Hepatic de Novo Synthesis of Glucose 6-Phosphate Is Not Affected in Peroxisome Proliferator-activated Receptor α-Deficient Mice but Is Preferentially Directed toward Hepatic Glycogen Stores after a Short Term Fast*

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Apart from impaired β-oxidation, Ppara-deficient (Ppara−/−) mice suffer from hypoglycemia during prolonged fasting, suggesting alterations in hepatic glucose metabolism. We compared hepatic glucose metabolism in vivo in wild type (WT) and Ppara−/− mice after a short term fast, applying novel isotopic methods. After a 9-h fast, mice were infused with [U-13C]glucose, [2-13C]glycerol, [1-13C]galactose, and paracetamol for 6 h, and blood and urine was collected in timed intervals. Plasma glucose concentrations remained constant and were not different between the groups. Hepatic glycogen content was 69 ± 11 and 90 ± 31 μmol/g liver after 15 h of fasting in WT and Ppara−/− mice, respectively. The gluconeogenic flux toward glucose-6-phosphate was not different between the groups (i.e. 157 ± 9 and 153 ± 9 μmol/kg/min in WT and Ppara−/− mice, respectively). The gluconeogenic flux toward plasma glucose, however, was decreased in PPARα−/− mice (i.e. 142 ± 9 versus 124 ± 13 μmol/kg/min) (p < 0.05), accounting for the observed decrease (−15%) in hepatic glucose production in Ppara−/− mice. Expression of the gene encoding glucose-6-phosphate hydrodrolase (G6ph) was lower in the PPARα−/− mice compared with WT mice. In conclusion, Ppara−/− mice were able to maintain a normal total gluconeogenic flux to glucose-6-phosphate during moderate fasting, despite their inability to up-regulate β-oxidation. However, this gluconeogenic flux was directed more toward glycogen, leading to a decreased hepatic glucose output. This was associated with a down-regulation of the expression of G6ph in PPARα-deficient mice.

Fuel selection to meet the body’s energy demand is of crucial importance during feeding-fasting transitions. The liver plays a central role in this switch. It changes from glucose uptake and glycogen synthesis during feeding to glucose production by gluconeogenesis (GNG)† and glycolysis during fasting. The origin of hepatic glucose production (HGP) shifts from mainly glycolysis to GNG as fasting prolongs. These changes in hepatic glucose metabolism are accompanied by adaptation of hepatic fatty acid metabolism (i.e. from fatty acid synthesis to fatty acid oxidation). This adaptation allows the optimization of fuel substrate utilization. These metabolic changes are, at least in part, effected by the reciprocal action of insulin and glucagon. However, the discovery of nuclear hormone receptors and the (partial) elucidation of their mode of action as ligand-activated regulators of gene expression has considerably complicated the picture. One member of the nuclear hormone receptors is of particular importance in mediating the adaptive response to fasting (i.e. peroxisome proliferator-activated receptor α (PPARα)). PPARα is a fatty acid-activated transcription factor that up-regulates the expression of a variety of genes that encode proteins involved in β-oxidation and lipoprotein metabolism (1). Lack of this receptor in Ppara−/− mice results in the inability to up-regulate hepatic fatty acid oxidation and ketogenesis upon fasting in the face of increased concentrations of free fatty acids in the circulation (2). It also became apparent that mice lacking PPARα develop hypoglycemia after a prolonged fast (2).

The etiology of hypoglycemia in fasting Ppara−/− mice is still unclear. It has been hypothesized that it reflects decreased GNG secondary to impaired hepatic fatty acid β-oxidation (2). Surprisingly, a recent study suggested increased HGP in long term (24-h) fasted Ppara−/− mice despite the development of hypoglycemia (3). Furthermore, glycogen content of the liver in Ppara−/− mice after a prolonged fast was not reduced in comparison with wild type (WT) mice but, unexpectedly, tended to be higher in the Ppara−/− mice. In contrast, glycogen content of the liver did not increase in Ppara−/− mice upon refeeding, whereas in WT mice, glycogen content strongly increased. These data suggest that the balance between HGP, glycogen synthesis, and GNG at the level of glucose-6-phosphate (G6P) is perturbed in Ppara−/− mice.

Partitioning of G6P can be studied in vivo using the glycoconjugate probe technique and mass isotopomer distribution analysis (MIDA) as described by Hellerstein and co-workers (4). Recently, we applied these stable isotope techniques to...
study partitioning of newly synthesized G6P while glucose-6-phosphatase activity was partially inhibited in 24-h fasted rats (5). We were able to show that when HGP was diminished by partial inhibition of glucose-6-phosphatase activity, quite surprisingly, de novo synthesis of G6P was unaffected. It appeared that newly synthesized G6P was partitioned away from plasma glucose to glycogen synthesis, and, as a consequence, the glycogen content of livers of treated rats increased severalfold. The importance of these observations, substantiating data from other groups (6), is that partitioning of G6P depends in part on the adaptive response of the liver to fasting (5). We have further miniaturized these methods for application in mice (7). In the present study, we addressed the following questions. 1) Is there a role of PPARs in the control of de novo synthesis of G6P? 2) What are the effects of PPARs deficiency on partitioning of newly synthesized G6P during fasting? We approached these questions experimentally in short term fasted Ppara−/− mice by infusion of [U-13C]glucose, [2-13C]glycerol, [1-2H]-galactose, and paracetamol, collecting serial blood and urine spots on filter paper, and measuring the mass isotopomer distribution in glucose and paracetamol-glucuronic acid. The fluxes were subsequently compared with expression of genes encoding enzymes involved in hepatic glucose metabolism and fatty acid oxidation, enabling us to delineate functional consequences of PPARs deficiency-induced changes in gene expression.

EXPERIMENTAL PROCEDURES

Animals—Male Ppara−/− mice and WT mice on a SV129 background were housed in a temperature-controlled (21 °C) room on a 10-h dark, 14-h light cycle. Experimental procedures were approved by the Ethics Committee of the State University Groningen. Mice were equipped with a permanent heart catheter that was attached to the skull with acrylic glue (8). Mice were allowed to recover from surgery for at least 4 days.

Fasting Experiments—The adaptive response to fasting in Ppara−/− and WT mice was compared first. Mice were fasted up to 24 h, and blood samples were taken at t = 0 (fed), 15, and 24 h by tail bleeding, and livers were removed at t = 0, 15, and 24 h for lipid analysis and RNA isolation. Hepatic in vivo carbohydrate metabolism was measured according to the protocol described by Van Dijk et al. (7). On the day of the experiment, mice were placed in individual metabolic cages. Filter paper was placed under the wired floor of the cage to collect urine samples. Food was removed 9 h before the start of the experiments. Body weight was 25.0 ± 1.9 g for WT mice and 24.1 ± 1.1 g for Ppara−/− mice at the time of the experiments. Mice received an infusion at a rate of 0.6 ml h−1 during 6 h of a solution consisting of [U-13C]glucose (13 μmol ml−1), [2-13C]glycerol (160 μmol ml−1), [1-2H]-galactose (33 μmol ml−1), and paracetamol (1 mg ml−1). Blood glucose during the experiment was measured using EuroFlash™ test strips (LifeScan Benelux, Beerse, Belgium), and bloodspots obtained by tail bleeding for gas chromatography-mass spectrometry (GC-MS) measurements were collected before the infusion and hourly afterward until 6 h after the start of the infusion. The filter paper placed under the wired floor of the cage was replaced at hourly intervals. A large blood sample was obtained by cardiac puncture under halothane anesthesia at the end of the experiment, and the liver was quickly excised and frozen immediately in liquid N2 for lipid analysis and RNA isolation.

Metabolite Concentrations and Enzyme Activities—Plasma was isolated by centrifugation, liver tissue was homogenized, and lipids were extracted using a modified Bligh & Dyer method (9). Plasma 3-hydroxybutyrate, lactate, free fatty acid, and liver triglyceride content were determined using commercially available kits (Roche Applied Science and Wako Chemicals GmbH, Neuss, Germany). Alanine concentrations were measured by ion exchange column chromatography followed by postcolumn ninhydrine derivatization (10, 11) on a Biochrom 20/30 automated amino acid analyzer (Amersham Biosciences). Glycerol concentrations were determined by GC-MS using 1,1,2,3,3,6,6-d7-glucose as internal standard. Samples were derivatized to their triacetate by adding 100 μl of pyridine and 200 μl of acetic anhydride to 50 μl of plasma and incubating the solution at 80 °C for 30 min. Samples were measured on a Trace-GC-MS (Finnigan Matt, San Jose, CA). Plasma insulin levels were determined by radioimmunoassay (RI-13K, Linco Research, St. Charles, MO). Total hepatic protein content was determined according to Lowry et al. (12). Hepatic glycogen content was determined in freeze clamped liver tissue after extraction in 1 ml KOH solution by sonication. The extract was incubated for 30 min at 90 °C, cooled, and brought to pH 4.5 by the addition of 3 μl acetic acid. Precipitated protein was removed by centrifugation. Glycogen was converted to glucose by treating the samples with amyloglucosidase, followed by assay of glucose at pH 7.4 with ATP, NADP+, hexokinase, and G6P dehydrogenase (13). Liver samples for the determination of G6P were treated by sonification in a 5% (w/v) HClO4 solution and centrifuged, and supernatant was neutralized to pH 7 by the addition of small amounts of a mixture of 2 ml KOH and 0.3 ml MOPS. G6P was determined fluorimetrically with NADP+ and G6P dehydrogenase (13). Hepatic ATP levels were measured using a bioluminescence assay kit (Roche Applied Science).

Hepatic mRNA Levels—Total RNA was isolated from liver tissue using the Trizol method (Invitrogen). RNA was converted to cDNA with M-Mulv-RT (Roche Applied Science) according to the manufacturer's protocol using random primers. cDNA levels of the genes of interest were measured by real time PCR using the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). An amount of cDNA corresponding to 20 ng of total RNA was amplified using the qPCR core kit (Eurogentec, Seraing, Belgium) according to the manufacturer's protocol using the appropriate forward and reverse primers (Invitrogen) and a template-specific 5′-carboxy-Ν2Ν′Ν′′-tetramethylrhodamine, 5′-carboxyfluorescein-labeled Double Double Oligonucleotide probe (Eurogentec). Calibration curves were run on serial dilutions of pooled cDNA solutions as used in the assay. The data were processed using the ABI Sequence Detector 6.3 system (Applied Biosystems). Quantified expression levels were within the linear part of the calibration curves. PCR results were normalized to β-actin mRNA levels.

The sequences of the primers and probes used in this study are listed in Table I.

Measurement of Mass Isotope Distribution by GC-MS—Analytical procedures for extraction of glucose and paracetamol-glucuronide from bloodspot and urine filter paper strips, respectively, derivatization of the extracted compounds, and GC-MS measurements of derivatives were initially according to the protocol described by Van Dijk et al. (5, 7). [U-13C]glucose was extracted by incubating a disk (6.5 mm) punched out of a bloodspot with ethanol/water (10/1, v/v) mixture. After drying the sample under a stream of N2, glucose was derivatized to its pentacetate-ester and aldonitrile pentaacetate-ester. The final derivatives were dissolved in 200 μl of ethyl acetate for injection. Paracetamol-glucuronolide (Par-GlcUA) was extracted from filter paper with methanol/water (3/1, v/v) and subsequently isolated by a Milton Roy high pressure liquid chro- matography system (Interscience, Breda, The Netherlands) on a Nucleosil 7C18 SP250/10 column (Bester, Amstelveen, The Netherlands) eluted with a gradient of 0.2% (v/v) ammonium formate in water (pH 4.5) and 40% (v/v) acetonitrile in water. The fraction containing the derivatized compound was dried and subsequently derivatized to its trimethylsilyl-ethyl-ester or oxidized to saccharic acid by nitric acid and derivatized to its tetracetate-diethyl-ester. After drying of the samples under a stream of N2, the dry residues were dissolved in 200 μl of ethyl acetate for injection. All samples were analyzed by GC-MS (SSQ7000; ThermoFinnigan, San Jose, CA) on an AT-3MS 30 m × 0.25-mm inner diameter (0.25-μm film thickness) capillary column (Alltech, Breda, The Netherlands). For all calculations of mass isotopomer distribution, Excalibur software (ThermoFinnigan, San Jose, CA) was used. Mass spectrometric analyses of glucose pentacetate, glucose aldonitrile pentaacetate, and saccharic acid diethyl-, tetracetate-ester were performed by positive ion chemical ionization with methane as reagent gas. Ions monitored for glucose pentacetate were m/z 331–337, ions for glucose aldonitrile pentaacetate were m/z 328–334, and ions for saccharic acid diethyl-ester tetracetate were m/z 375–381, all corresponding to the m−, m0−, m2− mass isotopomers. Mass spectrometric analyses for Par-GlcUA ethyl-, tetra-(trimethylsilyl)-ester were performed by electron impact ionization. The ions monitored for Par-GlcUA were m/z 331–337, corresponding to the m−, m0−, m2− mass isotopomers. Series of measurements were composed of experimental samples, control samples, and a dilution series obtained from a mixture of the last, most enriched, samples taken at the end of an experiment.

All measurements was accepted for further calculations when two conditions were met as described by Van Dijk et al. (7). First, for each derivative, the coefficient of variance of the fractional contribution of m−, m0−, and m2− to total ion abundance in control samples must be smaller than 1% for m− and 2% for m0− and m2−. Second, for each derivative, the fractional contribution of m−, m0−, and m2− to total ion
abundance measured in experimental samples must be within the range of constant response of the GC-MS as estimated from the values of the fractional contribution of m₁, m₂, and m₆ to total ion abundance of the inserted dilution series.

MIDA—The measured fractional isotopomer distribution by GC-MS (m₀–m₆) was corrected for the fractional distribution due to natural abundance of ¹³C. This was done by multiple linear regression as described by Lee et al. (14) to obtain the excess fractional distribution of mass isotopomers (M₀–M₆) due to incorporation of infused labeled compounds (i.e. [2-¹³C]glycerol, [U-¹³C]glucose, and [1-²H]galactose). This distribution was used in MIDA algorithms of isotope incorporation and dilution according to Hellerstein et al. (15) as described by Van Dijk et al. (5). Total rate of appearance of glucose into plasma (Ra(glc;whole body)) was calculated by isotope dilution as follows,

\[
\text{Ra(glc;whole body)} = \frac{\text{MPE(glc;M₆)}_{\text{infusate}}}{\text{MPE(glc;M₆)}_{\text{plasma}}} \times \frac{\text{MPE(glc;M₆)}_{\text{plasma}}}{\text{infusion(glc;M₆)}}
\]  (Eq. 1)

in which MPE(glc;M₆)_{infusate} represents the mole percent enrichment of infused [U-¹³C]glucose, MPE(glc;M₆)_{plasma} is the mole percent enrichment of plasma [U-¹³C]glucose, and infusion(glc;M₆) is the infusion rate of uniformly labeled [U-¹³C]glucose.

The total rate of appearance of UDPglc (Ra(UDPglc;whole body)) was calculated according to the following,

\[
\text{Ra(UDPglc;whole body)} = \frac{\text{MPE(UDPglc;M₁)}_{\text{urine}}}{\text{MPE(pGlcUA;M₁)}_{\text{urine}}} \times \frac{\text{MPE(pGlcUA;M₁)}_{\text{urine}}}{\text{infusion(UDPglc;M₁)}}
\]  (Eq. 2)

### Table I
**Sequences of primers and probes used**

| Glucose metabolism | Sequence | GenBank™ no. |
|--------------------|----------|--------------|
| G6ph               | NM_008061|
| Forward            | CTG CCA GGG AGA ACT CAG CAA |
| Reverse            | GAG GAC CAA GGA AGC CAC AAT |
| Probe              | TCG TTC CCA TTC GCC TTC GCC T |
| G6pt               | NM_008063|
| Forward            | GAG GCC TTG TAG GAA GCA TTG |
| Reverse            | CCA TCC CAG CCA TCA TGA GTA |
| Probe              | CTC TNT ATG GGA ACC CTC GCC ACG |
| Gk                 | L38990   |
| Forward            | CCT GGG CTT CAC CTT CTC CT |
| Reverse            | GAG GCC TTG AAG CCC TTG GT |
| Probe              | CAC GAA GAC ATC GAC AAG GCC ATC CTC CTC |
| Gp                 | BC013636|
| Forward            | GAA GGA GGC AAA CGG ATC AAC |
| Reverse            | TCA CAG TGT CCG ACT GGA TCT |
| Probe              | CCT CTC CAT CCG CTT CCA |
| Gs                 | AA537291|
| Forward            | GCT CTC CAG ACG ATT CTT GCA |
| Reverse            | GTG CCG TTC CTC TGA ATG ATC |
| Probe              | CCT CTA CCG GTT TTG TAA ACA ATC ACG CC |
|Pk                  | NM_013631|
| Forward            | CGT TTG TGC CAC ACA GAT GCT |
| Reverse            | CAT TGG CAA CAT CGG TCT TCT |
| Probe              | AGC ATC ATC ACT AAG GCT GCA CCA ATC CGG |
|Pepck               | NM_011146|
| Forward            | GTG TCA TCC GCA AGC TGA AGA |
| Reverse            | CTT TCG ATC CTG GCC ACA ATC |
| Probe              | CAA GTG TTG GGC TCT CAC TGA CCC |
|Chrebp              | AF156604|
| Forward            | GAT GGT GCG AAC AGC TCT TCT |
| Reverse            | CTG GGC TGT GTG ATG GTG AA |
| Probe              | CCA GGC TCC TCC TCG GAG CCC |

| Fatty acid oxidation | Sequence | GenBank™ no. |
|----------------------|----------|--------------|
| Ppara                | X57638   |
| Forward              | TAT TCG GCT GAA GCT GGT GTA C |
| Reverse              | CTG GCA TTT GTT CCG GTT CT |
| Probe                | CTG AAT CTT GCA GCT CCG ATC ACA CT |
| Pparαγ               | NM_011146|
| Forward              | CAC AAT GCC ATC AGG TTT GG |
| Reverse              | GCT GGT CGA TAT CAC TGG AGA TC |
| Probe                | CCA ACA GCT TCT CCT CCT CGG CCT G |
| CPT1a                | AF 017175|
| Forward              | CTC AGT GGG AGG GAC TCT TCA |
| Reverse              | GGC CTC TGT GGT ACA CGA CAA |
| Probe                | CCT GGG GAG GAC GAC ACC ATC CAA C |
| Mcad                 | NM_007382|
| Forward              | GCA GCC AAT GAT GTG TGC TTA C |
| Reverse              | CAC CCT TCT TCT CTG CTT TGG T |
| Probe                | CCC TCC GCA GGC TCT GAT GTG G |
| Hmgs                 | U12790   |
| Forward              | TGG TGG ATG GGA AGC TGT CTA |
| Reverse              | TTT TTG CGG TAG GCT GCA TAG |
| Probe                | CCA AAG CCC GCA GGT AGC ACT G |

| Fatty acid oxidation | Sequence | GenBank™ no. |
|----------------------|----------|--------------|
| Ppara                | X57638   |
| Forward              | TAT TCG GCT GAA GCT GGT GTA C |
| Reverse              | CTG GCA TTT GTT CCG GTT CT |
| Probe                | CTG AAT CTT GCA GCT CCG ATC ACA CT |
| Pparαγ               | NM_011146|
| Forward              | CAC AAT GCC ATC AGG TTT GG |
| Reverse              | GCT GGT CGA TAT CAC TGG AGA TC |
| Probe                | CCA ACA GCT TCT CCT CCT CGG CCT G |
| CPT1a                | AF 017175|
| Forward              | CTC AGT GGG AGG GAC TCT TCA |
| Reverse              | GGC CTC TGT GGT ACA CGA CAA |
| Probe                | CCT GGG GAG GAC GAC ACC ATC CAA C |
| Mcad                 | NM_007382|
| Forward              | GCA GCC AAT GAT GTG TGC TTA C |
| Reverse              | CAC CCT TCT TCT CTG CTT TGG T |
| Probe                | CCC TCC GCA GGC TCT GAT GTG G |
| Hmgs                 | U12790   |
| Forward              | TGG TGG ATG GGA AGC TGT CTA |
| Reverse              | TTT TTG CGG TAG GCT GCA TAG |
| Probe                | CCA AAG CCC GCA GGT AGC ACT G |
in which MPE(gal;M_{1})_{urine} is the mole percent enrichments of infused [1-2H]galactose, (pGlcUA;M_{1})_{urine} is the mole percent enrichments of hepatic UDP-glucose as measured in Par-GlcUA, and infusion(gal;M_{1}) is the infusion rate of [1-2H]galactose.

Ra(UDPglc;whole body) was calculated with the assumption of a constant and complete entry of infused galactose into the hepatic UDP-glucose pool. Furthermore, it was assumed that the fractional isotope distribution observed in urinary Par-GlcUA reflects the fractional isotope distribution in hepatic UDPglc.

Rates of endogenous plasma glucose (Ra(glc;endo)) and UDP-glucose (Ra(UDPglc;endo)) appearance were calculated as follows.

\[
Ra(glc;endo) = Ra(glc;whole body) - \text{infusion}(glc;M_6) \tag{Eq. 3}
\]

and

\[
Ra(UDPglc;endo) = Ra(UDPglc;whole body) - \text{infusion}(gal;M_6) \tag{Eq. 4}
\]

Fractional gluconeogenic contribution to both plasma glucose (f(glc)) and hepatic UDP-glucose (f(UDPglc)) were calculated using MIDA of glucose and Par-GlcUA derivatives, respectively, as described previously (see Refs. 16 and 17). The absolute gluconeogenic flux into both plasma glucose (GNG(glc)) and hepatic UDP-glucose (GNG(UDPglc)) were calculated as follows.

\[
GNG(glc) = f(glc) \times Ra(glc;whole body) \tag{Eq. 5}
\]

and

\[
GNG(UDPglc) = f(UDPglc) \times Ra(UDPglc;whole body) \tag{Eq. 6}
\]

The total absolute gluconeogenic flux is the sum of both components corrected for recycling,

\[
\text{Total GNG} = (1-c(glc)) \times GNG(glc) + (1-c(UDPglc)) \times GNG(UDPglc) \tag{Eq. 7}
\]

and

\[
c(glc) = \frac{\text{MPE(pGlcUA;M_{6})}_{urine}}{\text{MPE(gal;M_{6})}_{urine}} \times \frac{\text{MPE(glc;M_{6})}_{plasma}}{\text{MPE(pGlcUA;M_{6})}_{urine}} \tag{Eq. 8}
\]

in which MPE(pGlcUA;M_{6})_{urine} and MPE(gal;M_{6})_{plasma} are the mole percent enrichments of urinary p-GlcUA and plasma glucose, respectively, during an infusion of [U-13C]glucose,

\[
c(UDPglc) = \frac{\text{MPE(gal;M_{6})}_{plasma}}{\text{MPE(pGlcUA;M_{6})}_{urine}} \times \frac{\text{MPE(glc;M_{6})}_{plasma}}{\text{MPE(pGlcUA;M_{6})}_{urine}} \tag{Eq. 9}
\]

in which MPE(glc;M_{6})_{plasma} and MPE(pGlcUA;M_{6})_{urine} are the mole percent enrichments of plasma glucose and urinary p-GlcUA, respectively, during an infusion of [1-2H]-galactose.

Statistical Analysis—All values reported are mean ± S.D. Significance for metabolite and activity levels was determined using the nonparametric Mann Whitney test for unpaired data. Significance for fluxes calculated over time was determined using multiple measurement analysis of variance. Differences were considered significant at \( p < 0.05 \).

RESULTS

The Adaptive Response of Ppara\(^{-/-}\) Mice to Fasting—In Table II the effects of fasting are shown in Ppara\(^{-/-}\) and WT mice of various blood-borne compounds. Glucose concentration tended to be lower at 15 h of fasting but became significantly lower after 24 h of fasting in Ppara\(^{-/-}\) mice compared with WT mice. No differences were observed between WT and Ppara\(^{-/-}\) mice with respect to plasma lactate concentration; lactate concentrations decreased to a similar extent in both groups of mice. Lactate/pyruvate ratios after 24 h fasting were 21.4 ± 4.3 and 22.3 ± 5.0 in WT and Ppara\(^{-/-}\) mice, respectively. Free fatty acid concentrations were significantly higher at 15 h of fasting in Ppara\(^{-/-}\) mice and remained elevated up to 24 h of fasting. In contrast, the ketotic fasting response was diminished in Ppara\(^{-/-}\) mice. After an initial rise in the concentration of 3β-hydroxybutyrate in both groups of mice, the 3β-hydroxybutyrate concentration increased further in WT but not in Ppara\(^{-/-}\) mice. The changes in plasma glycerol concentration did not differ between Ppara\(^{-/-}\) or WT mice. After 24 h of fasting, a decrease in glycerol concentration was observed in both groups of mice. Alanine concentrations were 393 ± 61 μmol/liter in WT mice and 212 ± 27 μmol/liter (p < 0.05) in the Ppara\(^{-/-}\) mice at the end of the fasting period.

In Fig. 1 shows the changes in hepatic glycogen and triglyceride contents in Ppara\(^{-/-}\) and WT mice upon fasting. Liver weight was not different between groups (i.e. 0.9 ± 0.1 and 1.0 ± 0.1 g in WT and Ppara\(^{-/-}\) mice, respectively). When fed, hepatic glycogen content was significantly lower in Ppara\(^{-/-}\) mice than in WT mice. Upon fasting, glycogen decreased more strongly in WT than in knockout mice. After 15 h of fasting, hepatic glycogen content in Ppara\(^{-/-}\) mice was 90 ± 28 μmol/g liver, compared with 69 ± 10 μmol/g liver in WT mice. This difference in response of the hepatic glycogen content was apparent also after 24 h of fasting (i.e. 9 ± 9 μmol/g liver in WT mice and 36 ± 10 μmol/g liver in Ppara\(^{-/-}\) mice). Hepatic G6P levels, determined after 15 h of fasting, were not significantly different (i.e. 322 ± 57 and 397 ± 51 μmol/g liver in the WT and Ppara\(^{-/-}\) mice, respectively). During fasting, hepatic triglyceride content increased in both group of mice. However, in Ppara\(^{-/-}\) mice, the increase in hepatic triglyceride content was significantly more pronounced than in WT mice.

| TABLE II |
| Plasma glucose, lactate, free fatty acid, 3β-hydroxybutyrate, and glycerol concentrations in WT and Ppara\(^{-/-}\) (−/−) mice, either fed or fasted for 15 and 24 h |
| Glucose | Lactate | Free fatty acid | 3β-Hydroxybutyrate | Glycerol |
|---|---|---|---|---|
| WT | −/− | WT | −/− | WT | −/− | WT | −/− |
| Fed | 6.3 ± 1.0 | 6.2 ± 0.6 | ND | | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.3 ± 0.0 | 0.3 ± 0.0 |
| Fasted 15 h | 3.5 ± 0.3 | 2.6 ± 0.5 | 3.0 ± 0.7 | 2.5 ± 0.5 | 0.4 ± 0.1 | 1.0 ± 0.4* | 0.4 ± 0.2 | 0.5 ± 0.1 | 0.5 ± 0.2 | 0.5 ± 0.1 |
| Fasted 24 h | 3.3 ± 0.4 | 2.1 ± 0.3* | 1.7 ± 0.4 | 1.4 ± 0.6 | 0.4 ± 0.1 | 0.9 ± 0.3* | 2.4 ± 0.4 | 0.7 ± 0.1* | 0.3 ± 0.0 | 0.4 ± 0.2 |

Note: Each value represents the mean ± S.D.; n = 5 or 6 per group. * difference \( p < 0.05 \) between Ppara\(^{-/-}\) and WT mice. ND, not determined.
In Fig. 3, the rates of de novo synthesis of G6P into plasma glucose (Fig. 3A), into UDP-glucose (Fig. 3B) and the total de novo synthesis of G6P (Fig. 3C) are shown during the final 3 h of the label infusion experiment. De novo synthesis of G6P into plasma glucose was constant during the experiment. In Ppara−/− mice, the absolute rate of GNG toward plasma glucose was significantly diminished compared with WT mice (i.e., 124 ± 13 versus 142 ± 9 μmol kg−1 min−1 (p < 0.05), respectively). A different observation was made with regard to the absolute rate of appearance of newly synthesized G6P into the UDP-glucose pool. In the course of the experiment, a slow but significant decline was observed in the rate of appearance of newly formed UDP-glucose. In Ppara−/− mice, the decline, from 70 ± 4 μmol kg−1 min−1 at 3 h to 60 ± 5 μmol kg−1 min−1 at 6 h after the start of the label infusion (p < 0.05), was less pronounced than in WT mice, in which it declined from 65 ± 11 μmol kg−1 min−1 at 3 h to 42 ± 5 μmol kg−1 min−1 at 6 h after the start of the label infusion (p < 0.05). At the end of the experiment, the rate of appearance of newly formed UDP-glucose in Ppara−/− mice was therefore significantly higher than in WT mice. Interestingly, the total rate of de novo synthesis of G6P remained constant during the second half of the experiment and was not different between the two groups (i.e., 153 ± 9 and 157 ± 9 μmol kg−1 min−1 in Ppara−/− and WT mice, respectively).

In Fig. 4, the partitioning of newly synthesized G6P into plasma glucose and UDP-glucose is given at 6 h after the start of the label infusion. As is clear from this figure, there was a significantly larger part of newly synthesized G6P diverted to UDP-glucose in Ppara−/− mice than in WT mice. At the end of
the experiment, the fractional contribution of newly formed G6P to UDP-glucose synthesis was 0.40 ± 0.04 in Ppara−/− mice and 0.29 ± 0.03 in WT mice, a significant increase of 32% in Ppara−/− mice as compared with WT mice.

**Hepatic Gene Expression Profiles**—Hepatic expression of genes involved in glucose and fat metabolism in fed and 15- and 24-h fasted mice are shown in Fig. 5. The expected response was observed in expression of genes involved in hepatic glucose and fatty acid oxidation during fasting in WT mice. Expression of Pepck, G6ph, G6pt, Gp, Cpt1a, Mcad, and Hmgs all increased upon fasting. Pepck expression was similarly increased after the 15-h fast compared with the fed situation in both groups but significantly less in the Ppara−/− than in WT mice after a 24-h fast. Only the expressions of G6ph and Gs were significantly affected at 15 h of fasting in Ppara−/− mice compared with WT mice. Hepatic expression of the other genes involved in glucose metabolism (i.e. G6pt, Gp, Gk, and the transcription factor Chreb) were not different in Ppara−/− mice when compared with WT mice after 15 h of fasting. Expression of genes involved in fatty acid oxidation were differently affected. Expression of Cpt1a did not differ between Ppara−/− and WT mice at 15 h of fasting, but expression of Mcad and particularly of Hmgs was significantly decreased in Ppara−/− mice when compared with WT mice. The hepatic expression of the Pparγ gene was increased under all circumstances tested in Ppara−/− mice in comparison with WT mice. Thus, after a 24-h fast, expression of most genes studied was decreased compared with the fed situation and more severely so in Ppara−/− mice. This raised the question of whether this effect of 24-h fasting could be aspecific and simply due to a severe energy shortage in livers of these animals. To try to clarify this, we measured hepatic ATP levels. Hepatic ATP levels showed a trend, albeit not significant, to a decrease after 24 h of fasting in the Ppara−/− mice compared with WT mice (i.e. 0.56 ± 0.13 versus 0.93 ± 0.39 nmol of ATP/mg of liver).

**DISCUSSION**

In the present study, we addressed the role of PPARα in the control of hepatic glucose metabolism (i.e. de novo synthesis of G6P and its partitioning). The results show that after a short term fast of 15 h, de novo synthesis of G6P was not affected by PPARα deficiency. However, newly synthesized G6P was partitioned away from plasma glucose to glycogen synthesis. Furthermore, deficiency of PPARα resulted in a reduced hepatic expression of G6ph, Gs, and, to a lesser extent, Gp.

The validity of the isotope model, with the application of glycoconjugsates and the MIDA approach, has been substantiated in various studies, although some controversy still remains (18). We have validated the application of MIDA in mice in a separate study (7). In that study, we observed major adaptations in whole body and hepatic glucose metabolism in 24-h fasted mice but not in 9-h fasted mice during the course of a 6-h infusion of stable isotopically labeled compounds.

The alterations in the adaptive response to fasting of Ppara−/− mice that we observed were in line with those reported by other investigators (2, 3). The hypoglycemia in Ppara−/− mice did not appear to be due to an enhanced glucose consumption by peripheral tissues (i.e. metabolic clearance of glucose was similar in WT and Ppara−/− mice). Differences exist with respect to the reported time course of development of hypoglycemia in fasted Ppara−/− mice. Kersten et al. (2) and Xu et al. (3) reported significantly lower plasma glucose concentrations after, respectively, 15 and 17 h of fasting in Ppara−/− mice compared with WT mice. In our hands, blood glucose concentrations decreased to a larger extent in WT mice than reported by Kersten et al. (2). As yet, no explanation can be given for this discrepancy.

During infusion of stable isotopically labeled compounds, no decrease was observed in plasma glucose concentration of either Ppara−/− or WT mice. This was different from the observations made in noninfused mice during the adaptive response. Differences in plasma insulin concentrations do not offer an explanation; they were low as would have been expected for fasting mice and did not differ from the values observed in 24-h fasted, noninfused WT and Ppara−/− mice. In our opinion, the absence of a decrease in plasma glucose concentration upon prolonged fasting in infused mice might have been due to the delivery of gluconeogenic substrates by the infusion of stable isotopically labeled compounds. The rates of infusion were considerable, particularly that of [2,13C]glycerol at −60 μmol kg−1 min−1. Whereas all of the infused glycerol would have been utilized for GNG to plasma glucose, however, only 15–20% of the amount of glucose produced through GNG can be accounted for by the glycerol infusion. Furthermore, Previs et al. (18) showed that infusion of this amount of glycerol did not increase endogenous glucose production in 30-h fasted BALBc mice. Their experiments, however, consisted of short term infusion (3 h) of labeled glycerol instead of 6 h as in our experiments and were performed under conditions in which blood glucose concentrations were normal. On the other hand, Xu et al. (3) concluded from their experiments that in Ppara−/− mice glycero appeared to be the preferred gluconeogenic substrate at the expense of lactate. Our results imply that de novo synthesis of G6P exhibits a high elasticity toward the supply of gluconeogenic substrates.

PPARα deficiency resulted in a lower endogenous rate of appearance of glucose than observed in WT mice. Whereas GNG would have been calculated based on its fractional contribution to plasma glucose alone, our results would have led us to infer that total gluconeogenic flux was inhibited in parallel with inhibition of glucose production. By analyzing both plasma glucose and urinary paracetamol-glucuronic acid, however, we were able to show that the decrease in hepatic glucose production was not associated with a decrease in the rate of de novo synthesis of G6P but with a more predominant partitioning of newly synthesized G6P into glycogen. In line with this observation, glycogen content of liver of Ppara−/− mice remained higher during fasting when compared with WT mice. The metabolic fate of newly synthesized G6P, therefore, seems to be without consequence for the rate of its de novo synthesis. Similar lack of feedback inhibition of de novo synthesis of G6P by its product has been documented by us (5) as well as by others (6). In contrast to our conclusions, Xu et al. (3) reported an increase in endogenous glucose production in Ppara−/−

![Fig. 4. Absolute (A) and fractional (B) partitioning of G6P to glucose (light gray) and to UDP-glucose (dark gray) during the last 3 h of the experiment in WT and Ppara−/− mice. Fluxes were determined as described under "Experimental Procedures." Each value represents the mean ± S.D.; n = 6 per group. *, p < 0.05 compared with WT mice.](image-url)
Figure 5. Gene expression of enzymes involved in fatty acid β-oxidation and glucose metabolism in the fed state or after 15 and 24 h of fasting in WT and Ppara<sup>−/−</sup> mice. Levels of cDNA were measured by real-time PCR as described under "Experimental Procedures." Data are expressed as relative to β-actin, and the results of the fed wild type animals are defined as 1. Each value represents the mean ± S.D.; n = 5 or 6 per group. *, p < 0.05 compared with WT mice. pepck, phosphoenolpyruvate carboxykinase; gs, glycogen synthase; cpt1α, carnitine palmitoyltransferase 1α; pk, pyruvate kinase; gp, glycogen phosphorylase; mcad, medium chain acyl-CoA dehydrogenase; g6ph, glucose-6-phosphate dehydrogenase; gk, glucokinase; hmg, 3-hydroxy-3-methylglutaryl-CoA synthase (mitochondrial); g6pt, glucose-6-phosphate translocase; chrebp, carbohydrate-responsive element-binding protein; ppar γ, peroxisome proliferator-activated receptor γ.

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