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Chronic intermittent hypoxia upregulates genes of lipid biosynthesis in obese mice

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Li, Jianguo, Dmitry N. Grigoryev, Shui Qing Ye, Laura Thorne, Alan R. Schwartz, Philip L. Smith, Christopher P. O'Donnell, and Vsevolod Y. Polotsky. Chronic intermittent hypoxia upregulates genes of lipid biosynthesis in obese mice. *J Appl Physiol* 99: 1643–1648, 2005. First published July 21, 2005; doi:10.1152/japplphysiol.00522.2005.—Obstructive sleep apnea (OSA), a condition tightly linked to obesity, leads to chronic intermittent hypoxia (CIH) during sleep. There is emerging evidence that OSA is independently associated with insulin resistance and fatty liver disease, suggesting that OSA may affect hepatic lipid metabolism. To test this hypothesis, leptin-deficient obese (*ob/ob*) mice were exposed to CIH during the light phase (9 AM–9 PM) for 12 wk. Liver lipid content and gene expression profile in the liver (Affymetrix 430 GeneChip with real-time PCR validation) were determined on completion of the exposure. CIH caused a 30% increase in triglyceride and phospholipid liver content ($P < 0.05$), whereas liver cholesterol content was unchanged. Gene expression analysis showed that CIH upregulated multiple genes controlling *1*) cholesterol and fatty acid biosynthesis [malic enzyme and acetyl coenzyme A (CoA) synthetase], *2*) predominantly fatty acid biosynthesis (acetyl-CoA carboxylase and stearoyl-CoA desaturases 1 and 2), and *3*) triglyceride and phospholipid biosynthesis (mitochondrial glycerol-3-phosphate acyltransferase). A majority of overexpressed genes were transcriptionally regulated by sterol regulatory element-binding protein (SREBP) 1, a master regulator of lipogenesis. A 2.8-fold increase in SREBP-1 gene expression in CIH was confirmed by real-time PCR ($P = 0.001$). Expression of major genes of cholesterol biosynthesis, SREBP-2 and 3-hydroxy-3-methylglutaryl-CoA reductase, was unchanged. In conclusion, we have shown that CIH may exacerbate preexisting fatty liver of obesity via upregulation of the pathways of lipid biosynthesis in the liver.

obstructive sleep apnea; fatty liver; mouse; gene expression

OBESITY CAN CAUSE MULTIPLE metabolic abnormalities, including insulin resistance and hyperlipidemia (2, 3). Increased central adiposity is also a major risk factor for obstructive sleep apnea (OSA) (5, 11, 26, 46). Recent studies suggest that the repetitive hypoxic stress that occurs with recurrent collapse of the upper airway in OSA may independently contribute to metabolic dysfunction and cause insulin resistance (17, 31, 32, 46). Thus obesity and OSA may interact to produce marked disturbances in metabolic function.

The development of insulin resistance in obese humans and animal models of obesity has been linked to fatty liver disease (4, 22, 25). For example, genetically obese (*ob/ob*) mice exhibit insulin resistance and hepatic steatosis (22). We recently showed that chronic intermittent hypoxia (CIH) exacerbates insulin resistance in the *ob/ob* mouse (30). It is possible that the increase in insulin resistance in *ob/ob* mice exposed to CIH is related to a worsening of hepatic steatosis due to upregulation of enzymes of lipid biosynthesis in the liver.

Human obesity is the strongest predictor of nonalcoholic steatic hepatitis (NASH) (1, 6, 12, 47). Nevertheless, adiposity is not always associated with NASH (1, 47), and predisposing factors for NASH in obese patients are unknown. Recent human data showed that OSA is associated with NASH independent of body weight (44), suggesting that CIH may be a risk factor for NASH in obese subjects with OSA. However, there are no data examining the impact of CIH on fat accumulation and pathways of lipid metabolism in the liver.

We hypothesized that CIH in the presence of preexistent obesity causes an increase in gene expression of enzymes of lipid biosynthesis and results in an elevation of lipid content in the liver. To examine our hypothesis, we explored the effects of CIH on hepatic gene expression and lipid content in *ob/ob* mice with preexistent obesity. Analyses were specifically focused on the pathways of lipid biosynthesis, including the sterol regulatory element-binding proteins (SREBPs), which act as master regulators of lipid biosynthesis in the liver (13, 21, 36, 37, 40).

METHODS

Animals. A total of 12 male obese C57BL/6J-*Lep*^{ob} (*ob/ob*) mice (Jackson Laboratory, Bar Harbor, ME) were used in the study, which was approved by the Johns Hopkins University Animal Use and Care Committee and complied with the American Physiological Society guidelines for animal studies. For all blood samples and injections, anesthesia was induced and maintained with 1–2% isoflurane administered through a face mask.

Experimental design. A gas control delivery system was designed to regulate the flow of room air, N_2, and O_2 into customized cages housing the mice, as previously described (30). A maximum of two mice were housed continuously in a single customized cage (27 × 17 × 17 cm) with constant access to food and water. A series of programmable solenoids and flow regulators altered the inspired O_2 fraction ($F_{I_{O_2}}$) over a defined and repeatable profile (43). During each period of intermittent hypoxia (IH), $F_{I_{O_2}}$ was reduced from 20.9 to 4.8–5.0% over a 30-s period and then rapidly restored to room air levels in the subsequent 30-s period. The use of multiple inputs into the cage produced a uniform nadir $F_{I_{O_2}}$ throughout the cage.

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Five obese mice were placed in the CIH chamber for 12 consecutive weeks. Food intake and body weight were monitored daily for each animal. All animals were kept in a controlled environment (22–24°C, 12:12-h light-dark cycle, lights on at 0900) on a standard chow diet with free access to water. A separate series of seven obese mice were exposed to intermittent room air (IA, control group) for 12 wk in identical chambers. The CIH and IA states were induced during the light phase alternating with 12 h of constant room air during the dark phase.

Sample collection. All serum and tissue samples were obtained during exposure to CIH or control conditions. Mice were fasted for 5 h before ensanguination and death. Arterial blood (1 ml) was obtained by direct cardiac puncture under 1–2% isoflurane anesthesia, and the serum was separated and frozen at −80°C. After blood withdrawal, the animals were euthanized with pentobarbital sodium (60 mg ip). Livers were surgically removed, dissected into three portions, and immediately frozen for future analysis.

Biochemical analyses. Serum cholesterol, phospholipids (PL), free fatty acids (FFA), and triglycerides (TG) were measured using test kits from Wako Diagnostics (Richmond, VA). The first portion of the liver was homogenized (EZ Connect, Omni International, Warrington, VA), and cholesterol, PL, and TG were extracted in chloroform-methanol (2:1) as described by Yokode et al. (48) and measured using kits from Wako Diagnostics. The second portion of the liver was homogenized in 150 mM NaCl, 20 mM Tris, pH 7.2, 1% Triton X-100, and 1 mM DTT. Total protein was measured using a D_C kit (Bio-Rad, Hercules, CA).

RNA isolation. Immediately after death, the liver tissue was snap frozen in liquid nitrogen for subsequent mRNA analysis. For the mRNA analysis, liver tissue was homogenized (EZ Connect) on ice. Total RNA was isolated using Trizol reagent (Life Technologies, Rockville, MD); additional RNA cleanup was performed with the RNeasy (Qiagen, Valencia, CA) purification kit.

cDNA was produced from total RNA using the Advantage RT for PCR kit (Clontech, Palo Alto, CA).

Gene microarrays in liver tissue. Gene microarrays of all five mice exposed to IH and the first five mice exposed to IA were performed. The Affymetrix GeneChip system was used as a gene expression profiling platform as described previously (19). Total RNA (5 μg) from each sample was converted to double-stranded cDNA using the SuperScript Choice system (Invitrogen, Carlsbad, CA). Each double-stranded cDNA was subsequently used as a template to make biotin-labeled cRNA using the BioArray HighYield RNA transcript kit (Enzo Life Science, Farmingdale, NY), and 15 μg of fragmented, biotin-labeled murine liver cRNA from each sample were hybridized to the GeneChip Mouse Genome 430A 2.0 array (Affymetrix, Santa Clara, CA) at 45°C for 16 h. The arrays were washed and stained in the Affymetrix GeneChip Fluidics Station 450 using the supplier's reagents. The fluorescent images were read using the Affymetrix GeneChip Scanner 3000 and converted to GeneChip Cell files (CEL) using the Affymetrix GeneChip Operating Software (version 1.0), with global normalization of target intensity set to 150. Quality control for generated expression profiles was conducted according to "Affymetrix Guideline for Assessing Sample and Array Quality" specifications. Gene expression values for 22,626 transcripts on the Mouse Genome 430A 2.0 array were calculated using robust microarray analysis (RMA, Bioconductor *affy* package) (19). Briefly, the RMA module of this package (18) was used for background correction across array normalization, and identification of the probe set fluorescence intensity values. Then gene expression profiles from normoxic and hypoxic livers were grouped (5 samples in each group) for direct comparison. A two-tailed *t*-test was performed, and change in gene expressions with $P < 0.05$ was used as a cutoff for selection of candidate genes. The normoxic-to-hypoxic fold-change ratio was computed on the basis of mean values of each group of samples, and 20% changes in expression were used as a cutoff. This cutoff level was successfully applied for selection of candidate genes in a variety of conditions (10, 20). The RMA-generated gene expression values were also analyzed using Significance Analysis of Microarrays software (45). The user-definable delta value was set to the Significance Analysis of Microarrays default, and significance of changes in gene expression was tested as described above (fold-change cutoff set to 1.2, simulating 20% cutoff) and at more stringent conditions (2-fold-change cutoff). (All data are deposited according to minimum information about microarray experiment standards and are available for download at http://www.ncbi.nlm.nih.gov/geo/, series entry GSE1873.)

Real-time PCR in liver tissue. The differentially expressed genes of lipid biosynthesis from gene microarrays were assessed by real-time PCR. cDNA was produced from total RNA using the Advantage RT for PCR kit (Clontech). cDNA was amplified in real-time RT-PCR with primers (Invitrogen) and Taqman probes (Applied Biosystems, Foster City, CA). The sequences of primers and probes were as follows: 5′-CCACTAGAGGTCGGCATGGT-3′ (forward), 5′-TCCCTTGAGGACCTTTGTCATT-3′ (reverse), and 5′-TGCTTGTCAGGCTCACCCTCT GGAA-3′ (probe) for SREBP-1; 5′-CCGAGATGCAGGGCAAAG-3′ (forward), 5′-GATGAAAGAA-CAATGAACAAGGCTTA-3′ (reverse), and 5′-TCCCCTGTGC-CTGAC-3′ (probe) for SREBP-2; 5′-CGCCCACGCAGCAAA-3′ (forward), 5′-GGGACCACTGGCTTCCATTA-3′ (reverse), and 5′-CACTGCTATCTACATCGC-3′ (probe) for 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase; 5′-AGCAGTGCTACAAGGT-GACCAA-3′ (forward), 5′-CTCCAGGGAACACGTAGGAATT-3′ (reverse), and 5′-TGCAATCTTTGCCAGCGGCAGTC-3′ (probe) for malic enzyme; 5′-TTGGCGACAAAGTTGCTTTTT-3′ (forward), 5′-CGGAGAACATTGCTGAACTG-3′ (reverse), and 5′-CACAC-CTGGACCAGGAGTTCA-3′ (probe) for acetyl-CoA synthetase; 5′-TGGATCCGCTTACAGAGACTTTT-3′ (forward), 3′-GCCGGAG-CATCTCATTCG-3′ (reverse), and 5′-CAGCACTGCCAGGCACCG-CA-3′ (probe) for acetyl-CoA carboxylase; 5′-CCCCTGCGGATCT-TCCTTAT-3′ (forward), 5′-AGGGTCGGCGTGTGTTTCT-3′ (reverse), and 5′-CACCGCGCCCACCACAAGTTCT-3′ (probe) for stearoyl-CoA desaturase (SCD)-1; 5′-AGCGGGCTGCAGAAACT-TAG-3′ (forward), 5′-GGCTGAGTAAGCGCCAGAGAT-3′ (reverse), and 5′-CCGGCCCACATAC-3′ (probe) for SCD-2; and 5′-CCTCTCAGTGGTAGTGGATACTCTGT-3′ (forward), 5′-GTGA-CCTTCGATTATGCGATCAT-3′ (reverse), and 5′-TCGTCATACC-CGTGGGCATCTCG-3′ (probe) for mitochondrial glycerol-3-phosphate acyltransferase (GPAT). The threshold cycle (C_t) was determined for every sample. The mRNA expression levels were normalized to 18S rRNA concentrations using the following formula

$$\text{Gene of interest/18S} = 2^{C_t(18S) - C_t(\text{gene of interest})}$$

Statistical analysis. Values are means ± SE. Comparisons of the results of biochemical assays and real-time PCR between the CIH and IA groups of mice were performed using unpaired *t-test* (between groups) with Bonferroni's correction. $P < 0.05$ was considered significant. See *Gene microarrays in liver tissue* for statistical analysis of microarrays.

RESULTS

Liver lipid content and serum lipid levels in obese mice exposed to CIH. Weight gain and food intake were identical over the 3 mo of exposure in CIH animals and in animals exposed to IA as a control (Table 1). CIH caused increases ($P < 0.05$) in TG and PL content of the liver, with no change in total cholesterol content of the liver (Fig. 1). CIH induced a trend to an increase in fasting serum cholesterol level (Table 1; $P = 0.08$). Fasting serum TG, PL, and FFA levels were not significantly different between the CIH and control groups (Table 1), but the study was not adequately powered to exclude serum lipid changes.

Table 1. *Body weight, food intake, fasting serum cholesterol, TG, FFA, and PL in ob/ob mice after exposure to IH or IA for 12 wk*

	IA (n = 7)	IH (n = 5)	P
Age, wk	25±1	24±1	0.094
Body weight, g	60.3±1.3	60.0±2.5	0.031
Daily food intake, g	4.6±0.8	4.9±0.3	0.05
Fasting serum cholesterol, mg/dl	141.7±18.0	203.6±25.6	0.615
Fasting serum TG, mg/dl	48.8±3.1	56.5±3.5	0.275
Fasting serum FFA, mmol/l	1.30±0.16	1.38±0.11	0.42
Fasting serum PL, mg/dl	334±38	365±37	0.25

Values are means ± SE. IA and IH, intermittent room air and hypoxia, respectively; TG, triglycerides; FFA, free fatty acids; PL, phospholipids. Statistical significance of the difference between IH and IA was determined by unpaired *t*-test with Bonferroni's correction.

Altered hepatic gene expression in obese mice exposed to CIH. Gene microarrays with total RNA from the livers of *ob/ob* mice exposed to CIH revealed upregulation (>1.2-fold) of 198 genes ($P < 0.05$) and downregulation (<0.8-fold) of 98 genes ($P < 0.05$, unpaired *t*-test). Analysis with SAM software using the same fold changes (>1.2 and <0.8) revealed upregulation of 135 genes and downregulation of 11 genes. The majority of upregulated genes represented metabolic pathways, especially pathways of lipid and carbohydrate metabolism (Fig. 2). Genes of proteolysis and peptidolysis, including a number of hydrolases and proteases, were significantly downregulated. With the use of a more restrictive criterion (>2.0 and <0.5), SAM analyses showed significant upregulation of 11 genes and significant downregulation of 1 gene (Table 2).

Regardless of the statistical approach, the hepatic microarray data consistently demonstrated upregulation of multiple genes controlling lipid biosynthesis (Table 2). There were increases in genes of fatty acid biosynthesis, including the rate-limiting enzyme acetyl-CoA carboxylase (1.3-fold, $P = 0.009$), as well as SCD-1 (1.45-fold, $P = 0.045$), fatty acid desaturase (1.6-fold, $P = 0.04$), and the rate-limiting enzyme of triglyceride and phospholipid biosynthesis mitochondrial GPAT (1.6-fold, $P = 0.035$). ATP citrate lyase, farnesyl diphosphate synthetase, malic enzyme, and SCD-2 were upregulated more than two-fold, which was confirmed by more stringent SAM analysis

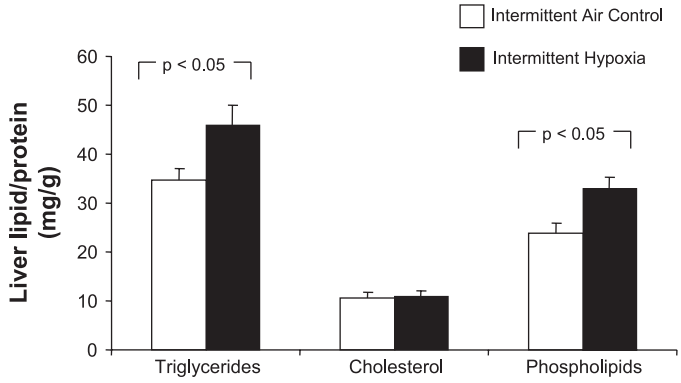

Fig. 1. Liver triglyceride, cholesterol, and phospholipid content normalized to protein concentration in liver extract from *ob/ob* mice exposed to intermittent hypoxia or intermittent room air (control) for 12 wk. Values are means ± SE. Statistical significance of the difference between animals exposed to intermittent hypoxia or intermittent room air was derived by an unpaired *t*-test with Bonferroni's correction.

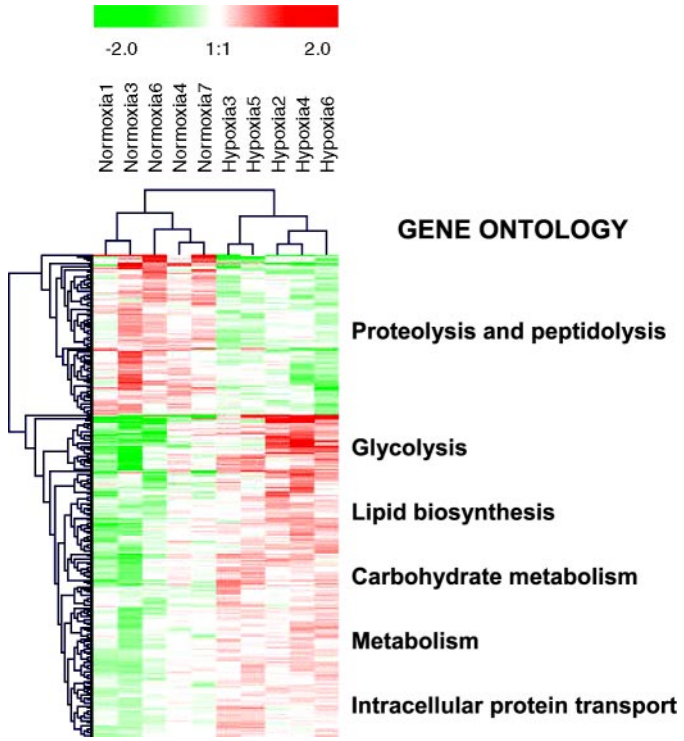

Fig. 2. Hierarchical clustering of gene expression profiles of liver tissue of *ob/ob* mice exposed to intermittent hypoxia or normoxia for 12 wk generated using the Affymetrix 430A 2.0 GeneChip. Normoxic-to-hypoxic fold-change ratio was computed on the basis of mean values of each group of samples, and 20% change in expression was used as cutoff. Genes, expression of which was significantly affected, were clustered using MeV (34). Each column represents an individual mouse and is labeled according to the mouse identification number. Regional hierarchical clustering (*top dendrogram*) correctly grouped experimental conditions into 2 large normoxia and hypoxia clusters. Hierarchical clustering of genes identified 2 major clusters (down- and upregulated by hypoxia). Genes from these clusters were linked to gene ontology terms using MAPPFinder (7), and most representative gene ontologies for each cluster are listed (*right*). Red represents upregulation and green represents downregulation of gene expression in response to hypoxia, with color intensity corresponding to the fold-change amplitude (scale at *top*).

(Table 2). Furthermore, increases in SCD-2 and malic enzyme were confirmed by positive results with two different probes for each gene. The genes of lipid metabolism overexpressed by CIH belonged to pathways regulated by SREBP-1 (Table 2), whereas the genes of the SREBP-2-dependent pathway of cholesterol biosynthesis were unchanged. However, expression of the rate-limiting enzyme of cholesterol biosynthesis HMG-CoA reductase was not examined, because the Affymetrix GeneChip 430A 2.0 array lacked a probe for this gene. Although the gene microarrays did not detect significant increases in any SREBP mRNA levels, there was a trend for CIH to increase SREBP-1 (1.7-fold, $P = 0.06$). Selective upregulation of genes of lipid biosynthesis by CIH was particularly striking, given that numerous other pathways remained unchanged, including apoptosis, amino acid phosphorylation, regulation of transcription, and GTPase-mediated signal transduction.

Verification of upregulated genes of lipid biosynthesis with real-time PCR. Expression data derived from gene microarray analysis for selected genes was verified by real-time PCR (Fig. 3). Specifically, we confirmed and quantified significant increases in the following genes of lipid biosynthesis in response to CIH:

Table 2. *Genes exhibiting a significant change in expression in livers of ob/ob mice exposed to IH for 12 wk*

Probe Set ID No.	GenBank Accession No.	Gene Name	Fold Change	*q*, %
1419528_at	NM_009286.1	Sulfotransferase, hydroxysteroid preferring 2	5.96	8.33
1415822_at	NM_009128.1	SCD-2*	3.98	8.33
1425326_at	AF332052.1	ATP citrate lyase*	3.73	8.33
1420447_at	NM_023135.1	Sulfotransferase family 1E, member 1	3.13	8.33
1423418_at	BI247584	Farnesyl diphosphate synthetase*	2.90	8.33
1451122_at	BC004801.1	Isopentenyl-diphosphate Δ-isomerase	2.34	8.33
1423078_a_at	AK005441.1	Sterol-C4-methyl oxidase-like	2.33	8.33
1430307_a_at	AK006387.1	Malic enzyme, supernatant*	2.14	8.33
1417017_at	NM_007809.1	Cytochrome *P*-450, family 17, subfamily a, polypeptide 1	2.07	8.33
1415823_at	BB459479	SCD- 2*	2.05	8.33
1416632_at	NM_008615.1	Malic enzyme, supernatant*	2.01	8.33
1424352_at	BC025936.1	Similar to cytochrome *P*-450, 4a10	−3.72	8.33

Normoxic-to-hypoxic fold-change ratio was computed on the basis of mean values of each group of samples, >2-fold change in expression was used as the cutoff. Data were analyzed using significance analysis of microarrays software (45). The TM4 Microarray Software Suite, developed by TIGR (www.tigr.org), was used to evaluate and visualize the similarity of gene expression profiles. *q,* False discovery rate. SCD, stearoyl-CoA desaturase. *Transcriptional regulating by sterol regulatory element-binding protein 1.

SCD-2 (15-fold), acetyl-CoA synthetase and carboxylase (5-fold), malic enzyme (4-fold), mitochondrial GPAT (2.3-fold), and SCD-1 (1.8-fold). PCR showed no change in SREBP-2 and HMG-CoA reductase. SREBP-1, which showed a trend for increased RNA expression in the microarrays, increased by threefold in response to CIH exposure according to PCR (Fig. 3).

DISCUSSION

The purpose of this study was to determine whether CIH, a primary physiological disturbance in OSA, exacerbates preexistent hepatic lipid dysregulation in genetically obese *ob/ob* mice. The data demonstrate that exposure to CIH caused a consistent upregulation of hepatic genes important in regulating metabolic processes (Fig. 2), in particular genes of lipid metabolism (Table 2, Fig. 3). Furthermore, SREBP-1, a master regulator of enzymes controlling TG and PL synthesis (8, 13, 14, 23, 24, 35, 36, 42), was upregulated threefold. Consistent with the upregulation of SREBP-1 and key regulatory enzymes such as SCD, acetyl-CoA carboxylase, and mitochondrial GPAT, we demonstrated increased TG and PL levels in the livers of animals exposed to CIH. Thus CIH exposure increased the accumulation of TG and PL in the liver, despite the presence of underlying hepatosteatosis.

Murine obesity is associated with increased SREBP-1 levels in the liver (39). The increase in SREBP-1 mRNA in our study may represent increases in both isoforms of the gene, SREBP-1a and SREBP-1c. Other groups reported that overexpression of truncated active portions of SREBP-1a and SREBP-1c in transgenic mice caused fatty liver, hyperlipidemia, and insulin resistance (15, 40). We have shown that upregulation of SREBP-1 by CIH in obese mice was associated with transcriptional activation of genes of lipid biosynthesis (Table 2, Fig. 3) and progression of hepatic steatosis. Thus our data suggest a novel mechanism by which obesity and CIH may interact to exacerbate fatty liver disease. However, protein and functional studies need to be implemented to confirm that changes induced by CIH at the mRNA level have physiological significance.

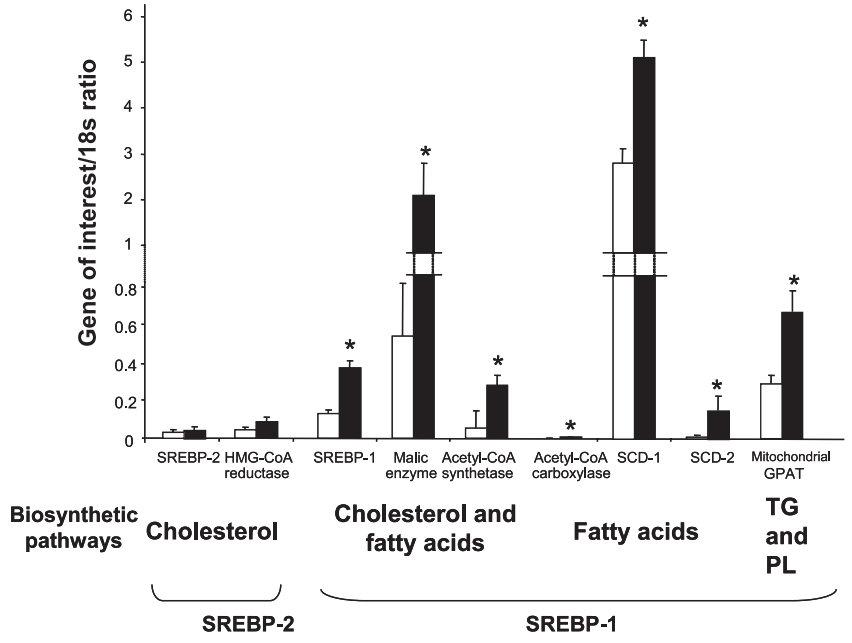

Fig. 3. Expression of genes of lipid metabolism by real-time PCR in livers of *ob/ob* mice after exposure to intermittent hypoxia (solid bars) or intermittent room air (open bars) for 12 wk. SREBP, sterol regulatory element-binding protein; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; SCD, stearoyl-CoA desaturase; GPAT, mitochondrial glycerol-3-phosphate acyltransferase; TG, triglycerides, PL, phospholipids. Values are means ± SE. *Significant difference between animals exposed to intermittent hypoxia and those exposed to intermittent room air ($P < 0.05$, unpaired *t*-test).

Our present study does not determine the upstream pathways that mediate the effects of CIH on SREBP-1, although we can suggest at least two putative mechanisms. One mechanism may involve the effects of CIH on circulating insulin levels. Indeed, CIH has been shown to increase serum insulin levels (30) and insulin to activate SREBP-1 (9). A second potential mechanism may involve a direct impact of hypoxia on SREBP-1. Hypoxia was reported to upregulate an SREBP-1 analog in yeast (16), and a similar effect may occur in mammalian cells.

One of the important genes upregulated by CIH and controlled by SREBP-1 was SCD (Table 2, Fig. 3). SCD introduces one double bond into saturated fatty acetyl-CoA in conjunction with NADPH (28, 29). NADPH is provided by malic enzyme (41), which was also upregulated by exposure to CIH. Monounsaturated fatty acids synthesized by SCD can be used as major substrates for the synthesis of hepatic TG and PL (27, 29). Therefore, overexpression of SCD in response to CIH may account for increases in TG and PL content in the liver, leading to progression of hepatic steatosis.

We did not observe increases in liver cholesterol content or in the major regulatory proteins that control cholesterol production in the liver. For example, neither SREBP-2, the predominant regulator of cholesterol synthetic pathways (13, 21, 36, 37, 40), nor HMG-CoA reductase, a rate-limiting enzyme of cholesterol biosynthesis, was upregulated in response to CIH. A trend to an increase in serum cholesterol is likely related to mechanisms other than biosynthesis; for instance, CIH may decrease cholesterol uptake from the circulation. Finally, serum TG and FFA levels were not elevated in response to CIH, presumably because our studies were conducted in the fasting state, which inhibits serum TG levels, increases serum FFA levels (13, 38), and potentially overrides the metabolic actions of hypoxia.

Several limitations of the study need to be acknowledged. *1*) Mice were exposed to a relatively severe IH stimulus with 5% nadir $F_{I_{O_2}}$. However, we previously demonstrated that C57BL/6J mice exposed to sleep-induced hypoxia can routinely achieve $\leq$5% $F_{I_{O_2}}$ before spontaneous arousal from sleep (33, 43). Thus the use of a constant 5% $F_{I_{O_2}}$ represents a severe, but nevertheless physiological, stimulus. *2*) Exposure to 5% nadir $F_{I_{O_2}}$ is likely to produce arousal if the animals are asleep when the hypoxic stimulus is initiated. Thus it is possible that the metabolic effects of CIH are in part, or entirely, caused by the stress of sleep fragmentation, rather than by hypoxia per se. Future studies specifically utilizing a stimulus of nonhypoxic sleep fragmentation are necessary to determine whether disruption of sleep can independently impact on liver metabolism. *3*) The study was not adequately powered to exclude serum lipid changes, and larger sample sizes are needed to address this issue in future protocols.

In conclusion, we have shown that CIH exacerbates preexisting fatty liver disease in genetically obese mice. Furthermore, our data show that hepatic steatosis resulting from CIH exposure is associated with upregulation of the pathways of lipid biosynthesis in the liver. We speculate that upregulation of hepatic biosynthetic pathways may occur in obese patients with OSA, which would contribute to the progression of insulin resistance in these individuals.

GRANTS

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REFERENCES

1. **Adams LA, Angulo P, and Lindor KD.** Nonalcoholic fatty liver disease. *CMAJ* 172: 899–905, 2005.
2. **Anonymous.** *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report.* Washington DC: Public Health Service, Department of Health and Human Services, 1998.
3. **Anonymous.** Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. *Arch Intern Med* 158: 1855–1867, 1998.
4. **Choudhury J and Sanyal AJ.** Insulin resistance in NASH. *Front Biosci* 10: 1520–1533, 2005.
5. **Dancey DR, Hanly PJ, Soong C, Lee B, Shepard J Jr, and Hoffstein V.** Gender differences in sleep apnea: the role of neck circumference. *Chest* 123: 1544–1550, 2003.
6. **Del Gaudio A, Boschi L, Del Gaudio GA, Mastrangelo L, and Munari D.** Liver damage in obese patients. *Obes Surg* 12: 802–804, 2002.
7. **Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, and Conklin BR.** MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biol* 4: R7, 2003.
8. **Ericsson J, Jackson SM, Kim JB, Spiegelman BM, and Edwards PA.** Identification of glycerol-3-phosphate acyltransferase as an adipocyte determination and differentiation factor 1- and sterol regulatory element-binding protein-responsive gene. *J Biol Chem* 272: 7298–7305, 1997.
9. **Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard C, Le L, X, Berthelier-Lubrano C, Spiegelman B, Kim JB, Ferre P, and Foufelle F.** ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol Cell Biol* 19: 3760–3768, 1999.
10. **Grigoryev DN, Finigan JH, Hassoun P, and Garcia JG.** Searching for gene candidates in acute lung injury. *Crit Care* 8: 440–447, 2004.
11. **Guilleminault C, Stoohs R, Kim YD, Chervin R, Black J, and Clerk A.** Upper airway sleep-disordered breathing in women. *Ann Intern Med* 122: 493–501, 1995.
12. **Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, Patel N, Madan A, Amarapurkar A, and Hafeezunnisa.** Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol* 19: 854–858, 2004.
13. **Horton JD, Bashmakov Y, Shimomura I, and Shimano H.** Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci USA* 95: 5987–5992, 1998.
14. **Horton JD, Goldstein JL, and Brown MS.** SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109: 1125–1131, 2002.
15. **Horton JD, Shimomura I, Ikemoto S, Bashmakov Y, and Hammer RE.** Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. *J Biol Chem* 278: 36652–36660, 2003.
16. **Hughes AL, Todd BL, and Espenshade PJ.** SREBP pathway responds to sterols and functions as an oxygen sensor in fission yeast. *Cell* 120: 831–842, 2005.
17. **Ip MS, Lam B, Ng MM, Lam WK, Tsang KW, and Lam KS.** Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med* 165: 670–676, 2002.
18. **Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, and Speed TP.** Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* 31: e15, 2003.
19. **Irizarry RA, Gautier L, and Cope LM.** An R package for analysis of Affymetrix oligonucleotide arrays. In: *The Analysis of Gene Expression Data: Methods and Software,* edited by Parmigiani RIG, Garret ES, and Ziegler S. Berlin: Springer, 2003, p. 102–119.
20. **Jison ML, Munson PJ, Barb JJ, Suffredini AF, Talwar S, Logun C, Raghavachari N, Beigel JH, Shelhamer JH, Danner RL, and Gladwin MT.** Blood mononuclear cell gene expression profiles characterize the oxidant, hemolytic, and inflammatory stress of sickle cell disease. *Blood* 104: 270–280, 2004.
21. **Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, and Brown MS.** Diminished hepatic response to fasting/refeeding and liver X receptor

agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem* 277: 9520–9528, 2002.
22. **Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, and Diehl AM.** Metformin reverses fatty liver disease in obese, leptin-deficient mice. *Nat Med* 6: 998–1003, 2000.
23. **Magana MM, Lin SS, Dooley KA, and Osborne TF.** Sterol regulation of acetyl coenzyme A carboxylase promoter requires two interdependent binding sites for sterol regulatory element binding proteins. *J Lipid Res* 38: 1630–1638, 1997.
24. **Magana MM and Osborne TF.** Two tandem binding sites for sterol regulatory element binding proteins are required for sterol regulation of fatty-acid synthase promoter. *J Biol Chem* 271: 32689–32694, 1996.
25. **Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, and Rizzetto M.** Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 37: 917–923, 2003.
26. **Millman RP, Carlisle CC, McGarvey ST, Eveloff SE, and Levinson PD.** Body fat distribution and sleep apnea severity in women. *Chest* 107: 362–366, 1995.
27. **Miyazaki M, Kim YC, Gray-Keller MP, Attie AD, and Ntambi JM.** The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. *J Biol Chem* 275: 30132–30138, 2000.
28. **Ntambi JM.** Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *J Lipid Res* 40: 1549–1558, 1999.
29. **Ntambi JM and Miyazaki M.** Regulation of stearoyl-CoA desaturases and role in metabolism. *Prog Lipid Res* 43: 91–104, 2004.
30. **Polotsky VY, Li J, Punjabi NM, Rubin AE, Smith PL, Schwartz AR, and O'Donnell CP.** Intermittent hypoxia increases insulin resistance in genetically obese mice. *J Physiol* 552: 253–264, 2003.
31. **Punjabi NM, Ahmed MM, Polotsky VY, Beamer BA, and O'Donnell CP.** Sleep-disordered breathing, glucose intolerance, and insulin resistance. *Respir Physiol Neurobiol* 136: 167–178, 2003.
32. **Punjabi NM, Sorkin JD, Katzel LI, Goldberg AP, Schwartz AR, and Smith PL.** Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. *Am J Respir Crit Care Med* 165: 677–682, 2002.
33. **Rubin AE, Polotsky VY, Balbir A, Krishnan JA, Schwartz AR, Smith PL, Fitzgerald RS, Tankersley CG, Shirahata M, and O'Donnell CP.** Differences in sleep-induced hypoxia between A/J and DBA/2J mouse strains. *Am J Respir Crit Care Med* 168: 1520–1527, 2003.
34. **Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich E, Borisovsky I, Liu Z, Vinsavich A, Trush V, and Quackenbush J.** TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* 34: 374–378, 2003.
35. **Sheng Z, Otani H, Brown MS, and Goldstein JL.** Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc Natl Acad Sci USA* 92: 935–938, 1995.
36. **Shimano H.** Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res* 40: 439–452, 2001.
37. **Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, and Goldstein JL.** Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest* 98: 1575–1584, 1996.
38. **Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, and Yamada N.** Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J Biol Chem* 274: 35832–35839, 1999.
39. **Shimomura I, Bashmakov Y, and Horton JD.** Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J Biol Chem* 274: 30028–30032, 1999.
40. **Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, and Brown MS.** Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev* 12: 3182–3194, 1998.
41. **Simpson ER and Estabrook RW.** Mitochondrial malic enzyme: the source of reduced nicotinamide adenine dinucleotide phosphate for steroid hydroxylation in bovine adrenal cortex mitochondria. *Arch Biochem Biophys* 129: 384–395, 1969.
42. **Tabor DE, Kim JB, Spiegelman BM, and Edwards PA.** Identification of conserved *cis*-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. *J Biol Chem* 274: 20603–20610, 1999.
43. **Tagaito Y, Polotsky VY, Campen MJ, Wilson JA, Balbir A, Smith PL, Schwartz AR, and O'Donnell CP.** A model of sleep-disordered breathing in the C57BL/6J mouse. *J Appl Physiol* 91: 2758–2766, 2001.
44. **Tanne F, Gagnadoux F, Chazouilleres O, Fleury B, Wendum D, Lasnier E, Lebeau B, Poupon R, and Serfaty L.** Chronic liver injury during obstructive sleep apnea. *Hepatology* 41: 1290–1296, 2005.
45. **Tusher VG, Tibshirani R, and Chu G.** Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98: 5116–5121, 2001.
46. **Vgontzas AN, Papanicolaou DA, Bixler EO, Hopper K, Lotsikas A, Lin HM, Kales A, and Chrousos GP.** Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab* 85: 1151–1158, 2000.
47. **Wanless IR and Lentz JS.** Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 12: 1106–1110, 1990.
48. **Yokode M, Hammer RE, Ishibashi S, Brown MS, and Goldstein JL.** Diet-induced hypercholesterolemia in mice: prevention by overexpression of LDL receptors. *Science* 250: 1273–1275, 1990.