MetS			
Waist circumference (cm)	113 ± 4	85 ± 9	116 ± 8
Systolic blood pressure (mmHg)	133 ± 2	130 ± 5	134 ± 3
Diastolic blood pressure (mmHg)	83 ± 2	78 ± 3	86 ± 2†
fS triglycerides (mmol/l)	1.7 ± 0.2	1.1 ± 0.2	1.9 ± 0.2†
fS HDL cholesterol (mmol/l)	1.5 ± 0.1	2 ± 0.3	1.3 ± 0.1†
fP glucose (mmol/l)	5.9 ± 0.2	5.3 ± 0.2	6.1 ± 0.3
Other parameters			
fS insulin (mU/l)	12 ± 2	6 ± 1	15 ± 3†
fS C-peptide (nmol/l)	0.9 ± 0.1	0.6 ± 0.1	1.1 ± 0.1‡
fS FFA (μmol/l)	588 ± 45	509 ± 90	628 ± 49
fS adiponectin (μg/ml)	9.7 ± 1	12.3 ± 2.1	8.4 ± 1§
fP PAI-1 (ng/ml)	31 ± 3	24 ± 6	35 ± 3
fS LDL cholesterol (mmol/l)	2.7 ± 0.2	2.7 ± 0.3	2.7 ± 0.2
fS ALT (units/l)	87 ± 13	97 ± 21	82 ± 17
fS AST (units/l)	53 ± 6	55 ± 9	53 ± 8
MCV (fl)	90 ± 1	90 ± 1	90 ± 1
Daily alcohol dose	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.2

Data are means ± SE. *$P < 0.001$, †$P < 0.05$, ‡$P < 0.01$, §$0.1 > P > 0.05$, for subjects without (NAFL−) versus with (NAFL+) NAFLD. ALT, alanine aminotransferase; ASP, aspartate aminotransferase; fP, fasting plasma, fS, fasting serum; MCV, mean corpuscular volume; MetS, metabolic syndrome; NA, not applicable.

RESULTS

The NAFL+ group had significantly higher concentrations of fasting serum insulin, C-peptide, and triglycerides and

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TABLE 2
Nonparametric correlations (Spearman's r) between hepatic gene expression (as expressed relative to housekeeping gene mRNA expression) and the amount of liver fat and liver fat adjusted for BMI

Gene	Liver fat	Liver fat adjusted for BMI	NAFL−	NAFL+	Fold difference
PAI1	0.32	0.05	2.12 ± 0.9	1.85 ± 0.28	0.9
PPARγ	0.39	0.22	0.13 ± 0.01	0.17 ± 0.01	1.3
PPARγ2	0.64*	0.34	0.05 ± 0.01	0.15 ± 0.02†	2.8
PGC1	−0.19	−0.19	5.6 ± 1.37	3.29 ± 0.56‡	0.6
PPARα	0.06	−0.05	4.58 ± 0.97	4.31 ± 0.52	0.9
PPARδ	0.29	0.1	0.18 ± 0.03	0.24 ± 0.03	1.3
ADIPOR1	−0.08	−0.14	1.23 ± 0.1	1.29 ± 0.13	1.1
ADIPOR2	0.05	0.03	3.01 ± 0.53	3.03 ± 0.46	1.0
HMGCS2	−0.07	−0.17	6.36 ± 0.71	5.96 ± 0.44	0.9
IGFBP1	−0.11	−0.28	4.88 ± 1.38	6.76 ± 2.5	1.4
CD68	0.14	0.17	0.17 ± 0.03	0.2 ± 0.02	1.1
MCP-1	0.61§	0.45†	0.32 ± 0.05	0.57 ± 0.05§	1.8
MIP-1α	0.42†	0.36	0.03 ± 0.01	0.04 ± 0.01	1.6
SCD	0.18	0.22	0.44 ± 0.13	0.48 ± 0.09	1.1
ACSL4	0.53§	0.43†	0.11 ± 0.05	0.31 ± 0.07‡	2.8
CPT1A	0.24	0.11	2.69 ± 0.53	3.39 ± 0.44	1.3
ACACA	0.27	0.2	1.39 ± 0.31	1.89 ± 0.18	1.4
FATP5	−0.16	−0.22	8.57 ± 0.87	7.81 ± 0.7	0.9
FABP4	0.83¶	0.76¶	0.002 ± 0.001	0.009 ± 0.001§	3.9
FABP5	0.74*	0.62§	0.016 ± 0.003	0.039 ± 0.008†	2.5
LPL	0.73*	0.66*	0.002 ± 0.001	0.006 ± 0.002	3.6

Data are means ± SE. mRNA concentration of selected genes in the liver in control subjects and subjects with NAFL. *$P < 0.001$, †$P < 0.05$, ‡$0.1 > P > 0.05$, §$P < 0.01$, ¶$P < 0.0001$ for correlation or control versus NAFL.

lower concentrations of HDL cholesterol than the NAFL+ group (Table 1). Five of the eight NAFL− patients had slightly elevated serum alanine aminotransferases and/or aspartate aminotransferases without any known cause.

Liver fat and markers of insulin resistance. In all subjects, liver fat correlated with BMI (Spearman's rank correlation coefficient $r = 0.46$, $P < 0.05$). Liver fat was positively correlated with fasting serum insulin ($r = 0.59$, $P < 0.01$), C-peptide ($r = 0.83$, $P < 0.0001$), and serum triglycerides ($r = 0.53$, $P < 0.01$) and negatively with serum HDL cholesterol ($r = -0.59$, $P < 0.01$). After adjusting for BMI, liver fat was significantly correlated with fasting serum insulin ($r = 0.45$, $P < 0.05$), C-peptide (0.63, $P < 0.01$), and HDL cholesterol ($r = -0.41$, $P < 0.05$) concentrations. Serum adiponectin did not correlate with BMI but was inversely related with liver fat (unadjusted for BMI, $r = -0.49$, $P < 0.05$; adjusted for BMI, $r = -0.44$, $P < 0.05$). Serum PAI-1 concentrations were also correlated with liver fat ($r = 0.41$, $P < 0.04$) but not with BMI ($r = 0.19$, NS).

Liver fat and hepatic gene expression. The correlation coefficients between liver fat and gene expression are shown in Table 2. Liver fat was significantly associated with increased expression of PPARγ2, MCP-1, and MIP-1α (Table 2; Figs. 1 and 2) and ACSL4, FABP4, FABP5, and LPL (Table 2; Figs. 2 and 3). When adjusted for BMI, the relationships between liver fat and MCP-1, ACSL4, FABP4, FABP5, and LPL expression remained statistically significant.

NAFL and hepatic gene expression. Data on gene expression in the subjects divided into groups based on NAFL are shown in Table 2. The NAFL+ group had significantly increased hepatic expression of PPARγ2, MCP-1, FABP4, FABP5, and LPL compared with the NAFL− group.

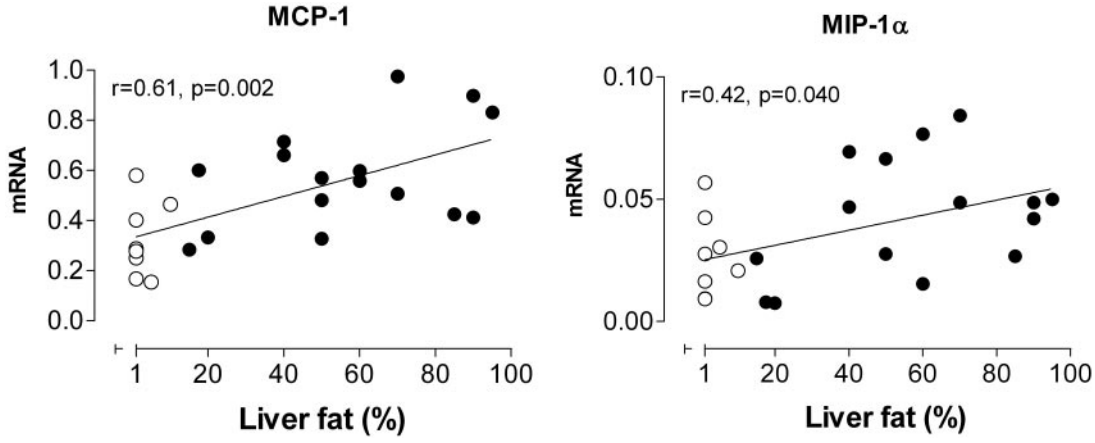

FIG. 1. The relationship between liver fat and hepatic mRNA concentrations of MCP-1 and MIP-1α expressed relative to the mean of housekeeping genes (RPLP0 and TBP) and the percentage of liver fat in the entire study group. ○, without NAFLD; ●, with NAFLD.

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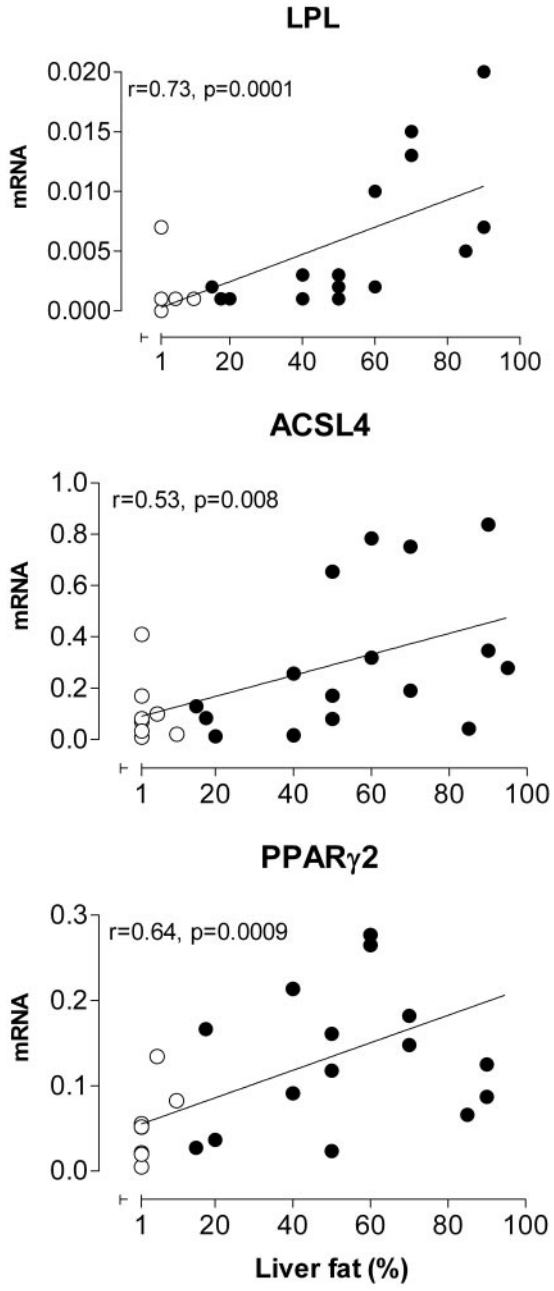

FIG. 2. The relationship between liver fat and hepatic mRNA concentrations of LPL, ACSL4, and PPARγ2 expressed relative to the mean of housekeeping genes (RPLP0 and TBP) and the percentage of liver fat in the entire study group. ○, without NAFLD; ●, with NAFLD.

DISCUSSION

We quantified expression of genes thought to be important in fatty acid trafficking, synthesis and storage, and inflammation in human liver samples with varying amounts of fat. We found the expression of PPARγ2, of two monocyte-attracting chemokines (MCP-1 and MIP-1α) and of four genes associated with fatty acid metabolism (ACSL4, FABP4, FABP5, and LPL) to be increased in proportion to liver fat content. Interestingly, of these genes, PPARγ2, FABP4, and LPL are normally expressed, especially in adipose tissue. The relationships between liver fat and MCP-1, ACSL4, FABP4, FABP5, and LPL expression remained significant even when adjusted for BMI, although statistical adjustment for the impact of obesity is inferior to study of weight-matched groups with and without NAFL. When the subjects were divided into those with NAFL using the cutoff point of 10% liver fat, those with NAFL had higher expression of PPARγ2, MCP-1, FABP4, and FABP5.

Studies examining gene expression in human NAFL are very limited. Younossi et al. (17) compared gene expression using microarray between 12 steatosis patients and 7 obese controls. Ten of the 5,220 genes examined were differentially expressed in the liver. None was related to inflammation, insulin action, or fatty acid transport or synthesis (17). The steatosis (NAFL) subjects did not, however, differ with respect to serum insulin concentrations or the homeostasis model assessment insulin resistance index from the nonsteatosis control subjects. Younossi et al. (18) also reported results of microarray analysis comprising 5,220 genes in 29 patients with NASH, 14 subjects with steatosis alone, and 7 obese and 6 nonobese control subjects. A total of 34 genes were differentially expressed between obese patients with NASH and nonobese subjects. Altered expression of four of these genes was verified using real-time PCR. Results regarding differences between subjects with and without steatosis were not reported. Chiappini et al. (19) recently reported microarray data comparing hepatic gene expression between obese steatotic ($n = 9$) and significantly leaner subjects. Features of insulin resistance or degree of alcohol consumption were not reported. Of 110 differentially expressed genes, expression of one mitochondrial (mtDNA) and three inflammatory genes (SIGIRR, TOLLIP, and STIPEC) were verified using real-time PCR in a larger group of 40 liver donors, but characteristics of these subjects were not reported.

The chemokines MCP-1 and MIP-1α are low–molecular weight proteins secreted by several tissues. These chemokines primarily stimulate leukocyte recruitment. Haukeland et al. (20) reported, using immunohistochemistry, that MCP-1 was expressed in normal human livers and in livers from patients with simple steatosis and NASH. Positive staining was found in bile duct epithelial cells, endothelial cells, leukocytes, and hepatocytes (20). Our data support these observations by showing a significant positive correlation between liver fat and MCP-1 gene expression.

CD68 is a transmembrane glycoprotein that is highly expressed by human monocytes and tissue macrophages. In the present study, expression of CD68 was not associated with liver fat and did not differ between those with and without NAFL. Although this was somewhat unexpected, it is noteworthy that the subjects did not have histological signs of inflammation as determined by histopathological examination. Possibly, expression of macrophage markers could be increased in more advanced forms of NAFLD.

FABPs are members of the superfamily of lipid-binding proteins with tissue-specific distribution (21). They regulate fatty acid uptake and are involved in intracellular trafficking of long-chain fatty acids for oxidation and storage. In the present study, expression of both FABP4 and FABP5 were closely positively correlated with liver fat content. FABP4 is considered to be adipocyte specific (22), although it is expressed also in stimulated human monocytes and macrophages (23). FABP5 (epidermal FABP) is expressed in many tissues, such as brain, kidney, adipose tissue, and liver (24). In livers of *ob/ob* mice, FABP expression has been found to be increased twofold com-

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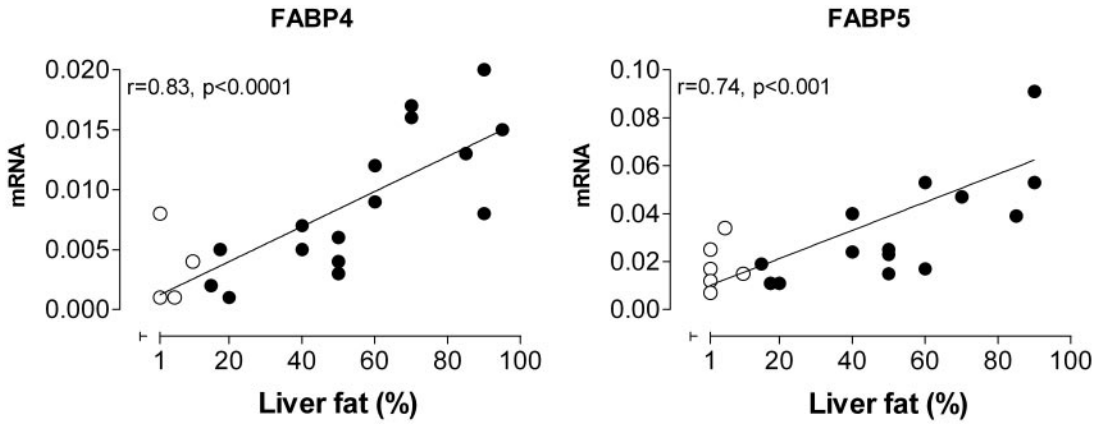

FIG. 3. The relationship between liver fat and hepatic mRNA concentrations of FABP4 and FABP5 expressed relative to the mean of housekeeping genes (RPLP0 and TBP) and the percentage of liver fat in the entire study group. ○, without NAFLD; ●, with NAFLD.

pared with that in lean control mice (25), in keeping with the present data in humans. In these mice, FATP has been reported to be unchanged, as in the present study (25). Mice lacking both FABP4 and FABP5 are protected from diet-induced obesity and accumulation of fat in the liver (13), implying that these proteins may play a role in regulating liver fat.

LPL is a rate-limiting enzyme for intravascular hydrolysis of lipoprotein-rich triglyceride particles, which is expressed at high levels in adipose tissue, heart and skeletal muscle, kidney, and the mammary gland and at lower levels in the liver, adrenal gland, and brain (26). In the liver, LPL has been shown to be upregulated by liver X receptor (LXR) agonists (27). In addition, LPL expression is induced in the liver by tumor necrosis factor-α (28). A study performed in rats suggested LPL activity in the liver and in peripheral tissues to be reciprocally regulated (29). Liver-specific overexpression of LPL in mice causes hepatic steatosis and insulin resistance (30). In humans, LPL activity in adipose tissue is high in obese, insulin-resistant subjects (31). It is thus possible that LPL is reciprocally regulated even in humans, a combination that would favor fat accumulation in the liver.

Acyl-CoA synthetases catalyze the initial step of acyl-CoA formation from long-chain fatty acids. These enzymes have been classified by their preferences for short, medium, long, and very long chain fatty acids, although there is considerable overlap (32). Isoforms 1, 3, 4, and 5 are abundant in the liver (33). Overexpression of ACSL isoforms has been shown to increase fatty acid uptake (34). Rat ACSL4 is a membrane-associated long-chain acyl-CoA synthetase that can activate both saturated and unsaturated fatty acids from 14 to 26 carbons (35). Increased ACSL4 expression in the liver could thus promote fatty acid uptake, although the exact function of this isoform in the liver is unexplored.

Studies using animal models, such as lipoatrophic A-ZIP/F-1 mice with PPARγ ablation (15), leptin-deficient mice with liver-specific disruption of PPARγ (36), and mice with hepatic overexpression of either PPARγ1 (37) or PPARγ2 (38), have established that increased PPARγ activity can cause hepatic steatosis. In mice, PPARγ and a fatty liver can be induced by synthetic and natural ligands, such as those generated by a high-fat diet (39). The 2.8-fold increase in PPARγ2 expression in the NAFL patients is consistent with these data. We have previously found in two cross-sectional studies that liver fat content is positively correlated with the percentage of calories originating from fat and especially saturated fat (40,41). A high-fat diet compared with a eucaloric low-fat diet increases liver fat content in humans (42). The possibility that high relative fat intake contributed to the increased PPARγ2 expression in the NAFL group remains to be tested. In mice, saturated fats also appear to increase hepatic steatosis and obesity via activation of SREBP1 and SCD (43–45). Disruption of SCD alone is sufficient to protect against steatosis and obesity (43). Although SREBP1c expression was not measured, expression of two of its target lipogenic mRNAs, ACACA and SCD, was not increased in the NAFL+ compared with the control group (43). No measurement of fatty acid oxidation was performed, but the mRNA concentration of CPT1A, the rate-limiting enzyme for mitochondrial β-oxidation, was unchanged.

PPARγ activation changes the transcription of hundreds of genes in vitro in cell lines, such as 3T3-L1 adipocytes. However, in a recent study examining the effect of chronic rosiglitazone treatment in type 2 diabetic patients on gene expression, only 13 of 50 genes, which have been most frequently reported to be regulated by PPARγ agonism in animal studies, were altered. Nevertheless, some of the genes in which expression was studied in the present study could represent PPARγ targets. These include the adiponectin receptors in addition to LPL, FABP4, and FABP5, which were discussed above. Whereas FABP4 is a classic PPARγ-regulated gene in adipose tissue (46), FABP5 has also been shown to be overexpressed in the liver in response to adenovirus-induced overexpression of PPARγ in PPARα$^{-/-}$ mice (37). Such treatment induced profound adipogenic transformation of the gene expression pattern in hepatocytes. Rosiglitazone has been shown to increase the mRNA and protein levels of ADIPOR2 in HepG2 cells (47), but expression of both ADIPOR1 and ADIPOR2 were unchanged in the present study. It may seem paradoxical that PPARγ agonists markedly lower liver fat content in humans (48), although PPARγ2 is overexpressed in the human fatty liver. The reason for this paradox is poorly understood, but possibly, the decrease in FFA flux and increase in serum adiponectin via PPARγ agonism in adipose tissue could be more important regulators of liver fat than direct effect of these drugs in the liver.

In conclusion, the human steatotic liver due to NAFL overexpresses PPARγ2 and genes normally found abun-

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dantly in adipose tissue, such as FABP4 and LPL, the chemokine genes MCP-1 and CCL3, and two other genes facilitating fatty acid transport and synthesis, FATP5 and ACSL4. These changes in the human fatty liver bear some resemblance to the adipogenic transformation described in the PPARα$^{-/-}$ overexpressing PPARγ in the liver. This conclusion awaits verification of the now described alterations in gene expression functionally and at the level of protein expression.

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