Beyond lipids, pharmacological PPARα activation has important effects on amino acid metabolism as studied in the rat

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Sheikh K, Camejo G, Lanne B, Halvarsson T, Landergren MR, Oakes ND. Beyond lipids, pharmacological PPARα activation has important effects on amino acid metabolism as studied in the rat. Am J Physiol Endocrinol Metab 292: E1157–E1165, 2007. First published December 12, 2006; doi:10.1152/ajpendo.00254.2006.—PPARα agonists have been characterized largely in terms of their effects on lipids and glucose metabolism, whereas little has been reported about effects on amino acid metabolism. We studied responses to the PPARα agonist WY 14,643 (30 μmol·kg⁻¹·day⁻¹ for 4 wk) in rats fed a saturated fat diet. Plasma and urine were analyzed with proton NMR. Plasma amino acids were measured using HPLC, and hepatic gene expression was assessed with DNA arrays. The high-fat diet elevated plasma levels of insulin and triglycerides (TG), and WY 14,643 treatment ameliorated this insulin resistance and dyslipidemia, lowering plasma insulin and TG levels. In addition, treatment decreased body weight gain, without altering cumulative food intake, and increased liver mass. WY 14,643 increased plasma levels of 12 of 22 amino acids, including glucogenic and some ketogenic amino acids, whereas arginine was significantly decreased. There was no alteration in branched-chain amino acid levels. Compared with the fat-fed control animals, WY 14,643-treated animals had raised plasma urea and ammonia levels as well as raised urine levels of N-methylisocitramide and dimethylglycine. WY 14,643 induced changes in a number of key genes involved in amino acid metabolism in addition to expected effects on hepatic genes involved in lipid catabolism and ketone body formation. In conclusion, the present results suggest that, in rodents, effects of pharmacological PPARα activation extend beyond control of lipid metabolism to include important effects on whole body amino acid mobilization and hepatic amino acid metabolism.

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Diet. Up until and including day 1, all rats ate ad libitum a carbohydrate-rich (chow) diet. Fat feeding commenced on day 2 for groups 2 and 3.

Dosing. On day 9, gavaging, performed once daily at 1300, was commenced with either vehicle or WY 14,643.

Measurements. Body weight and food intake were recorded daily for each rat. On days 1 and 36, in the conscious 3-h fasted state, blood samples were collected at 1000 from the tail vein into vials containing potassium-EDTA (Microvette CB1000; Sarstedt, Nümbrecht, Germany). Plasma was separated and stored at −20°C, awaiting analysis of glucose, insulin, triglycerides (TG), and free fatty acid (FFA) (measured as described in Ref. 27), and in a subset of the samples amino acids and proton NMR spectra were measured as well (n = 7, chow-fed controls; n = 4–5, fat-fed controls; and WY 14,643-treated animals). Animals were killed on day 36 by anesthetic overdose; the liver was weighed and urine collected. Samples of liver were frozen and stored at −70°C for mRNA and urine samples stored at −20°C for NMR analysis.

Sample Preparation and 1H-NMR Spectroscopy

Plasma samples, 400 μl, were transferred to the insert of a 1.5-ml Eppendorf tubes containing 10,000 MW cutoff filters (Ultrafree-MC; Millipore) that were centrifuged at 13,000 rpm for 3 h at 4°C, using a 5417R Eppendorf Centrifuge (Eppendorf, Hamburg, Germany). Plasma filtrate (150 μl) was mixed with 150 μl phosphate buffer, pH 7.3, in D2O containing 0.25 mg/ml sodium 3-trimethylsilyl-[2,2,3,3-2H4]-1-propionate (TSP) as internal reference. Urine samples, 50–60 μl, were diluted with phosphate buffer to a total volume of 180 μl.

The mixtures were transferred to NMR tubes and analyzed at 25°C on a 600-MHz Inova Varian (Palo Alto, CA) spectrometer with a 3-mm pulsed-field-gradient triple-resonance probe. Total number of scans was 256, with four steady-state scans. Acquisition time was 4 s, with a relaxation delay of 1 s. Spectral width was 8,468.3 Hz. Experiments were run with a presaturation pulse sequence to suppress water signals. The TSP signal was used as internal reference at δ0H = 0 ppm. Spectra were phased and baseline corrected using the Varian Vnmr software. Two-dimensional T1TROCSY NMR experiments (3, 21) were performed for metabolite identification.

Multivariate Data Analysis

Multivariate data analysis in combination with 1H NMR was applied for metabolic profiling, as previously described (4, 25, 33).

Urine samples. Data reduction was performed on each spectrum by introducing 210 bins; small spectral regions of width were 0.04 ppm. Acquisition time was 4 s, with a relaxation delay of 1 s. Spectral width was 8,468.3 Hz. Experiments were run with a presaturation pulse sequence to suppress the water signal. The TSP signal was used as internal reference at δ0H = 0 ppm. Spectra were phased and baseline corrected using the Varian Vnmr software. Two-dimensional T1TROCSY NMR experiments (3, 21) were performed for metabolite identification.

Liver Samples

RNA was isolated from ~50 mg liver. The procedure used for isolation, cDNA synthesis and analysis with Affymetrix arrays (Affymetrix, Santa Clara, CA), was performed as previously described (1), with the following modifications. First, strand synthesis was performed for 1 h using Superscript reverse transcriptase (Invitrogen, Stockholm, Sweden). DNA ligase was from Invitrogen. The in vitro transcript reaction was allowed to proceed for 16 h, and fragmentation of cRNA proceeded for 35 min at 94°C. The chip used was Rat Genome 230 2.0 Array (Affymetrix), and the chips were visualized by the addition of streptavidin phycoerythrin using an Affymetrix Gene Chip Fluidics Station 450/250. The Transfac database (BIOBASE; Biological Databases/Biologische Datenbanken) was used to search for putative peroxisome proliferator response element (PPRE) sequence motifs among significantly regulated genes.

Statistical Tests

Differences in group means were evaluated using Student’s t-test with Bonferroni correction. P < 0.05 was considered statistically
Table 1. Basic plasma factors, body weights, and liver weights in chow-fed control, fat-fed control, and fat-fed WY 14,643-treated rats before assignment to diet groups (start) and at the end of the treatment period (end)

<table>
<thead>
<tr>
<th>Plasma Level</th>
<th>Chow Control Start</th>
<th>Chow Control End</th>
<th>Fat Control Start</th>
<th>Fat Control End</th>
<th>Fat WY 14,643 Start</th>
<th>Fat WY 14,643 End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>7.1 ± 0.4</td>
<td>7.1 ± 0.21</td>
<td>7.2 ± 0.18</td>
<td>7.7 ± 0.29*</td>
<td>6.6 ± 0.12</td>
<td>7.4 ± 0.13</td>
</tr>
<tr>
<td>Insulin, nM</td>
<td>0.23 ± 0.03</td>
<td>0.23 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.61 ± 0.07*</td>
<td>0.15 ± 0.03</td>
<td>0.35 ± 0.04†</td>
</tr>
<tr>
<td>TG, mM</td>
<td>0.62 ± 0.10</td>
<td>0.97 ± 0.14</td>
<td>0.81 ± 0.07</td>
<td>2.19 ± 0.22*</td>
<td>0.51 ± 0.06</td>
<td>0.89 ± 0.11†</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.52 ± 0.06</td>
<td>0.69 ± 0.06</td>
<td>0.42 ± 0.04</td>
<td>0.74 ± 0.08</td>
<td>0.5 ± 0.06</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>Urea, mM</td>
<td>NM</td>
<td>7.6 ± 0.3</td>
<td>NM</td>
<td>6.1 ± 0.5*</td>
<td>NM</td>
<td>7.5 ± 0.6†</td>
</tr>
<tr>
<td>Ammonia, mM</td>
<td>NM</td>
<td>103 ± 20</td>
<td>NM</td>
<td>128 ± 23*</td>
<td>NM</td>
<td>160 ± 15†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>382 ± 6</td>
<td>436 ± 9</td>
<td>391 ± 6</td>
<td>447 ± 10</td>
<td>395 ± 5</td>
<td>435 ± 11†</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>NM</td>
<td>11.2 ± 0.4</td>
<td>NM</td>
<td>12.1 ± 0.4</td>
<td>NM</td>
<td>18.9 ± 0.9†</td>
</tr>
</tbody>
</table>

Data presented as means ± SE (n = 7–10/group except for urea and ammonia, where n = 4–7/group). TG, triglycerides; FFA, free fatty acids; NM, not measured. *P < 0.05 chow control vs. fat-fed controls; †P < 0.05 treated fat-fed vs. fat-fed controls.

Results

Basic Characteristics of the Chow-Fed, Fat-Fed Control, and Fat-Fed WY 14,643-Treated Groups

Basic data for the three groups are presented in Table 1. In the fat-fed control animals, the plasma levels of insulin and TG were characteristically elevated compared with chow-fed controls. Treatment with WY 14,643 (30 μmol·kg⁻¹·day⁻¹) for 4 wk resulted in lower insulin levels, without changing the normoglycemic glucose levels, indicating an increase in insulin sensitivity. Treatment lowered plasma TG compared with the fat-fed controls. Both groups of fat-fed animals gained weight over the treatment period; however, the rate of gain was lower in the WY 14,643-treated group (31.3 g vs. 40.8 g) compared with the fat-fed group (48.8 g vs. 5.6 g/27 days) vs. the chow-fed group. WY 14,643 treatment increased liver weight in treated rats compared with both control groups, an expected PPARα agonist effect in rodents.

Metabolite Identification in Urine and Plasma by NMR

Representative NMR spectra for plasma and urine samples are shown in Fig. 1. Labels are included for peaks corresponding to known and identified metabolites. PCA plots (not shown) and manual addition of standards were used to identify metabolites of interest in urine and plasma. Several group-specific elevations in metabolites were identified: taurine and N-methyl nicotinamide (MNA) in WY 14,643-treated rats, betaine/trimethylaminoxide (TMAO) in fat-fed rats, and citrate in chow-fed rats. Peaks that were identified by PCA were then inspected in the original NMR spectrum and manually compared with spectra from the other groups for visual confirmation.

Urine NMR Analysis

Fat-fed control group vs. chow-fed group. NMR analysis of urine revealed that hippuric acid and citrate relative concentrations were reduced in animals on a fat diet, whereas betaine/TMAO was excreted in higher concentrations in the fat-fed control group compared with the chow-fed control animals (Fig. 2).

Fig. 2. Relative peak areas of metabolites identified in NMR spectra of urine samples. Cit, citrate. Data are means ± SE (n = 7–10/group). *P < 0.05 vs. chow control; †P < 0.05 vs. fat control.
Plasma HPLC Analysis

Fat-fed control group vs. chow-fed group. The results of the HPLC analysis of amino acids in plasma are summarized in Fig. 4. The high-fat diet was associated with significantly higher plasma concentrations of the amino acids aspartate, threonine, serine, glutamate, proline, alanine, valine, methionine, isoleucine, leucine, and lysine, whereas glutamine and arginine were found in lower concentrations. The plasma concentration of urea was lowered, whereas ammonia concentration was raised in the fat-fed controls compared with the chow-fed animals (Table 1).

WY 14,643-treated group vs. fat-fed control group. As suggested by the initial NMR analysis, HPLC measurements of plasma revealed increased concentrations of glycine, threonine, glutamine, and lysine with WY 14,643 treatment. In addition, plasma levels of the following amino acids were also elevated by treatment: taurine, citrulline, ornithine, phenylalanine, tyrosine, tryptophan, serine, and histidine. Arginine was the only amino acid present in lower concentrations compared with fat-fed controls. WY 14,643 treatment increased levels of both urea and ammonia (Table 1) compared with fat-fed controls.

Liver Transcriptomics

The results from the NMR analysis of urine and plasma, as well as the HPLC analysis, suggested multiple effects on amino acid metabolism. Therefore, we explored whether these changes were associated with alterations in expression of genes involved in nitrogen metabolism in the liver.

Chow-fed group vs. fat-fed control group. Results of the hepatic gene array analysis for genes involved in amino acid metabolism, together with the enzyme name abbreviations, are summarized in Table 2. Of the 25 genes reported, only five genes exclusively involved in amino acid metabolism were significantly different in fat- and chow-fed control groups.

WY 14,643-treated group vs. fat-fed control group. WY 14,643 treatment of the fat-fed rats changed a large number of genes, with a substantial contribution of genes previously reported to be involved in mitochondrial and peroxisomal fatty acid oxidation. In addition, treatment with WY 14,643 induced a number of alterations in mRNA level of genes involved in amino acid metabolism (Table 2). The majority of these genes could be related to the following three metabolically distinct functions: 1) reduced catabolism of several essential and non-essential amino acids, as illustrated in Fig. 5; 2) increased polyamine synthesis (Fig. 6); and 3) enhanced ketone body production with upregulation of both 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGC2; the rate limiting step in ketone body production) and 3-hydroxy-3-methylglutaryl CoA lyase.

All genes in Table 2 were scanned, within 4,000 base pairs of the coding regions, for PPRE-like motifs using the Transfac database. As expected, this search identified the established functional PPRE (34) on the ketone body enzyme HMGC2. In addition, putative PPREs were identified only in the promoter regions of four of the remaining 23 genes; these were argininosuccinate lyase (ASL), phosphoserine aminotransferase, cystathionine γ-lyase (CTH), and alanine-glyoxylate aminotransferase 2.

DISCUSSION

Rats were fed a high-fat diet to provide an animal model of dyslipidemia and insulin resistance, allowing studies of the pharmacodynamic effects of the PPARα agonist WY 14,643. As expected, WY 14,643 increased liver mass, raised plasma ketone body levels, and lowered plasma TG as well as insulin levels. We used NMR and HPLC analysis of plasma and urine as well as analysis of hepatic mRNA to obtain an integrated view of the metabolic consequences of WY 14,643 treatment.
Principal component analysis of the NMR data revealed distinct metabolic effects of diet and treatment.

Published work examining effects of the PPAR agonists (12) has almost exclusively focused on lipid and glucose metabolism. The lack of information concerning effects on in vivo amino acid metabolism prompted the current study. Important lism. The lack of information concerning effects on in vivo has almost exclusively focused on lipid and glucose metabo-
tinct metabolic effects of diet and treatment. Principal component analysis of the NMR data revealed dis-

<table>
<thead>
<tr>
<th>Enzyme (Affymetrix probe set identity)</th>
<th>Gene Name</th>
<th>Fat Control/ Chow Control</th>
<th>Fat WY 14,643/Fat Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea production</td>
<td>CPS1</td>
<td>NS 0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Argininosuccinate synthetase, 1370964_at</td>
<td>ASS NS 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Argininosuccinate lyase, 1368916_at, 1367754_s_at</td>
<td>ASL NS 0.6, 0.7</td>
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<tr>
<td>Polyamine production</td>
<td>Ornithine decarboxylase 1, 1370163_at</td>
<td>ODC1 NS 2.3</td>
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</tr>
<tr>
<td></td>
<td>4-Aminobutyrate aminotransferase, 1369799_at</td>
<td>ABAT NS 0.56</td>
<td></td>
</tr>
<tr>
<td>Essential amino acid degradation</td>
<td>2-Aminoisobutyric acid 3-hydroxy-6-semialdehyde decarboxylase, 1383111_at</td>
<td>ACM5D 2.3 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Histidine ammonia lyase, 1387307_at</td>
<td>HAL NS 0.27</td>
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<tr>
<td></td>
<td>Cystathionine γ-lyase, 1367838_at</td>
<td>CTH 2.3 0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cystathionine β-synthase, 1387178_a_at</td>
<td>CBS NS 0.58</td>
<td></td>
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<tr>
<td></td>
<td>Phenylnalanine hydroxylase, 1387034_at</td>
<td>PAH NS 0.59</td>
<td></td>
</tr>
<tr>
<td>Nonessential amino acid degradation</td>
<td>Tyrosine aminotransferase, 1369790_at</td>
<td>TAT NS 2.1</td>
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<tr>
<td></td>
<td>4-Hydroxyphenylpyruvic acid dioxygenase, 1368188_at</td>
<td>HPD NS 0.61</td>
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<tr>
<td></td>
<td>Alanine aminotransferase, glutamic pyruvic transaminase 1, 1387052_at</td>
<td>AAT1 1.7 0.59</td>
<td></td>
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<tr>
<td></td>
<td>Glutaminase 2 (liver), 1370375_at</td>
<td>GLS2 NS 0.52</td>
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<td>Ornithine aminotransferase, 1367729_at</td>
<td>OAT NS 0.49</td>
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<tr>
<td>Phospholipid metabolism</td>
<td>Sarcosine dehydrogenase, 1397744_at, 1372323_at</td>
<td>SARDH NS 0.5, 0.64</td>
<td></td>
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<td></td>
<td>Dimethylglycine dehydrogenase, 1370936_at</td>
<td>DMGDH NS 0.6</td>
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<tr>
<td>Serine metabolism</td>
<td>Phosphoserine aminotransferase 1, 1372665_at</td>
<td>PSAT1 NS 5.2</td>
<td></td>
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<tr>
<td>Miscellaneous</td>
<td>Glycine methyltransferase, 1387672_at</td>
<td>GNMT 2.2 0.41</td>
<td></td>
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<td></td>
<td>Alanine-glyoxylate aminotransferase 1, 1387215_at</td>
<td>AGT1 NS 2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alanine-glyoxylate aminotransferase 2, 1368659_at</td>
<td>AGT2 NS 1.4</td>
<td></td>
</tr>
<tr>
<td>Ketone body production</td>
<td>Acetyl-CoA acetyltransferase 1, 1367763_at, 1383416_at</td>
<td>ACAT1 NS 5.5, 5.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-Hydroxy-3-methylglutaryl-CoA synthase, 1389161_at, 1370310_at</td>
<td>HMGCS2 NS 2.1, 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-Hydroxy-3-methylglutaryl-CoA lyase, 1367767_at</td>
<td>HMGCL NS 2.9</td>
<td></td>
</tr>
</tbody>
</table>

Numbers show statistically significant fold changes (P < 0.05) in mRNA transcription relative to control groups (n = 5/group). NS, not significant. Note that, in some cases, individual genes have been assessed using 2 probe sets.

Different situation in the liver tissue. Thus, of the detected changes in hepatic gene expression associated with amino acid metabolism, the majority were compatible with the observed hepatic growth, suggesting a reduction in the capacity of each gram of tissue to catabolize amino acids and an enhanced capacity for polyamine synthesis. Overall, our findings are consistent with an extension of the role of PPARα beyond the control of fatty acid and glucose to important effects on amino acid metabolism in rodents.

The treatment-induced reduction of plasma arginine concentration may be a key observation in unraveling the amino acid metabolic response to WY 14,643. This may be related to the considerable (56%) increase in liver mass. Since arginine is a so-called conditionally essential amino acid, with dietary requirements increasing during periods of rapid growth, the reduced arginine concentration may be a consequence of the observed rapid hepatic growth or the result of a PPARα-mediated reduction in arginine synthesis. The observation that the precursors citrulline and ornithine were markedly increased, whereas arginine was reduced, is compatible with a treatment-induced limitation in arginine synthesis. Some support for this possibility is provided by the mRNA results in the liver with arginosuccinate synthetase (ASS) and ASL expression, involved in the conversion of citrulline to arginine, both being downregulated by WY 14,643. These results confirm the findings of Kersten et al. (16). However, the liver is most likely not a significant contributor to the systemic arginine pool in...
adult mammals. Instead, arginine synthesis is thought to be performed mostly by the kidney using citrulline taken up from the plasma (38). Whether similar transcriptional effects occur in the kidney and are responsible for lowering the plasma arginine levels remains to be determined. Furthermore, since fatty acids or their metabolic products are the endogenous ligands of PPARα (15), the intriguing possibility exists that PPARα activation may also have mediated the reduction in arginine level observed in response to high-fat feeding.

In contrast to the lowered plasma levels of arginine, WY 14,643 treatment induced increases in the plasma concentrations of 12 of the 22 measured amino acids. These increases could have resulted from either increased rates of entry into the plasma compartment or reduced rates of plasma clearance. In this regard, future tracer kinetic studies will be decisive, although the increased levels of plasma urea and ammonia may be consistent with an elevation in hepatic amino acid catabolism. Two effects of treatment might be expected to have resulted in a large increase in urea production. First, the increased plasma levels of several amino acids, including glutamine, the major transporter of systemic nitrogen, could increase catabolism by mass action. Second, the increase in hepatic mass should increase the urea production capacity. The fact that treatment resulted in only modest increases in urea and ammonia, however, could be explained by the opposing effect of treatment to reduce the per gram rate of hepatic amino acid catabolism, as suggested by the mRNA data discussed below.

The hepatic enlargement induced by WY 14,643 treatment is associated with cell proliferation (35), and we interpret the majority of the observed changes in hepatic expression of genes involved in amino acid metabolism as supporting this growth and cell division. First, a number of changes were compatible with suppression of hepatic amino acid catabolism, a situation favoring conservation of amino acids for local synthetic processes, including protein and purine synthesis. A reduction in the hepatic per gram rate of general amino acid degradation was suggested by the downregulation of three genes involved in urea production, carbamoyl-phosphate synthetase 1, ASS, and ASL (Fig. 5), in agreement with previous findings in mice (16). In addition, we observed reduction of mRNA expression for groups of genes, including those for rate-limiting enzymes, mediating the degradation of the essential amino acids phenylalanine, histidine, methionine, and tryptophan and those for the degradation of the nonessential amino acids alanine, tyrosine, glutamine, and ornithine, as illustrated in Fig. 5. It is interesting to note that 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase is an important branch point in tryptophan metabolism, with its downregulation tending to favor production of nicotinate mononucleotide, a precursor for NAD (Fig. 5). An elevated flux through this pathway is indeed
consistent with the observed increase in urine levels of MNA, a breakdown product of NAD. These findings concerning tryptophan metabolism confirm the results of previous studies of PPARα agonists in rodents (31, 37).

In addition to changes that may translate to conservation of amino acids for synthesis, there was also evidence that WY 14,643 induced a coordinated regulation of gene expression for enhancement of polyamine synthesis, a key event enabling cell division. This included several changes, illustrated in Fig. 6A, that could increase the capacity to provide ornithine for polyamine synthesis, including an upregulation of ornithine decarboxylase 1. However, polyamine synthesis would not be increased solely on the basis of these changes. A corresponding increase in propylamine supply, provided by the conversion of decarboxylated adenosylmethionine to 5-methylthioadenosine, would also be required for the conversions of putrescine to...
spermidine and spermidine to spermine (Fig. 6A). Indeed, a second group of gene expression changes, illustrated in Fig. 6B, could indirectly support such an increase in the supply of propylamine. Thus, mRNA for two enzymes, cystathionine β-synthase and CTH, mediating the initial steps in the transulfuration sequence, which results in irreversible loss of the essential amino acid methionine, was reduced by WY 14,643. This could tend to conserve methionine for either methionine-homocysteine cycling or methionine-methyl thioribose cycling (producing propylamine). Increased diversion of adenosylmethionine through the latter cycle might well be favored in situations of rapid growth (13), and indeed, the observed reductions in mRNA levels of enzymes performing the transmethylation reactions [glycine methyltransferase, sarcosine dehydrogenase (SARDH), and dimethylglycine dehydrogenase (DMGDH)] provide support for this scenario. It is interesting to note that, consistent with a reduction in DMGDH (and/or SARDH) function, we observed a substantial increase in DMG concentration in urine of treated rats (Fig. 2).

To our knowledge there is only one previous study that reports effects of a PPARα agonist on plasma amino acid levels in rodents (28). A high dose of clofibrate (300 mg·kg⁻¹·day⁻¹) was given to rats fed a standard chow diet for 2 wk. The treatment induced only modest changes, lowering four out of a total of 13 amino acids measured (arginine not reported). One possible explanation for the difference between the results of this previous study and the present study is that compound specific effects not mediated via PPAR activation may be involved. Along these lines, clofibrate is known to be a direct inhibitor of branched-chain α-keto acid dehydrogenase kinase at pharmacologically relevant concentrations, whereas WY 14,643 is not (18). Indeed, elevated branched-chain amino acid oxidation has been proposed to be responsible for the state of muscle wasting seen in clofibrate-treated animals due to impaired protein synthesis resulting from a lack of branched-chain amino acids (28). Regarding the generality of the current findings, we have recently observed a similar amino acid response to a structurally unrelated PPARα agonist in Chow-fed mice (Oakes ND, unpublished observations).

The fact that a number of amino acids were elevated by WY 14,643, including several essential amino acids (lysine, tryptophan, threonine, and phenylalanine), is consistent with a treatment-induced increase in protein turnover. The mechanism for this amino acid mobilization is at present unknown. However, the data suggest several alternative possibilities. First, reduced arginine availability may be a key triggering event, since there are striking similarities between the present response to WY 14,643 (involving rapid hepatic growth) and the plasma amino acid profile in transgenic mice that have an arginine deficiency due to overexpression of arginase (8). During rapid growth, these mice have elevated plasma levels of many of the amino acids, including glycine, serine, threonine, tryptophan, ornithine, and phenylalanine, like those observed in our experiment. A second explanation for an increased protein turnover may be related to the treatment-induced reduction in plasma insulin levels leading to an increase in net protein degradation (24). It should be noted, however, that PPARα agonists have been shown to increase insulin sensitivity (39), which could tend to cancel the effect of the insulin level. A third possible explanation is that increased protein degradation may have resulted from the lipid-lowering action of WY 14,643. Thus, it has been shown that high plasma levels of FFAs and ketone bodies can decrease protein degradation (11, 26). The lower plasma lipid levels could have triggered an increase in whole body protein degradation. Finally, the increased amino acid mobilization resulting from WY 14,643 treatment may be associated with the elevated demands for hepatic liver protein synthesis. The relevance of the current findings to humans will require clinical studies of PPARα agonists.

An important issue concerns the nature of the relationship between PPARα activation, the hepatic enlargement, and the alterations in amino acid metabolism. In unpublished experiments conducted in our laboratory using NMR imaging and the same experimental design, it was found that the hepatic enlargement occurs very early after start of treatment (+27 ± 3% after only 2 days). In our view, the available evidence may suggest that the changes in amino acid metabolism are mainly associated with the liver enlargement. First, transcriptional activation is mediated by the binding of PPARα to response elements usually located in the promotor region of the gene (20). However, a search of the significantly regulated hepatic genes did not yield evidence for the existence of an abundance of PPREs associated with the set of genes related to amino acid handling. An additional line of evidence that the changes in amino acid metabolism were related to hepatic growth is suggested by similarities with another, completely independent situation, the regeneration following partial (usually 70%) hepatectomy in rats with restoration of liver size within 2 wk. An early and indeed essential step in this process is increased hepatic polyamine synthesis (2). In terms of PPARα activation, one study (9) has shown activation of polyamine synthesis occurring only 5 h following a single dose of clofibrate and, moreover, that chronic pharmacological inhibition of this pathway substantially reduced the treatment-induced hepatomegaly. Another similarity is that partial hepatectomy induces an increase in total circulating amino acid concentration involving a number of individual species, including serine and glycine (5, 14). Furthermore, plasma arginine levels have been reported to be markedly reduced during hepatic regeneration (5). Additional work is needed to evaluate the relevance of our findings to the clinical situation, where PPARα agonism does not appear to induce hepatic enlargement (6). However, the current findings are important given that a large fraction of the substantial literature concerning PPARα pharmacology and biology is based upon studies performed in rodents.

In conclusion, in addition to expected effects on lipid and glucose metabolism, WY 14,643 induced substantial alterations in systemic amino acid metabolism in fat-fed rats. Although further study is required to establish the generality of these findings, the results suggest that PPARα activation has important metabolic effects on the handling of all major substrate classes.

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