Sixteen hours of fasting differentially affects hepatic and muscle insulin sensitivity in mice

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Abstract **Fasting readily induces hepatic steatosis. Hepatic steatosis is associated with hepatic insulin resistance. The purpose of the present study was to document the effects of 16 h of fasting in wild-type mice on insulin sensitivity in liver and skeletal muscle in relation to *1*) tissue accumulation of triglycerides (TGs) and *2*) changes in mRNA expression of metabolically relevant genes. Sixteen hours of fasting did not show an effect on hepatic insulin sensitivity in terms of glucose production in the presence of increased hepatic TG content. In muscle, however, fasting resulted in increased insulin sensitivity, with increased muscle glucose uptake without changes in muscle TG content. In liver, fasting resulted in increased mRNA expression of genes promoting gluconeogenesis and TG synthesis but in decreased mRNA expression of genes involved in glycogenolysis and fatty acid synthesis. In muscle, increased mRNA expression of genes promoting glucose uptake, as well as lipogenesis and β-oxidation, was found. In conclusion, 16 h of fasting does not induce hepatic insulin resistance, although it causes liver steatosis, whereas muscle insulin sensitivity increases without changes in muscle TG content. Therefore, fasting induces differential changes in tissue-specific insulin sensitivity, and liver and muscle TG contents are unlikely to be involved in these changes.**—Heijboer, A. C., E. Donga, P. J. Voshol, Z-C. Dang, L. M. Havekes, J. A. Romijn, and E. P. M. Corssmit. **Sixteen hours of fasting differentially affects hepatic and muscle insulin sensitivity in mice. *J. Lipid Res.* 2005.** 46: **582–588.**

Supplementary key words glucose metabolism • steatosis • insulin action • transcription factors • isotopes

Fasting increases hepatic triglycerides (TGs) in rodents (1). This fasting-induced hepatic steatosis results from repartitioning of FFAs, released from adipose tissue, to the liver. In the liver, FFAs can either be used for β-oxidation in mitochondria or reesterified into TG. TG can be stored or secreted as VLDL. In turn, TG-rich VLDL particles are lipolyzed by LPL and deliver FFAs to other tissues, such as skeletal muscle (2), where FFAs are used for β-oxidation. If muscle FFA uptake exceeds β-oxidation, excessive TG storage will be the consequence (3).

Evidence is accumulating indicating that accumulation of TG is involved in tissue-specific insulin resistance. For instance, studies in transgenic mice with targeted disturbances in peripheral fatty acid/TG partitioning showed that there is an inverse relationship between hepatic TG stores and hepatic insulin sensitivity (4, 5). In muscle, TG accumulation is also associated with insulin resistance, characterized by a decrease in insulin-stimulated glucose uptake (6). There is a lot of evidence on the action of fatty acid derivatives as agonists and antagonists for nuclear transcription factors, such as peroxisome proliferator-activated receptors (PPARs) and sterol-regulatory element binding proteins (SREBPs) (7, 8). These transcription factors profoundly alter the expression of enzymes/proteins involved in glucose and lipid metabolism (8–13) and have interactions with hormones such as insulin (14, 15). Therefore, these transcription factors could be molecular links between intracellular fatty acid/TG accumulation and insulin resistance. Because hepatic steatosis is associated with hepatic insulin resistance, we postulated that fasting also induces hepatic insulin resistance. The effects of fasting on muscle TG accumulation and insulin sensitivity have not been studied. Therefore, the aim of the present study was to evaluate the effects of 16 h of fasting

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Abbreviations: ACC, acetyl-C7oA carboxylase; BGU, body glucose uptake; BHQ1, BlackHoleQuencher-1; Ct, critical threshold; 2-DG, 2-deoxyglucose; DGAT, diacylglycerol acyltransferase; GAPDH, glyceraldehyde phosphate dehydrogenase; GLUT4, glucose transporter 4; GP, glycogen phosphorylase; G6P, glucose-6-phosphatase; HGP, hepatic glucose production; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator-activated receptor; RT-PCR, real-time polymerase chain reaction; SREBP, sterol-regulatory element binding protein; TG, triglyceride.

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on hepatic and muscle insulin sensitivity in wild-type mice in vivo in relation to *1*) tissue TG accumulation and *2*) changes in mRNA expression of transcription factors and related proteins involved in glucose and lipid metabolism.

MATERIALS AND METHODS

Animals

Male, 12–16 week old C57Bl/6 mice were used in all experiments. Mice were kept in a temperature- and humidity-controlled environment and had free access to standard mouse chow and water. Control mice were fasted for 4 h with food withdrawn at 5:00 AM, and the experimental mice were fasted overnight with food withdrawn at 5:00 PM (16 h fasted). All experiments were performed at 9:00 AM. All animal experiments were approved by the Animal Ethics Committee of the Leiden University Medical Center and Netherlands Organization for Applied Scientific Research (TNO) Prevention and Health (Leiden, The Netherlands).

Hyperinsulinemic euglycemic clamp

Hyperinsulinemic euglycemic clamps of the two experimental groups were performed side by side on the same day. The hyperinsulinemic euglycemic clamp procedure was performed as described previously (4, 16). In short, a continuous infusion of D-[^{14}C]glucose (0.3 μCi/kg/min; Amersham, Little Chalfont, UK) was started and blood samples were taken (after 60 and 80 min of tracer infusion) to determine basal glucose kinetics. Subsequently, a hyperinsulinemic study started with a bolus of insulin (100 mU/kg Actrapid; Novo Nordisk, Chartres, France) followed by continuous infusion of insulin (6.8 mU/h) and of D-[^{14}C]glucose. A variable infusion of 12.5% D-glucose (in PBS) solution was also started and adjusted to maintain blood glucose levels constant at ~8 mmol/l, measured via tail bleeding (Freestyle, TheraSense; Disetronic Medical Systems BV, Vianen, The Netherlands). During the last hour of the experiment, blood samples (75 μl) were taken every 20 min to determine plasma [^{14}C]glucose and insulin concentrations.

To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-D-[^{3}H]glucose (2-[^{3}H]DG; Amersham) was administered as a bolus (1 μCi) 40 min before the end of the clamp procedure.

After the last blood sample was taken, mice were killed and liver and muscle were taken out, immediately frozen using liquid N_2, and stored at −20°C until further analysis.

Analytical procedures

Plasma levels of ketone bodies, glucose, and free fatty acids were determined using commercially available kits (#310-A Sigma GPO-Trinder kit and #315-500; Sigma, St. Louis, MO; FFA; Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin concentrations were measured by radio immunoassay using rat insulin standards (Sensitive Rat Insulin Assay; Linco Research, St. Charles, MO). For determination of plasma D-[^{14}C]glucose, plasma was deproteinized with 20% trichloroacetic acid, dried to remove water, resuspended in demiwater, and counted with scintillation fluid (Ultima Gold; Packard, Meridien, CT) on dual channels for separation of ^{14}C and ^{3}H, as described earlier (17).

Tissue analysis

Liver and muscle samples were homogenized (~10%, w/v) in water. Lipids were extracted according to Bligh and Dyer's method (18). In short, a solution was made of each 200 μg sample of protein in 800 μl of water, then 3 ml of methanol-chloroform (2:1) was added and mixed thoroughly, after which 500 μl of chloroform, 100 μl of internal standard, and 1 ml of demiwater were added. After centrifugation, the chloroform layer was collected and dried. The remaining pellet was dissolved in 50 μl of chloroform and put on a high-performance TLC plate. With high-performance TLC analysis, TGs, cholesterol, and cholesteryl esters were separated. The amount of TG in the tissues was quantified by scanning the plates with a Hewlett-Packard Scanjet 4c and by integration of the density using Tina® version 2.09 software (Raytest, Staubenhardt, Germany).

For determination of tissue 2-DG uptake, the homogenate of muscle was boiled and the supernatant was subjected to an ion-exchange column to separate 2-DG-6-phosphatase from 2-DG, as described previously (16, 17, 19).

Calculations

Under steady-state conditions for plasma glucose concentrations, the rate of glucose disappearance equals the rate of glucose appearance [body glucose uptake (BGU)]. The latter was calculated as the ratio of the rate of infusion of D-[^{14}C]glucose (dpm/min) and the steady-state plasma [^{14}C]glucose specific activity (dpm/μmol glucose). Hepatic glucose production (HGP) was calculated as the difference between the rate of glucose disappearance and the infusion rate of exogenous D-glucose.

The hepatic insulin sensitivity index was calculated as the ratio of the relative suppression of HGP during the hyperinsulinemic condition to the change in plasma insulin levels from basal to hyperinsulinemic conditions. The whole body insulin sensitivity index was calculated as the ratio of BGU to plasma insulin levels during hyperinsulinemic conditions.

Muscle-specific glucose uptake was calculated from tissue 2-DG content, which was expressed as percentage of 2-DG of the dosage per gram of tissue, as previously described (19).

Real-time polymerase chain reaction

Real-time polymerase chain reaction (RT-PCR) was used to measure mRNA expression levels in skeletal muscle [glucose transporter 4 (GLUT4), PPARγ coactivator-1 (PGC1), PPARγ, diacylglycerol acyltransferase 1 (DGAT1), DGAT2, SREBP1c, FAS, acetyl-CoA carboxylase (ACC)1, and PPARα] and in liver [phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6P), glycogen phosphorylase (GP), PGC1, PPARγ, SREBP1c, FAS, ACC1, PPARα, DGAT1, and DGAT2] of mice after 4 and 16 h of starvation. Two other groups of mice, which were not subjected to a hyperinsulinemic clamp, were killed after 4 or 16 h of fasting, and liver and skeletal muscle were taken out for further analysis.

Muscle and liver were homogenized in 1.2 ml of RNA-Bee (Tel-Test, Inc.), and total RNA was extracted according to Chomczynski and Sacchi (20). The amount of RNA was determined by spectrophotometry at a wavelength of 260 nm. The quality was checked by the ratio of absorption at 260 nm and absorption at 280 nm. cDNA was obtained from total RNA.

For RT-PCR, forward and reverse primers and TaqMan probe (**Table 1**) were designed from mouse-specific sequence data (Entrez, National Institutes of Health; and Ensembl, Sanger Institute) using computer software (Primer Express; Applied Biosystems). For each of the genes, a Basic Local Alignment Search Tool search was done to reveal that sequence homology was obtained only for the target gene.

All TaqMan probes were 5′-6-carboxyfluorescein and 3′-BlackHoleQuencher-1 (BHQ1) labeled, except for glyceraldehyde phosphate dehydrogenase (GAPDH) (5′-VIC and 3′-BHQ1; Applied Biosystems) and cyclophiline (5′-TET and 3′-BHQ1).

Each oligonucleotide set was optimized to determine the optimal primer concentrations and probe concentration and verify the efficiency of the amplification.

TABLE 1. Primer and probe sequences of genes used for mRNA quantification

Gene	Forward Primer	Reverse Primer	TaqMan Probe
SREBP1c	5′-GGAGCCATGGATTGCACATT-3′	5′-CCTGTCTCACCCCCAGCATA-3′	5′-CAGCTCATCAACAACCAAGACAGTGACTTCC-3′
FAS	5′-GGCATCATTGGGCACTCCTT-3′	5′-GCTGCAAGCACAGCCTCTCT-3′	5′-CCATCTGCATAGCCACAGGCAACCTC-3′
ACC1	5′-GCCATTGGTATTGGGGCTTAC-3′	5′-CCCGACCAAGGACTTTGTTG-3′	5′-CTCAACCTGGATGGTTCTTTGTCCCAGC-3′
DGAT1	5′-CTGGGCATTCACAGCCATG-3′	5′-TTCCCTTGGAAGAATCGGC-3′	5′-CTCAGGTCCCACTGGCCTGGATTGT-3′
DGAT2	5′-TGACTGGAACACGCCCAA-3′	5′-ACGGCCCAGTTTCGCA-3′	5′-CCACTGCGATCTCCTGCCACCTTT-3′
PPARα	5′-CCTCAGGGTACCACTACGGAGT-3′	5′-GCCGAATAGTTCGCCGAAA-3′	5′-AAGCCCTTACAGCCTTCACATGCGTG-3′
PPARγ	5′-TACATAAAGTCCTTCCCGCTGAC-3′	5′-GTGATTTGTCCGTTGTCTTTCCT-3′	5′-CAAGATCGCCCTCGCCTTGGCTT-3′
PGC1	5′-TTTTTGGTGAAATTGAGGAATGC-3′	5′-CGGTAGGTGATGAAACCATAGCT-3′	5′-GTCTCCATCATCCCGCAGATTTACGG-3′
GLUT4	5′-ACCTGTAACTTCATTGTCGGCAT-3′	5′-ACGGCAAATAGAAGGAAGACGTA-3′	5′-GGACCCATAGCATCCGCAACATACTGG-3′
PEPCK	5′-CCATGAGATCTGAGGCCACA-3′	5′-GTATTTGCCGAAGTTGTAGCCG-3′	5′-CAAGGGCAAGATCATCATGCACGACC-3′
G6P	5′-CAGGTCGTGGCTGGAGTCTT-3′	5′-GACAATACTTCCGGAGGCTGG-3′	5′-TGAAAGTTTCAGCCACAGCAATGCCTG-3′
GP	5′-GCGGTGAACGGTGTAGCAA-3′	5′-CTTGTCTGGTTCTAGCTCGCTG-3′	5′-CCACTCGGACATCGTGAAGACCCAAGTA-3′
GAPDH™/ Cyclophiline	5′-CAAATGCTGGACCAAACACAA-3′	5′-GCCATCCAGCCATTCAGTCT-3′	5′-CCGGTTCCCAGTTTTTTATCTGCACTGCC-3′

ACC, acetyl-CoA carboxylase; DGAT, diacylglycerol acyltransferase 1; GLUT4, glucose transporter 4; G6P, glucose-6-phosphatase; GP, glycogen phosphorylase; PEPCK, phosphoenolpyruvate carboxykinase; PGC1, peroxisome proliferator-activated receptor γ coactivator-1; PPAR, peroxisome proliferator-activated receptor; SREBP1c, sterol-regulatory element binding protein 1c.

PCR amplification was performed in a total reaction volume of 12.5 μl. The reaction mixture consisted of qPCR™ MasterMix (Eurogentec), the optimal primer and probe concentrations of the target gene and the endogenous control, nuclease-free water, and cDNA. An identical cycle profile was used for all genes: 50°C for 2 min, 95°C for 10 min, followed by 95°C for 15 s and 60°C for 1 min for 40 cycles.

Data were analyzed using a comparative critical threshold (Ct) method in which the amount of target normalized to the amount of endogenous control (GAPDH/cyclophiline) and relative to the control sample is given by $2^{-\Delta\Delta Ct}$ (Applied Biosystems). For each gene, all samples were run together, allowing relative comparisons of the samples of a given gene.

Statistics

The data are presented as means ± SD. The data were analyzed using a nonparametric Mann-Whitney *U* test for independent samples. Differences were considered statistically significant at $P \leq 0.05$.

RESULTS

Body weight and plasma parameters

Body weight and basal and hyperinsulinemic plasma concentrations are shown in **Table 2**. Body weight was significantly lower in 16 h fasted mice compared with control mice ($P < 0.05$). Plasma insulin and FFA concentrations were not significantly different between the groups, whereas basal plasma glucose concentrations were lower and plasma ketone bodies were higher in 16 h fasted mice ($P < 0.01$). During the hyperinsulinemic euglycemic clamp procedure, there were no differences in plasma glucose and FFA concentrations between the two groups, whereas insulin concentrations were lower in the 16 h fasted animals ($P < 0.01$).

HGP

Basal HGP was not significantly different between the 16 h fasted mice and the control mice (38 ± 7 versus 43 ± 9 μmol/kg/min, respectively). Liver insulin sensitivity index also was not significantly different between 16 h fasted and control mice (38 ± 29 versus 25 ± 11; NS), as seen in **Fig. 1**.

Glucose uptake

Basal BGU was not significantly different between the 16 h fasted mice and the control mice (38 ± 7 versus 43 ± 9 μmol/kg/min, respectively). Interestingly, whole body insulin sensitivity index was higher in 16 h fasted compared with control mice (45 ± 21 versus 15 ± 4; $P < 0.01$), reflecting increased whole body insulin sensitivity after 16 h of fasting (**Fig. 2**).

Muscle-specific glucose uptake was significantly higher under hyperinsulinemic conditions in 16 h fasted compared with control mice (4.0 ± 2.6% versus 1.3 ± 0.2% glucose uptake/g tissue; $P < 0.01$) (**Fig. 3**).

TABLE 2. Body weight and plasma glucose, insulin, FFA, and ketone body concentrations in 4 h fasted (control) and 16 h fasted mice

Group	Body Weight	Glucose		Insulin		FFA		Ketone Bodies
		Basal	Hyperinsulinemia	Basal	Hyperinsulinemia	Basal	Hyperinsulinemia	
	g	*mmol/l*		*ng/ml*		*mmol/l*		
4 h fasted(control)	27 ± 2	5.9 ± 0.7	8.7 ± 1.2	0.8 ± 0.5	4.0 ± 1.0	1.0 ± 0.2	0.5 ± 0.2	0.50 ± 0.12
16 h fasted	24 ± 2[a]	4.9 ± 0.9[a]	7.6 ± 1.4	0.5 ± 0.4	2.3 ± 1.3[b]	1.2 ± 0.5	0.5 ± 0.3	1.21 ± 0.25[b]

Plasma glucose, insulin, and FFA levels were measured during the hyperinsulinemic euglycemic clamp procedure in basal as well as hyperinsulinemic conditions. Body weight was measured just before the hyperinsulinemic euglycemic clamp procedure. Values represent means ± SD of at least nine mice per group. Ketone bodies were measured in basal conditions (mean ± SD of seven mice per group).

[a] $P < 0.05$ compared with control mice.

[b] $P < 0.01$ compared with control mice.

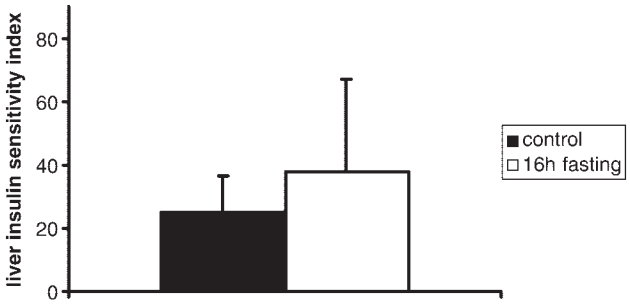

Fig. 1. Liver insulin sensitivity index in 16 h fasted and control mice. Data are means ± SD for at least nine animals per group.

Tissue lipid levels

Hepatic TG content was 6-fold higher in 16 h fasted mice compared with control mice (71 ± 19 versus 12 ± 7 μg/mg protein; $P < 0.01$), whereas muscle TG content did not differ between the two groups (25 ± 7 versus 28 ± 13 μg/mg protein; NS) (**Fig. 4**).

mRNA expression levels

Hepatic mRNA expression levels of transcription factors and related proteins involved in gluconeogenesis and in TG synthesis increased during 16 h of fasting, whereas mRNA expression levels of transcription factors and related proteins involved in glycogenolysis and fatty acid synthesis decreased. The expression levels of G6P and PPARα mRNA were not significantly different (**Table 3**).

Muscle mRNA expression levels of transcription factors and related proteins involved in glucose uptake, fatty acid synthesis, TG synthesis, and β-oxidation increased during 16 h of fasting, whereas SREBP1c (which has a role as a sensor of nutritional status) decreased (**Table 4**).

DISCUSSION

This study indicates that fasting does not result in changes in hepatic insulin sensitivity with regard to HGP in vivo. However, fasting increases muscle insulin sensitivity in vivo, reflected by an increased ability of insulin to stimulate muscle glucose uptake. In liver, the increased TG accumulation was not associated with changes in insulin sensitivity. Moreover, the increase in muscle insulin sensitivity occurred without changes in muscle TG content. Therefore, changes in liver and muscle TG content are unlikely to be involved in changes in insulin sensitivity during conditions of fasting. Studies in transgenic mice with targeted disturbances in peripheral fatty acid/TG distribution showed that there appears to be an inverse dose-effect relationship between hepatic TG stores and hepatic insulin sensitivity (4, 5). However, it does not seem possible to expand this theory to cases of fasting and fasting-induced hepatic steatosis.

The increase in muscle insulin sensitivity during fasting is a new and interesting finding. Previous studies showed an inverse relationship between intramuscular TG content and insulin action (21, 22). However, because we observed an increase in muscle insulin sensitivity without changes in muscle TG content, it is unlikely that changes in muscle TG play a role in the increased muscle-specific insulin sensitivity during fasting.

During the hyperinsulinemic period of the clamp procedure, we observed significantly lower insulin concentrations in 16 h fasted mice compared with control mice. As both groups received the same amount of insulin during hyperinsulinemic conditions by infusion, this difference suggests an increased insulin clearance during fasting. Be-

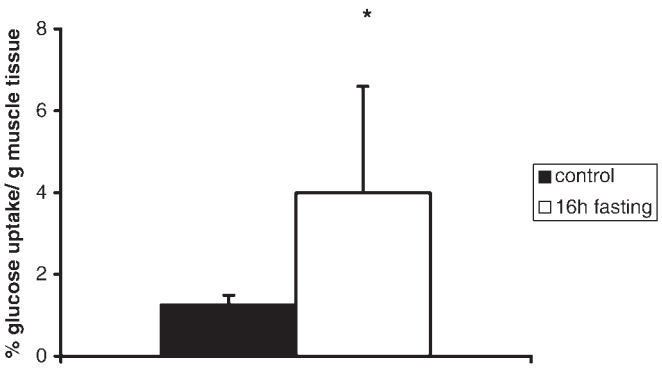

Fig. 3. Muscle-specific glucose uptake under hyperinsulinemic conditions in 16 h fasted and control mice. Data are means ± SD for at least eight animals per group. * $P < 0.01$ versus control mice.

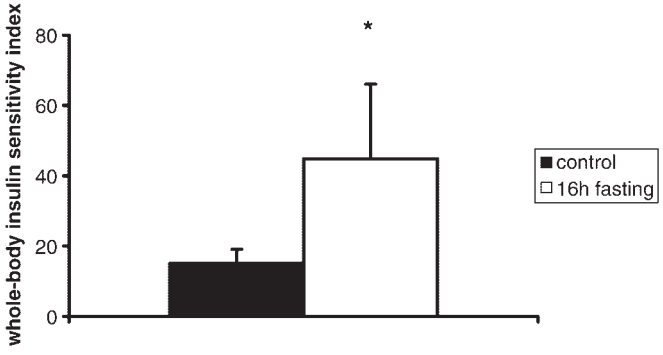

Fig. 2. Whole body insulin sensitivity index in 16 h fasted and control mice. Data are means ± SD for at least nine animals per group. * $P < 0.01$ versus control mice.

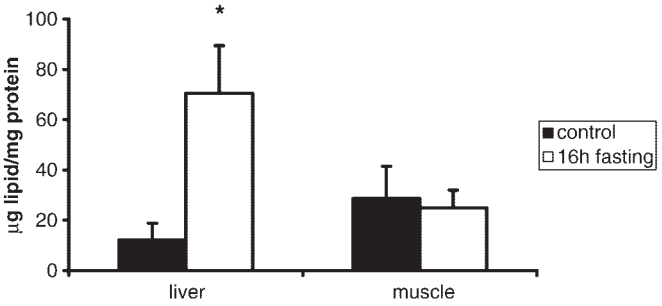

Fig. 4. Triglyceride content determined in liver and skeletal muscle of 16 h fasted and control mice. Data are means ± SD for at least six animals per group. * $P < 0.01$ versus control mice.

TABLE 3. mRNA expression levels of different proteins in liver of control (n = 4) and 16 h fasted (n = 4) mice

mRNA Source	Control	16 h Fasted
	%	
Glucose production		
G6P	100 ± 7	138 ± 23
PEPCK	100 ± 6	184 ± 9[a]
GP	100 ± 6	59 ± 1[a]
PGC1	100 ± 10	380 ± 32[a]
Fatty acid synthesis		
SREBP1c	100 ± 12	34 ± 1[a]
FAS	100 ± 11	12 ± 1[a]
ACC1	100 ± 14	21 ± 1[a]
TG synthesis		
PPARγ	100 ± 9	179 ± 4[a]
DGAT1	100 ± 1	269 ± 6[a]
DGAT2	100 ± 7	113 ± 4[b]
β-Oxidation		
PPARα	100 ± 15	96 ± 4

ACC, acetyl-CoA carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; TG, triglyceride. Values are expressed as means ± SD.
[a] $P < 0.01$ compared with control mice.
[b] $P < 0.05$ compared with control mice.

cause of this difference, we corrected the data on insulin sensitivity for the insulin concentrations.

Another purpose of the present study was to relate the observed in vivo metabolic changes to changes in the transcriptional regulation of genes involved in glucose metabolism, lipogenesis, and β-oxidation in liver and muscle. In the liver, there were changes in the expression of regulatory transcription factors favoring gluconeogenesis, β-oxidation, and TG synthesis, with negative effects on glycogenolysis and fatty acid synthesis. Total HGP is the sum of glycogenolysis and gluconeogenesis. Liver glycogen stores are limited; consequently, during starvation, the relative contribution of gluconeogenesis to total glucose production increases, whereas that of glycogenolysis decreases (23). PGC1 promotes gluconeogenesis by stimulation of PEPCK (24, 25). Our results document a significant increase in the expression of PGC1 and PEPCK. GP is an enzyme involved in glycogenolysis, and its expression is decreased. G6P is involved in glucose production, with sources from both gluconeogenesis and glycogenolysis. The expression of G6P is not significantly altered by fasting, which seems to reflect the absence of changes in HGP in 16 h fasted compared with control mice.

TABLE 4. mRNA expression levels of different proteins in skeletal muscle of control (n = 4) and 16 h fasted (n = 4) mice

mRNA Source	Control	16 h Fasted
	%	
Glucose uptake		
GLUT4	100 ± 2	157 ± 12[a]
PGC1	100 ± 17	166 ± 36[b]
Nutritional status		
SREBP1c	100 ± 8	3 ± 0[a]
Fatty acid synthesis		
FAS	100 ± 10	123 ± 8[b]
ACC1	100 ± 26	194 ± 35[a]
TG synthesis		
PPARγ	100 ± 1	364 ± 12[a]
DGAT1	100 ± 12	193 ± 15[a]
DGAT2	100 ± 4	270 ± 20[a]
β-Oxidation		
PPARα	100 ± 7	278 ± 48[a]

ACC, acetyl-CoA carboxylase. Values are expressed as means ± SD.
[a] $P < 0.01$ compared with control mice.
[b] $P < 0.05$ compared with control mice.

Our study showed a reduced expression level of SREBP1c. This decrease in SREBP1c mRNA during fasting is in accordance with findings of others in mouse liver (26). Hepatic expression levels of enzymes involved in fatty acid synthesis (FAS and ACC1) decreased during fasting. The expression of these enzymes is stimulated by SREBP1c (27). Although this was not reported in mice before, these findings are in accordance with those of Hillgartner, Charron, and Chesnut (28) in fasted chickens. Because a decrease in the expression of SREBP1c, FAS, and ACC1 during 16 h of fasting results in decreased suppression of gluconeogenesis and reduced fatty acid synthesis, leaving pyruvate as a substrate for gluconeogenesis, these changes seem to be of physiological importance in the adaptive response of glucose and lipid metabolism to fasting.

The observed increase in liver TG accumulation in our study is in agreement with the observed increase of hepatic mRNA expression levels of PPARγ and of DGAT1 and DGAT2, all three favoring hepatic TG synthesis (29). The increase in hepatic PPARγ is in agreement with the findings of others (26, 29, 30).

PPARα, which is known as the "fasting gene," controls the expression of numerous genes related to lipid metabolism in the liver, including genes involved in β-oxidation, fatty acid uptake, and transport. Therefore, it is surprising that PPARα mRNA was not increased after 16 h of fasting, whereas this was found by others in wild-type mice (9). Because these mice had another background (SV129), and others found a decrease in PPARα mRNA after 48 and 72 h of fasting in wild-type mice (1) with the same background as our mice (C57Bl6), it seems likely that these differences in background explain this discrepancy.

In muscle, RT-PCR results demonstrate the fasting-induced expression of genes and enzymes favoring glucose uptake. mRNA expression levels of PGC1 and GLUT4 were increased after 16 h of fasting. PGC1 can control the level of endogenous GLUT4 gene expression in multinucleate myotubes via coactivating MEF2C (31). Moreover, Hammarstedt et al. (32) showed a high correlation ($r = 0.91$) between GLUT4 mRNA and PGC1 mRNA in human skeletal muscle. Therefore, our findings support their hypothesis that PGC1 is associated with increased GLUT4 expression and insulin sensitivity.

The observed decrease in muscle SREBP1c mRNA in our study is in agreement with recently published results (33). These studies found a decrease in SREBP1c mRNA in different rat muscle types that was related to the duration of fasting and consistent with a role for SREBP1c as a sensor of nutritional status in skeletal muscle. Although SREBP1c stimulates the expression of genes involved in lipid metabolism (such as FAS and ACC1) in tissues like the liver, not much is known about the regulatory role of SREBP1c in skeletal muscle. Because our study shows an

increase in FAS and ACC1 mRNA during 16 h of fasting but decreased expression of SREBP1c, our data imply that muscle FAS and ACC1 are not stimulated by SREBP1c.

The mRNA expression levels of PPARγ, another lipogenesis-promoting transcription factor in skeletal muscle, strongly increased during 16 h of fasting. This contrasts with a previous study (34), which found no alterations of PPARγ mRNA expression level during fasting in rat skeletal muscle. However, these rats were fasted for 46 h, whereas our mice were fasted for only 16 h. Because mRNA expression levels of DGAT1 and DGAT2, which are involved in muscle TG synthesis (35), were significantly increased after fasting in the absence of muscle TG accumulation in this period, it can be speculated that prolonged fasting will be accompanied by muscle TG accumulation.

In the present study, PPARα mRNA, which is involved in β-oxidation, was increased after fasting. This is in agreement with a recent finding (36) showing increased muscle glucose uptake in mice treated with a PPARα agonist (WY14,643).

In conclusion, 16 h of fasting in wild-type mice results in hepatic steatosis without changes in hepatic insulin sensitivity. In muscle, however, 16 h of fasting increased insulin sensitivity without changes in muscle TG content. Therefore, fasting induces differential changes in tissue-specific insulin sensitivity. In addition, changes in liver and muscle TG content are unlikely to be involved in changes in insulin sensitivity during fasting.

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REFERENCES

1. Hashimoto, T., W. S. Cook, C. Qi, A. V. Yeldandi, J. K. Reddy, and M. S. Rao. 2000. Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. *J. Biol. Chem.* **275:** 28918–28928.
2. Teusink, B., P. J. Voshol, V. E. Dahlmans, P. C. Rensen, H. Pijl, J. A. Romijn, and L. M. Havekes. 2003. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. *Diabetes.* **52:** 614–620.
3. Lewis, G. F., A. Carpentier, K. Adeli, and A. Giacca. 2002. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr. Rev.* **23:** 201–229.
4. Voshol, P. J., G. Haemmerle, D. M. Ouwens, R. Zimmermann, R. Zechner, B. Teusink, J. A. Maassen, L. M. Havekes, and J. A. Romijn. 2003. Increased hepatic insulin sensitivity together with decreased hepatic triglyceride stores in hormone-sensitive lipase-deficient mice. *Endocrinology.* **144:** 3456–3462.
5. Reitman, M. L. 2002. Metabolic lessons from genetically lean mice. *Annu. Rev. Nutr.* **22:** 459–482.
6. Cooney, G. J., A. L. Thompson, S. M. Furler, J. Ye, and E. W. Kraegen. 2002. Muscle long-chain acyl CoA esters and insulin resistance. *Ann. NY Acad. Sci.* **967:** 196–207.
7. Yokoyama, C., X. Wang, M. R. Briggs, A. Admon, J. Wu, X. Hua, J. L. Goldstein, and M. S. Brown. 1993. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell.* **75:** 187–197.
8. Hertz, R., J. Magenheim, I. Berman, and J. Bar-Tana. 1998. Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4alpha. *Nature.* **392:** 512–516.
9. Kersten, S., J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli. 1999. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J. Clin. Invest.* **103:** 1489–1498.
10. Fruchart, J. C., P. Duriez, and B. Staels. 1999. Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. *Curr. Opin. Lipidol.* **10:** 245–257.
11. Guerre-Millo, M., P. Gervois, E. Raspe, L. Madsen, P. Poulain, B. Derudas, J. M. Herbert, D. A. Winegar, T. M. Willson, J. C. Fruchart, R. K. Berge, and B. Staels. 2000. Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J. Biol. Chem.* **275:** 16638–16642.
12. Martin, G., K. Schoonjans, B. Staels, and J. Auwerx. 1998. PPAR-gamma activators improve glucose homeostasis by stimulating fatty acid uptake in the adipocytes. *Atherosclerosis.* **137 (Suppl.):** 75–80.
13. Desvergne, B., and W. Wahli. 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20:** 649–688.
14. Shulman, G. I. 2000. Cellular mechanisms of insulin resistance. *J. Clin. Invest.* **106:** 171–176.
15. Chakravarty, K., P. Leahy, D. Becard, P. Hakimi, M. Foretz, P. Ferre, F. Foufelle, and R. W. Hanson. 2001. Sterol regulatory element-binding protein-1c mimics the negative effect of insulin on phosphoenolpyruvate carboxykinase (GTP) gene transcription. *J. Biol. Chem.* **276:** 34816–34823.
16. Voshol, P. J., M. C. Jong, V. E. Dahlmans, D. Kratky, S. Levak-Frank, R. Zechner, J. A. Romijn, and L. M. Havekes. 2001. In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. *Diabetes.* **50:** 2585–2590.
17. Goudriaan, J. R., V. E. Dahlmans, B. Teusink, D. M. Ouwens, M. Febbraio, J. A. Maassen, J. A. Romijn, L. M. Havekes, and P. J. Voshol. 2003. CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J. Lipid Res.* **44:** 2270–2277.
18. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Biophys.* **37:** 911–917.
19. Rossetti, L., and A. Giaccari. 1990. Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. *J. Clin. Invest.* **85:** 1785–1792.
20. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156–159.
21. Pan, D. A., S. Lillioja, A. D. Kriketos, M. R. Milner, L. A. Baur, C. Bogardus, A. B. Jenkins, and L. H. Storlien. 1997. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes.* **46:** 983–988.
22. Kraegen, E. W., P. W. Clark, A. B. Jenkins, E. A. Daley, D. J. Chisholm, and L. H. Storlien. 1991. Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes.* **40:** 1397–1403.
23. Corssmit, E. P., J. A. Romijn, and H. P. Sauerwein. 2001. Regulation of glucose production with special attention to nonclassical regulatory mechanisms: a review. *Metabolism.* **50:** 742–755.
24. Rhee, J., Y. Inoue, J. C. Yoon, P. Puigserver, M. Fan, F. J. Gonzalez, and B. M. Spiegelman. 2003. Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. *Proc. Natl. Acad. Sci. USA.* **100:** 4012–4017.
25. Yoon, J. C., P. Puigserver, G. Chen, J. Donovan, Z. Wu, J. Rhee, G. Adelmant, J. Stafford, C. R. Kahn, D. K. Granner, C. B. Newgard, and B. M. Spiegelman. 2001. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature.* **413:** 131–138.
26. Horton, J. D., Y. Bashmakov, I. Shimomura, and H. Shimano. 1998. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc. Natl. Acad. Sci. USA.* **95:** 5987–5992.
27. Shimano, H., J. D. Horton, I. Shimomura, R. E. Hammer, M. S. Brown, and J. L. Goldstein. 1997. Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J. Clin. Invest.* **99:** 846–854.
28. Hillgartner, F. B., T. Charron, and K. A. Chesnut. 1996. Alterations in nutritional status regulate acetyl-CoA carboxylase expression in avian liver by a transcriptional mechanism. *Biochem. J.* **319:** 263–268.
29. Gavrilova, O., M. Haluzik, K. Matsusue, J. J. Cutson, L. Johnson, K. R. Dietz, C. J. Nicol, C. Vinson, F. J. Gonzalez, and M. L. Reitman.

2003. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J. Biol. Chem.* **278:** 34268–34276.
30. Matsusue, K., M. Haluzik, G. Lambert, S. H. Yim, O. Gavrilova, J. M. Ward, B. Brewer, Jr., M. L. Reitman, and F. J. Gonzalez. 2003. Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *J. Clin. Invest.* **111:** 737–747.
31. Michael, L. F., Z. Wu, R. B. Cheatham, P. Puigserver, G. Adelmant, J. J. Lehman, D. P. Kelly, and B. M. Spiegelman. 2001. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. *Proc. Natl. Acad. Sci. USA.* **98:** 3820–3825.
32. Hammarstedt, A., P. A. Jansson, C. Wesslau, X. Yang, and U. Smith. 2003. Reduced expression of PGC-1 and insulin-signaling molecules in adipose tissue is associated with insulin resistance. *Biochem. Biophys. Res. Commun.* **301:** 578–582.
33. Bizeau, M. E., P. S. MacLean, G. C. Johnson, and Y. Wei. 2003. Skeletal muscle sterol regulatory element binding protein-1c decreases with food deprivation and increases with feeding in rats. *J. Nutr.* **133:** 1787–1792.
34. Samec, S., J. Seydoux, A. P. Russell, J. P. Montani, and A. G. Dulloo. 2002. Skeletal muscle heterogeneity in fasting-induced upregulation of genes encoding UCP2, UCP3, PPARgamma and key enzymes of lipid oxidation. *Pflugers Arch.* **445:** 80–86.
35. Cases, S., S. J. Smith, Y. W. Zheng, H. M. Myers, S. R. Lear, E. Sande, S. Novak, C. Collins, C. B. Welch, A. J. Lusis, S. K. Erickson, and R. V. Farese, Jr. 1998. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc. Natl. Acad. Sci. USA.* **95:** 13018–13023.
36. Kim, H., M. Haluzik, Z. Asghar, D. Yau, J. W. Joseph, A. M. Fernandez, M. L. Reitman, S. Yakar, B. Stannard, L. Heron-Milhavet, M. B. Wheeler, and D. LeRoith. 2003. Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes.* **52:** 1770–1778.