Uncoupling protein-2 modulates the lipid metabolic response to fasting in mice

Anthony R. Sheets,1 Péter Fülöp,1,2 Zoltán Derdák,1 Andrea Kassai,1,2 Edmond Sabo,3 Nicholas M. Mark,1 György Paragh,2 Jack R. Wands,1 and György Baffy1

1Division of Gastroenterology and Liver Research Center, 2Department of Pathology, Brown Medical School and Rhode Island Hospital, Providence, Rhode Island; and 3First Department of Medicine, University of Debrecen Medical and Health Science Center, Debrecen, Hungary

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Sheets AR, Fülöp P, Derdák Z, Kassai A, Sabo E, Mark NM, Paragh G, Wands JR, Baffy G. Uncoupling protein-2 modulates the lipid metabolic response to fasting in mice. Am J Physiol Gastrointest Liver Physiol 294: G1017–G1024, 2008. First published February 21, 2008; doi:10.1152/ajpgi.00016.2008.—Uncoupling protein-2 (UCP2) regulates insulin secretion by controlling ATP levels in β-cells. Although UCP2 deficiency improves glycemic control in mice, increased expression of UCP2 interferes with glucose-stimulated insulin secretion. These observations link UCP2 to β-cell dysfunction in type 2 diabetes with a perplexing evolutionary role. We found higher residual serum insulin levels and blunted lipid metabolic responses in fasted ucp2−/− mice, supporting the concept that UCP2 evolved to suppress insulin effects and to accommodate the fuel switch to fatty acids during starvation. In the absence of UCP2, fasting initially promotes peripheral lipolysis and hepatic fat accumulation at less than expected rates but culminates in protracted steatosis, indicating diminished hepatic utilization and clearance of fatty acids. We conclude that UCP2-mediated control of insulin secretion is a physiologically relevant mechanism of the metabolic response to fasting.

prolonged fasting; lipolysis; steatosis; insulin secretion

UNCOUPLING PROTEIN (UCP) 2, a member of the anion carrier protein superfamily, is a widely distributed constituent of the mitochondrial inner membrane (6, 35). Although UCP2 was identified over 10 years ago by molecular cloning, its biological role and evolutionary aspects remain debated (6, 29, 35, 40). Based on its significant homology with the brown adipose tissue-specific UCP1 (thermogenin), UCP2 was predicted to regulate energy balance. Subsequent observations did not confirm this prediction, and UCP2 appears to have a complex function yet to be fully explored (6, 29, 35, 40). Similar to other uncoupling proteins, UCP2 lowers the mitochondrial membrane potential by mediating proton leak across the inner membrane (6). As a result, UCP2 has the ability to interfere with the function of F1F0 ATP synthase and to alter the matrix ATP-to-ADP ratio (6).

Soon after its discovery, the importance of UCP2 was recognized in pancreatic β-cells, where UCP2 acts as a negative regulator of glucose-stimulated insulin secretion (GSIS) (30, 51). Pancreatic β-cells sense blood glucose through its metabolism that results in increased levels of intracellular ATP and triggers GSIS to maintain glucose homeostasis (41). Because of more efficient oxidative phosphorylation and increased rates of mitochondrial ATP synthesis, UCP2 deficiency is associated with enhanced β-cell insulin secretory capacity in ucp2−/− mice and allows the partial correction of diabetes in leptin-deficient ob/ob mice (51). In contrast, overexpression of UCP2 in β-cells reduces intracellular ATP levels, leading to diminished GSIS (21). Because increased UCP2 expression of β-cells is associated with obesity, UCP2 has been linked to impaired insulin production in type 2 diabetes (44, 51). Consequently, however, the evolutionary meaning of UCP2 in β-cells has been questioned, and its effect on GSIS was even labeled a “surprising whim of nature” (40). Nonetheless, the obesity epidemic is a recent (and essentially human-specific) development, and one may reasonably assume that the ability of UCP2 to interfere with the regulation of insulin in β-cells has a true physiological function.

UCP2 expression is elevated in skeletal muscle during periods of fasting (4, 37), and this “starvation paradox” served as a major argument against the role of UCP2 in thermogenesis or energetic inefficiency (13). Most recently, increased UCP2 expression was also described in the whole pancreas and in islets of starved mice, raising speculations that upregulation of UCP2 in β-cells reflects a physiological mechanism for suppression of GSIS in the fasting state (3, 10). Under fed conditions, glucose is preferentially metabolized to generate ATP, whereas surplus carbohydrates are converted to triacylglycerols and stored as peripheral fat. Under fasting conditions, glucose is not sufficiently available, and lipids become the predominant source of fuel (1, 15). Thus hepatic glycogen stores are rapidly depleted while triacylglycerols are released from white adipose tissue and transported as nonesterified fatty acids to liver mitochondria for β-oxidation (1, 15). Acetyl-CoA, the end product of β-oxidation, is then used for ketogenesis and reesterification in very low-density lipoproteins (VLDL) to supply the energy demand of extrahepatic tissues (1). With the exception of hepatocellular uptake of fatty acids (47), these metabolic pathways are under tight hormonal regulation in which insulin has a pivotal inhibitory role that needs to be suppressed in fasting (45).

Because fatty acids stimulate both the expression (30, 36) and the activity of UCP2 in β-cells (28), higher levels of circulating fatty acids may signal the upregulation and activation of UCP2 in fasting and contribute to the suppression of insulin secretion, thus suggesting an amplification loop through which UCP2 plays a role in normal β-cell physiology. Moreover, recent work found that the half-life of UCP2 is unusually

Address for reprint requests and other correspondence: G. Baffy, 150 S. Huntington Ave., Rm. A646, Boston, MA 02130 (e-mail: gyorgy.baffy@va.gov).

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short (~30 min), making UCP2 a suitable candidate for regulating rapid biological responses (43).

Here we investigated a specific aspect of lipid metabolism by testing the hypothesis that modulation of insulin secretion by UCP2 is necessary for the fasting-induced fuel switch to fatty acid oxidation. To this end, we analyzed fasting-induced changes in the lipid metabolism of ucp2<sup>−/−</sup> mice and conclude that the physiological response to fasting requires the presence of UCP2.

**MATERIALS AND METHODS**

**Animals and study design.** Founders of ucp2<sup>−/−</sup> mice were obtained from Dr. Bradford B. Lowell (Harvard Medical School, Boston, MA). Genotypes were determined as described (51). Male ucp2<sup>−/−</sup> mice and their wild-type littermates (n = 4–10) at the age of 12–14 wk were fasted by removing food at 8:00 A.M. for either 24 or 72 h. Control groups were fed ad libitum with regular chow diet. All animals had unlimited access to drinking water. At the time of death, animals were weighed, their blood glucose was measured from the tail vein, and the liver and epidymal fat pads were removed, weighed, and snap-frozen or processed for histological studies. Blood was obtained by cardiac puncture for additional biochemical assays. All experiments were performed by the approval of the Lifespan Animal Welfare Committee of Rhode Island Hospital.

**Biochemical measurements.** We used commercially available kits to determine plasma levels of nonesterified fatty acids (Wako, Richmond, VA), triacylglycerols, and β-hydroxybutyrate (β-OHB) (StanBio, Boerne, TX). Plasma insulin levels were measured by enzyme-linked immunosorbent assay (Linco, St. Charles, MO). Total liver tissue lipid content was determined by the chloroform-methanol extraction method as originally described (2).

**Histological studies and image analysis.** Liver tissue pieces were embedded and frozen in Tissue-Tek medium (Sakura, Torrance, CA) and sectioned at 4 μm thickness. To estimate the extent of hepatic lipid accumulation, we prepared digitized images of liver slides stained with oil-red-O (MicroPublisher 3.3 RTV; Qimaging, Burnaby, British Columbia). We recorded the area of oil-red-O staining above a constant optical density threshold using Image Pro Plus 5.1 (MediaCybernetics, Silver Spring, MD) to calculate the percentage of area stained positive for oil-red-O. Constant optical conditions were maintained along the entire morphometric evaluation.

**Western blot analysis.** Liver tissue lysates were prepared as described (22). Equal amounts of protein were size-fractionated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (PerkinElmer Life Sciences, Waltham, MA), and immunoblots were developed using polyclonal rabbit antibodies against phosphorylated hormone-sensitive lipase (HSL) (Cell Signaling, Danvers, MA), peroxisome proliferator-activated receptor (PPAR)-α (Sigma), PPAR-γ, and sterol regulatory element-binding protein-1c (SREBP-1c) (Santa Cruz, Santa Cruz, CA). Secondary donkey antibody (Santa Cruz) was conjugated with horseradish peroxidase and immunoblots detected by enhanced chemiluminescence (PerkinElmer). Equal loading was confirmed using mouse anti-β-actin antibody (Sigma) and secondary goat antibody (Santa Cruz).

**Real-time quantitative PCR.** Total RNA was extracted from snap-frozen liver tissue specimens with TRIzol reagent (Invitrogen, Carlsbad, CA), and contaminating genomic DNA was removed with DNase I, RNase-free (Roche, Indianapolis, IN), before reverse transcription using the first-strand cDNA synthesis kit (Roche). PCR was performed using an iCycler iQ Multi-Color Real Time PCR Detection System (Bio-Rad, Hercules, CA) and SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). We used the TATA box binding protein (TBP) as reference gene (49). The full-length mouse TBP gene (957 bp) was amplified and cloned into pCR2.1 vector to create standard curves by serial dilutions. Sample cDNAs (equivalent of 5 ng total RNA) were used as template with gene-specific primers (Table 1). Each sample was normalized using its TBP mRNA content, and data are given as relative abundance over fed controls.

**Statistical analysis.** We presented the results as means ± SE. The data were analyzed with an unpaired Student’s t-test or ANOVA when multiple comparisons were made. Association between categorical groups was evaluated by the Fisher’s exact probability, Mann-Whitney U-test, and binomial exact calculations. Differences with calculated P values <0.05 were regarded as significant.

**RESULTS**

**Biochemical indicators reveal blunted response to fasting in ucp2<sup>−/−</sup> mice.** We fasted ucp2<sup>−/−</sup> mice and wild-type littersmates for up to 72 h to analyze the impact of UCP2 deficiency on the metabolic response to fasting. In previous studies, serum insulin levels were found to be elevated in ucp2<sup>−/−</sup> mice (51). Importantly, hyperinsulinemia in ucp2<sup>−/−</sup> mice is not the result of insulin resistance, since it is associated with mild hypoglycemia and a normal insulin tolerance test (51). We confirmed threefold higher serum insulin levels in ucp2<sup>−/−</sup> mice during the fed state when compared with wild-type controls (Fig. 1A). These differences are known to persist at lower serum insulin levels following an overnight fast (51), but the effect of UCP2 deficiency in response to longer periods of fasting has not been reported before. Serum insulin levels in ucp2<sup>−/−</sup> mice remained higher even after a 24-h fast, whereas this difference disappeared when fasting continued for a total of 72 h (Fig. 1A). Although ambient blood glucose levels and fasting-induced hypoglycemia in this cohort of ucp2<sup>−/−</sup> mice were not significantly different from the wild type, blood glucose levels

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Table 1. Primer sequences of genes used for mRNA quantification

<table>
<thead>
<tr>
<th>Protein</th>
<th>NCBI Accession No.</th>
<th>Forward Primer (5’ to 3’)</th>
<th>Reverse Primer (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC-α</td>
<td>NM_133360</td>
<td>CAGGCGTACATCTCCTGAAGCA</td>
<td>GGCTATGTTGGACAGATGGA</td>
</tr>
<tr>
<td>AOX</td>
<td>NM_015729</td>
<td>TTATGCGGAGACAGAGATGGA</td>
<td>TATCTGGAGCTGTCTTCAACT</td>
</tr>
<tr>
<td>CPT1A</td>
<td>NM_013495</td>
<td>CCAGGCGGAGTGGAAGTGTGTT</td>
<td>GGAACACATACATCGTCCAG</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>NM_021282</td>
<td>GCAAAAGGAGCAGACACTTCAG</td>
<td>GCAAGAACATCAACAGAAAGCT</td>
</tr>
<tr>
<td>FAS</td>
<td>NM_007988</td>
<td>GCAGCATTGAGGCAGCCTCTCT</td>
<td>GCTGCAACAAAGGCTCTCACT</td>
</tr>
<tr>
<td>mHMGS</td>
<td>NM_008256</td>
<td>CTATCGCGAGAAAGAGGAGCAG</td>
<td>GAAAGGTGGTGTGTCAGCAG</td>
</tr>
<tr>
<td>MCAD</td>
<td>NM_007382</td>
<td>TGATCAACGGCCGACATTTCG</td>
<td>GAACTTCCGAGGGCCCAAG</td>
</tr>
<tr>
<td>MTTP</td>
<td>NM_008642</td>
<td>GGATGCTCTGCTGCTCTATGTTG</td>
<td>TGTAGAAGCTGCTGAGCAG</td>
</tr>
<tr>
<td>SCD-1</td>
<td>NM_009127</td>
<td>CCTTCGGGAAATGAAGGAGAAG</td>
<td>GAGGACACAGATCCCTTCGCACT</td>
</tr>
<tr>
<td>TBP</td>
<td>NM_016848</td>
<td>ACTGCTGGCAGAAATGGTGGAA</td>
<td>TGTGGCTGCTGCTTCTTACCT</td>
</tr>
</tbody>
</table>

ACC-α, acyl-CoA carboxylase-α; AOX, acyl-CoA oxidase; CPT1A, carnitine palmitoyl transferase 1A; CYP2E1, cytochrome P-450 2E1; FAS, fatty acid synthase complex; mHMGS, hydroxy-methyl-glutaryl CoA synthase (mitochondrial form); MCAD, medium-chain acyl-CoA dehydrogenase; MTTP, microsomal triacylglycerol transfer protein; SCD-1, stearoyl-CoA desaturase-1; TBP, TATA box-binding protein.
following the 72-h fast in ucp2−/− mice were lower compared with wild-type controls, consistent with a continued imbalance of metabolic regulation in UCP2 deficiency (Fig. 1B).

As expected, serum levels of fatty acids increased in wild-type mice after a 24-h fast, whereas this response was diminished in ucp2−/− mice (Fig. 1C). This genotypic difference was even more apparent after a 72-h fast, when fatty acid levels dropped below prefasting values in ucp2−/− mice, suggesting insufficient availability (Fig. 1C). Peripheral lipolysis is one of the earliest responses of lipid metabolism to fasting, accounting for elevated serum levels of fatty acids (24). During lipolysis, triacylglycerols stored in white adipose tissue are hydrolyzed into fatty acids that subsequently help coordinate the complex fasting response. HSL is traditionally viewed as a major determinant of this process (39). HSL is activated by phosphorylation, but this reaction is very sensitive to the inhibitory effect of insulin (8). We therefore hypothesized that higher serum insulin levels persisting in fasted ucp2−/− mice may be associated with diminished activation of HSL. To assess the lipolytic activity in peripheral adipose tissue, we used immunoblot analysis to determine the abundance of phosphorylated HSL in the epididymal fat pad of fasted animals. There was no genotypic difference between the weight of epididymal fat pads before and after fasting (not shown). Following a 24-h fast, amounts of phosphorylated HSL dramatically increased in wild-type mice, indicating release from insulin-mediated inhibition, but this change was attenuated in ucp2−/− mice (Fig. 1D). Thus fasting-induced lipolysis is diminished in ucp2−/− mice, coinciding with higher residual serum insulin levels. (Notably, the 72-h fast resulted in massive lipolysis regardless of the genotype, resulting in the disappearance of epididymal fat pads in most animals and preventing the systematic analysis of HSL activation at this extreme phase of fasting.)

Serum levels of triacylglycerols in wild-type mice became gradually lower as fasting continued for up to 72 h, consistent with dwindling peripheral fat stores due to increased hydrolysis and utilization (Fig. 1E). Interestingly, this trend was delayed in ucp2−/− mice, complementing changes in serum levels of fatty acids as described above. The genotypic difference disappeared after the 72-h fast when serum triacylglycerols levels were uniformly low, consistent with the overall depletion of peripheral fat stores. We then assessed fasting-induced ketogenesis by measuring serum levels of β-OHB. Importantly, although wild-type mice responded with a sharp and continuous increase in β-OHB production, ucp2−/− mice were unable to maintain the same trend in response to a 72-h fast, indicating impaired fasting-induced ketogenesis in the absence of UCP2 (Fig. 1F).

Fasting-induced hepatic steatosis is delayed in ucp2−/− mice. As a result of fasting-induced peripheral lipolysis, large amounts of fatty acids are transported to the liver where they provide an alternative energy source (24). Flux rates of fatty acids in starvation often exceed normal hepatocellular capacity for mitochondrial β-oxidation and result in transient accumulation of triacylglycerols, manifesting as steatosis (32, 47). Because fasting-induced hyperfattyacidemia is diminished in ucp2−/− mice, we predicted that steatosis would be less prominent in these animals. Indeed, reduced amounts of fat accumulated in livers of ucp2−/− mice after a 24-h fast as assessed by total tissue lipid extraction (Fig. 2A) and oil-red-O staining (Fig. 2, B and D). Surprisingly, however, steatosis became more severe in ucp2−/− mice after a 72-h fast when it already showed substantial resolution in wild-type mice. Increased hepatic fat accumulation in ucp2−/− mice was also indicated by elevated liver weight-to-body weight ratios after the 72-h fast (Fig. 2C). These findings suggest that hepatocytes in ucp2−/− mice fasted for a prolonged period fail to channel fatty acids efficiently into lipid catabolic pathways such as mitochondrial β-oxidation and ketogenesis, resulting in delayed hepatocellular lipid clearance.
Hepatic expression of key lipid regulatory molecules in fasted ucp2−/− mice. Lipid and energy metabolism in the liver is primarily regulated by insulin at the transcriptional level with the mediation of various transcription factors (45). To better understand the mechanisms by which UCP2 affects the metabolic fate of fatty acids in fasting, we analyzed the gene expression pattern of key lipid regulatory enzymes in the liver. Gene expression for carnitine palmitoyltransferase 1, the rate-limiting enzyme for mitochondrial fatty acid uptake (33), gradually increased with fasting in wild-type mice but showed no change in ucp2−/− mice (Fig. 3A). The pattern of gene expression was similar for medium chain-specific acyl-CoA dehydrogenase, a key enzyme for mitochondrial β-oxidation (14), indicating that mitochondrial capacity for fasting-induced lipid utilization is relatively diminished in hepatocytes of ucp2−/− mice (Fig. 3A). The pattern of gene expression was similar for medium chain-specific acyl-CoA dehydrogenase, a key enzyme for mitochondrial β-oxidation (14), indicating that mitochondrial capacity for fasting-induced lipid utilization is relatively diminished in hepatocytes of ucp2−/− mice (Fig. 3A). Fatty acid oxidation may occur through accessory pathways during starvation (42). Therefore, we also analyzed expression of acyl-CoA oxidase (AOX) and cytochrome P-450 2E1 (CYP2E1) to estimate peroxisomal β-oxidation and microsomal ω-oxidation, respectively. Both genes had lower expression in fasted ucp2−/− mice, indicating lack of compensation at the transcriptional level for diminished mitochondrial β-oxidation by accessory fatty acid oxidation pathways (Fig. 3A).

Gene expression for mitochondrial β-hydroxy-β-methylglutaryl-CoA synthase, a pivotal enzyme of ketogenesis (19), rose markedly upon fasting in wild-type mice but remained unchanged in ucp2−/− mice (Fig. 3A), in line with diminished β-OHB production. In addition, gene expression for microsomal triglyceride transfer protein, the protein responsible for exporting VLDL from hepatocytes (48), became progressively diminished in ucp2−/− mice, suggesting that impaired clearance of de novo synthesized triacylglycerols may contribute to protracted fasting-induced steatosis in the absence of UCP2 (Fig. 3A). These findings support the notion that UCP2 is required for proper induction of ketogenesis and VLDL transport in response to fasting.

Because anabolic pathways of lipid metabolism contribute to steatosis, we also assessed the impact of UCP2 deficiency on fasting-induced changes in hepatic expression of key lipogenic enzymes. We measured mRNA levels of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD), enzymes that are under positive transcriptional control by insulin and that mediate de novo production of fatty acids in the liver (7, 45). Consistent with higher insulin levels in UCP2 deficiency, mRNA levels for ACC-α, FAS, and SCD-1 in the fed state were higher in ucp2−/− mice than in wild-type controls (Fig. 3B). Gene expression of all three...
lipogenic enzymes markedly diminished in both genotypes in response to fasting, although the decline in mRNA levels was more robust in *ucp2<sup>-/-</sup>* mice (Fig. 3B). This finding is at variance with higher residual insulin levels found in UCP2 deficiency, suggesting that noninsulin regulatory pathways may contribute to the genotypic difference. Nonetheless, the gene expression profile suggests that catabolic rather than anabolic lipid pathways correlate with fasting-induced steatosis and serum metabolic parameters in *ucp2<sup>-/-</sup>* mice. Thus impairment of lipid breakdown appears to dominate the effect of UCP2 deficiency in our animal model.

To further analyze the metabolic response to fasting in *ucp2<sup>-/-</sup>* mice, we measured hepatic expression of key transcription factors that regulate lipid homeostasis. Peroxisome proliferator-activated receptors PPAR-α and PPAR-γ are nuclear hormone receptors naturally activated by fatty acids and their derivatives (25). PPAR-α regulates the genes of mitochondrial, peroxisomal, and microsomal fatty acid oxidation and ketogenesis (31), whereas PPAR-γ promotes adipocyte differentiation and lipid storage, with hepatic expression mostly seen in fatty livers related to obesity and starvation (20, 34). In agreement with observations that both PPAR-α and PPAR-γ are upregulated in the liver in response to fasting (50), hepatic PPAR-α and PPAR-γ levels increased following a 24-h fast in wild-type mice, whereas this response was attenuated in *ucp2<sup>-/-</sup>* mice (Fig. 3C). SREBP-1c controls de novo hepatic synthesis of fatty acids and triacylglycerols by inducing the expression of lipogenic genes such as ACC, FAS, and SCD-1 (7, 16). In contrast to PPAR-α and PPAR-γ, starvation normally results in downregulation of hepatic SREBP-1c expression (23). As predicted, liver tissue SREBP-1c levels in wild-type mice decreased in response to the 24-h fast, but remained unchanged in *ucp2<sup>-/-</sup>* mice (Fig. 3C). Thus UCP2 deficiency attenuates fasting-induced changes in the levels of major lipid regulatory transcription factors examined in our study. Notably, the 72-h fast resulted in uniformly low levels of PPAR-α, PPAR-γ, and SREBP-1c regardless of the genotype, suggesting general dysfunction of metabolic regulation at this extreme phase of fasting.

Fig. 3. Expression of key lipid regulatory molecules is altered in the liver of fasted *ucp2<sup>-/-</sup>* mice. Quantitative real-time PCR analysis of genes encoding for enzymes involved in metabolic pathways of lipid breakdown (A) and synthesis (B) was performed on whole liver tissue of fed and fasted *ucp2<sup>-/-</sup>* mice and their wild-type littermates (*n* = 4–10). Relative abundance of mRNA content over the mRNA of TATA box-binding protein expressed in arbitrary units referenced to fed control. CPT1, carnitine palmitoyl transferase-1 (mitochondrial fatty acid uptake); MCAD, medium-chain acyl-CoA dehydrogenase (mitochondrial β-oxidation); CYP2E1, cytochrome P-450 2E1 (microsomal ω-oxidation); AOX, acyl-CoA oxidase (peroxisomal β-oxidation); mtHMGS, mitochondrial β-hydroxy-β-methylglutaryl-CoA synthase (ketogenesis); MTTP, microsomal triglyceride transfer protein (very low-density lipoprotein transport); ACCa, acetyl-CoA carboxylase-α; FAS, fatty acid synthase complex; SCD-1, stearoyl-CoA desaturase-1 (lipogenesis). Open bars, wild-type mice; filled bars, *ucp2<sup>-/-</sup>* mice. Error bars denote SE. *Significantly different from wild-type mice (*P* < 0.05).

C: immunoblot analysis of peroxisome proliferator-activated receptor (PPAR)-α, PPAR-γ, and sterol regulatory element-binding protein-1c (SREBP-1c) in livers of fed and fasted *ucp2<sup>-/-</sup>* mice and their wild-type littermates. β-Actin served as loading control.
DISCUSSION

There is considerable effort to elucidate the molecular mechanisms that contribute to the rapidly growing prevalence of obesity and type 2 diabetes in developed societies (38). It has been argued that this epidemic reflects the maladaptation of a “thrifty” phenotype that originally evolved in our hunter-gatherer ancestors as a trait to promote efficient energy storage, but now it fails to meet rapid environmental changes such as continuous food availability and sedentary lifestyle (9, 17). Better understanding of how the metabolic response to fasting is regulated under physiological conditions may therefore identify molecular targets that erroneously promote this alarming trend.

In this study, we identified UCP2 as a modulator of the lipid metabolic response to fasting in mice since the complex biochemical response of the liver, which involves the efficient breakdown, conversion, and redistribution of fatty acids, is perturbed in UCP2 deficiency. Thus fasting-induced physiological changes in peripheral lipolysis and hepatic lipid utilization appear blunted in ucp2−/− mice as assessed by biochemical parameters and by expression of key lipid regulatory enzymes and transcription factors.

Peroxisomal β-oxidation and microsomal ω-oxidation are minor pathways of fatty acid oxidation (46) suggested to gain significance as a result of impaired mitochondrial β-oxidation or during increased delivery of fatty acids to the liver as it occurs in fasting (42). Although insufficient β-oxidation capacity of mitochondria in UCP2 deficiency could theoretically repartition the catabolism of fatty acids into peroxisomes and microsomes, and this mechanism has recently been described in the liver of subjects with nonalcoholic fatty liver disease (27), we found no signs for such compensation. In fact, lower gene expression of AOX and CYP2E1 in fasted ucp2−/− mice suggests that nonmitochondrial routes for fatty acid breakdown remain suppressed in this animal model.

The effect of fasting on the expression of lipid regulatory transcription factors has been well characterized. As a result of peripheral lipolysis and rapid delivery of fatty acids to the liver, fasting stimulates hepatic expression of both PPAR-α (26) and PPAR-γ (20). The impact of PPAR-α and PPAR-γ on hepatic lipid metabolism, however, is different. Although overexpression of PPAR-γ in the mouse liver causes lipid accumulation (50), PPAR-α promotes lipid utilization as demonstrated by marked hepatic steatosis and diminished fatty acid oxidation and ketogenesis in fasted PPAR-α knockout mice (18, 31). In our experiments, a 24-h fast resulted in increased expression of hepatic PPAR-α and PPAR-γ in wild-type mice, indicating a dynamic response of lipid metabolism impaired in the livers of ucp2−/− mice (Fig. 3C). Notably, fasting-induced changes in hepatic SREBP-1c expression did not correlate with the degree of steatosis in our animal model. Thus the 24-h fast resulted in decreased amounts of hepatic SREBP-1c in wild-type mice but remained unchanged in ucp2−/− mice (Fig. 3C), whereas genotype-specific changes in steatosis during starvation showed an opposing trend (Fig. 1). Together with the similarly discordant expression pattern of SREBP-1c target genes ACC-α, FAS, and SCD-1 (Fig. 3B), we conclude that SREBP-1c contributes little to the metabolic consequences of UCP2 deficiency in the fasting liver.

Our findings show that metabolic disparities between ucp2−/− and wild-type mice become increasingly prominent with prolonged starvation. Thus serum levels of fatty acids and β-OHB (Fig. 1) as well as the hepatic expression pattern of genes responsible for fatty acid oxidation, ketogenesis, and VLDL export (Fig. 3) are increasingly abnormal after a 72-h fast in the absence of UCP2. In addition, although initially delayed, fasting-induced hepatic lipid accumulation in ucp2−/− mice becomes more severe by 72 h, by which time steatosis is already resolving in wild-type mice (Fig. 2). It is likely that insufficient lipolysis in fasted ucp2−/− mice initially provides reduced amounts of fatty acids for hepatic uptake, accounting for less steatosis and for diminished impact of fatty acids on metabolic regulation. When fasting continues, steatosis rapidly resolves as hepatocytes process fatty acids to distribute lipid-derived energy in compensation for insufficiently available glucose (32, 47). In contrast, hepatic lipid utilization in response to fasting is apparently impaired in ucp2−/− mice, likely accounting for protracted steatosis.

Insulin plays a critical negative regulatory role both in fasting-induced peripheral lipolysis and in hepatic lipid breakdown (24, 45). It is known from previous work that GSIS is enhanced and hyperinsulinemia develops in ucp2−/− mice as a result of altered glucose sensing in pancreatic β-cells (51). Here we show that residual serum insulin levels remain higher in ucp2−/− mice even after a 24-h fast (Fig. 1A), indicating that UCP2 deficiency in β-cells may impair the fasting response of hepatic lipid metabolism. These data support recent speculations on the evolutionary role of UCP2 in suppressing GSIS of β-cells (3, 10) and suggest that insufficient suppression of insulin secretion may perturb the fasting response of lipid metabolism in ucp2−/− mice. However, plasma insulin levels in ucp2−/− mice after a 24-h fast are comparable to nonfasting baseline levels in wild-type mice, and some of the metabolic changes are most prominent after a 72-h fast when fasting insulin levels in ucp2−/− and wild-type mice are no longer different. Although insulin tolerance tests in our previous studies indicated no change in the peripheral insulin sensitivity of ucp2−/− mice (51), enhanced insulin signaling was recently described in the adipose tissue of mice treated with antisense oligonucleotides to UCP2 (11). Altered peripheral insulin action may therefore contribute to changes seen in the liver of fasted ucp2−/− mice, a possibility that warrants further studies.

In conclusion, fasting-induced changes in the lipid metabolism of mice gain from the presence of UCP2. Our findings lend experimental support to the concept that upregulation of UCP2 in β-cells is a physiologically important response to fasting (3, 10). Increasing UCP2 levels diminish insulin release from β-cells, thus facilitating peripheral lipolysis and hepatic lipid utilization in fasting. Accordingly, the augmented presence of UCP2 seems beneficial under these conditions. UCP2 upregulation and activation in β-cells may in fact involve an amplification loop driven by peripherally mobilized fatty acids during the initial phase of fasting. This regulatory mechanism, however, is misdirected in type 2 diabetes where steady abundance of UCP2 may result in β-cell dysfunction, thus contributing to profound metabolic disturbances. Moreover, enduring hyperglycemia and hyperfattyacidemia in type 2 diabetes may induce oxidative stress in β-cells, permanently activating the superoxide-UCP2 pathway (5, 29). There is growing evidence that inhibition of UCP2 by genetic ablation (51), antisense
oligonucleotides (12), RNA interference (44), or the herbal derivative genipin (52) may restore the insulin-secreting ability of β-cells and improve type 2 diabetes. Whether one needs to be concerned about the loss of physiological effects of UCP2 under these conditions, however, remains to be seen.

GRANTS

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REFERENCES


