Effects of fatty acids and growth hormone on liver fatty acid binding protein and PPARα in rat liver

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Carlsson, Linda, Daniel Lindén, Masoumeh Jalouli, and Jan Oscarsson. Effects of fatty acids and growth hormone on liver fatty acid binding protein and PPARα in rat liver. Am J Physiol Endocrinol Metab 281: E772–E781, 2001.—The aim of this study was to investigate the interaction between long-chain fatty acids (LCFA) and growth hormone (GH) in the regulation of liver fatty acid binding protein (LFABP) and peroxisome proliferator-activated receptor-α (PPARα). Cultured rat hepatocytes were given oleic acid (OA; 500 μM) and GH (100 ng/ml) for 3 days. LFABP mRNA increased 3.6-fold by GH and 5.7-fold by OA, and combined incubation with GH and OA increased LFABP mRNA 17.6-fold. PPARα mRNA was decreased 50% by GH, but OA had no effect. Hypophysectomized (Hx) female rats were treated with L-thyroxine, cortisol, GH, and dietary fat for 7 days. PPARα mRNA levels were three- to fourfold higher in Hx than in normal female rats. GH decreased PPARα mRNA 50% in Hx rats. Dietary triglycerides (10% corn oil) increased LFABP mRNA and cytosolic LFABP about twofold but had no effect on PPARα mRNA in Hx rats. GH and dietary triglycerides had an additive effect on LFABP expression. Dietary triglycerides increased mitochondrial hydroxymethylglutaryl-CoA synthase mRNA only in the presence of GH. The diet increased serum triglycerides in Hx rats, and GH treatment prevented this increase. Addition of cholesterol to the diet did not influence LFABP levels but mitigated increased hepatic triglyceride content. In summary, these studies show that GH regulates LFABP expression independently of PPARα. Moreover, GH has different effects on PPARα-responsive genes and does not counteract the effect of LCFA on the expression of these gene products.

Peroxisome proliferators, such as fibrates, increase LFABP and LFABP mRNA levels in vivo (1) and in cultured hepatocytes (8), and the LFABP promoter has been shown to contain a DR-1 peroxisome proliferator-responsive element (5). LCFA can activate the peroxisome proliferator response element via binding to and activation of PPARα (17, 27, 36). Incubation of a rat hepatoma cell line and primary cultures of rat hepatocytes with LCFA have been shown to increase LFABP mRNA levels (34). A diet enriched in triglyceride has been shown to increase the amount of hepatic LFABP and LFABP mRNA in rats (1, 2, 33, 47) and mice (26, 36), indicating that an increased hepatic uptake of LCFA in vivo also upregulates LFABP. Moreover, the use of PPARα-null mice has clearly indicated the importance of PPARα for the effect of LCFA on LFABP gene expression (18, 26).

PPARα mRNA expression is also increased by dietary triglycerides (26) and under hormonal control in the liver. Glucocorticoids have been shown to increase hepatic PPARα expression both in vivo and in vitro (29, 32, 35) (for review see Refs. 1, 5, 15, 43). Several enzyme activities, especially enzymes involved in fatty acid metabolism, are stimulated by LFABP, but it is not clear whether LFABP specifically enhances esterification or β-oxidation pathways (1, 24, 43). Overexpression of LFABP in a fibroblast cell line (L-cells) produces alterations in membrane phospholipids and intracellular cholesterol distribution, resulting in increased fluidity of plasma membranes (23, 49). Moreover, LFABP may have a role in the trafficking of LCFA to the nucleus (28) and subsequent activation of peroxisome proliferator-activated receptor-α (PPARα) (48), further emphasizing the importance of LFABP in LCFA metabolism.

Activation of both PPARα and classical hormones regulates LFABP expression in the liver at the mRNA level (1, 8, 9, 36, 37). Growth hormone (GH) increases LFABP mRNA and cytosolic LFABP levels in hypophysectomized (Hx) rats (6, 9). By use of cultured rat hepatocytes, it was shown that GH increases LFABP mRNA levels via increased transcription and that the effect of GH was dependent on the presence of insulin in the culture medium (9).

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42), and the increased PPARα expression was shown to increase the responsiveness to a peroxisome proliferator (29). In contrast, if there are a few reports that indicate that GH may decrease the PPARα activity, GH may interfere with PPARα signaling, as suggested from the finding that nuclear signal transducer and activator of transcription-5b inhibited PPARα-dependent reporter gene expression (52). Moreover, GH has been shown to decrease PPARα mRNA levels in cultured rat hepatocytes (51) and to counteract the increase in peroxisomal β-oxidation induced by peroxisome proliferators (44, 50). Thus GH and LCFA alone increase LFABP gene expression, but the interplay between these factors is not known.

The primary aim of the present study was to investigate the interaction between oleic acid (OA) and GH in cultured hepatocytes and the interaction between GH and dietary triglycerides in Hx rats on LFABP expression. The second aim was to study the regulation of LFABP by GH in cultured hepatocytes and the interaction between OA and GH. The interaction between oleic acid (OA) and GH may interfere with PPARα signaling, as suggested from the finding that nuclear signal transducer and activator of transcription-5b inhibited PPARα-dependent reporter gene expression (52). Moreover, GH has been shown to decrease PPARα mRNA levels in cultured rat hepatocytes (51) and to counteract the increase in peroxisomal β-oxidation induced by peroxisome proliferators (44, 50). Thus GH and LCFA alone increase LFABP gene expression, but the interplay between these factors is not known.

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MATERIALS AND METHODS

The Ethics Committee of Göteborg University approved this study. All chemicals used were from Sigma Chemical (St. Louis, MO), if not stated otherwise.

Animals. Female Sprague-Dawley rats from Møllegaard Breeding Center (Ejby, Denmark) were used. Hypophysectomy was performed at 50 days of age by Møllegaard Breeding Center. Intact, age-matched female rats served as controls in two experiments. The rats were maintained under standardized conditions of temperature (24–26°C) and humidity (50–60%) and with lights on between 0500 and 1900. The rats had free access to standard laboratory chow (rat and mouse standard diet, B&K Universal, Sollentuna, Sweden) and water. The standard laboratory chow contains (wt/wt) 42% carbohydrate, 20% protein, and 38% fat. Two different kinds of diets enriched in fat were used. In one experiment, corn oil alone (10% wt/wt; Mazola, CPC Food, Kristianstad, Sweden) was added to the powdered diet, and in the other experiment, cholesterol (2% wt/wt) and corn oil before it was mixed with the rat and mouse standard diet. The percentage of triglyceride in the diet was increased from 2.5 to ~12% (wt/wt). The fatty acid composition of corn oil triglycerides is 13% saturated, 29% monounsaturated, and 58% polyunsaturated fatty acids.

Hormonal treatment started 7–10 days after hypophysectomy. Hx rats were given cortisol phosphate (400 mg/kg, Solu-Cortef, Upjohn, Puurs, Belgium) and L-thyroxine (1 µg/g, T4, 100 µg/kg, Solu-Cortef, Upjohn, Puurs, Belgium) and water. The standard laboratory chow contains (wt/wt) 58% polyunsaturated fatty acids.

Probes. Total RNA was prepared according to Chomczynski and Sacchi (10). LFABP mRNA was measured using a 333-bp fragment, 26 nt upstream from ATG to nt 307 of rat LFABP cDNA (accession no. J00732.1) inserted in a pSP 72 vector (Promega, Madison, WI) (9). The pSP 72 vector was linearized with PvuII, and a [32P]UTP antisense RNA probe or a [35S]CTP antisense RNA probe was generated with T7 RNA polymerase (Maxiscript; Ambion, Austin, TX). For PPARα mRNA measurements, a 249-bp fragment, nt 76 from ATG to nt 324 of rat PPARα cDNA (accession no. M88592) was subcloned into a pBluescript II vector (Stratagene, La Jolla, CA). The full-length rat PPARα plasmid was kindly supplied by Dr. Jørgen Fantvold, Karolinska Institute, Stockholm, Sweden. The pBluescript plasmid was linearized with EcoRI, and a [35S]UTP or [32P]CTP antisense RNA probe was generated with T7 RNA polymerase (Maxiscript). A biotin-labeled PPARα antisense RNA probe was generated using Biotin-16-UTP (Enzo, Roche) in the reaction.

A plasmid containing rat mitochondrial (m)HMG-CoA synthase cDNA (pJA 118A) was kindly supplied by Dr. Fausto G. Hegardt, University of Barcelona, Spain. The pJA 118A plasmid was linearized with HincII. A biotin-labeled antisense mHMG-CoA synthase RNA probe of ~1,000 bp was generated using T3 RNA polymerase. The probe was labeled using Biotin-16-UTP and Strip-EZ RNA StripAble RNA probe synthesis kit (Ambion). A 163-bp fragment (nt 444–nt 606) of rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (Ambion) or an 80-bp fragment of 18S (Ambion) was used as internal controls of the gel ribonuclease protection assays and Northern blot.

Gel ribonuclease protection assays. Gel ribonuclease protection assays with the use of radioactive probes were performed as described by the manufacturer (RPA III kit, Ambion). [32P]-labeled antisense LFABP, PPARα, and GAPDH RNA probes were used. Protected fragments were separated on denaturing polyacrylamide 6% TBE-urea gels (Novex, San Diego, CA). The RNA probes were used.

Conclusion. The regulation of LFABP in the liver is not known.
Detection and quantification were performed with a Phospholmager and Image Quant software (Molecular Dynamics, Sunnyvale, CA).

Biotin-labeled PPARα and 18S RNA probes were also used. The protected fragments were transferred from the polyacrylamide 6% TBE-urea gels to Bright Star-Plus membranes (Ambion) by a semidy transfer system (Trans-blot cell; Bio-Rad). After the transfer, the protected fragments were cross-linked by ultraviolet irradiation (UVC Crosslinker, Hoefer) to the membrane, and detection was carried out using the Bright Star BioDetect Kit as described by the manufacturer (Ambion). The chemiluminescence was detected and quantified using Fluor-S-Multimager (Bio-Rad). The band intensity of the LFABP band or the PPARα band was divided by the intensity of the housekeeping GAPDH or 18S band. The amounts of the transcripts are expressed as the ratio between these bands.

Solution hybridization assays. Measurement of LFABP mRNA and PPARα mRNA levels was performed using solution hybridization assays as described previously (9, 12). In brief, 35S-labeled antisense RNA probes were hybridized to aliquots of RNA. After hybridization, the samples were treated with RNase T1 and A. Protected hybrids were precipitated and collected on glass fiber filters and counted in a scintillation counter. The LFABP standard (sense) used in the solution hybridization assay was generated after linearization with EcoRI. The PPARα standard (sense) used was generated after linearization with Asp718. The hybridization signal obtained with the antisense probe was compared with the signal of a standard curve of sense RNA. The results are expressed as picograms per microgram of total RNA.

Northern blot. Twenty micrograms of RNA were denatured with glyoxal and DMSO and run in a 1% agarose gel according to the protocol of the manufacturer (NorthernMax-Gly; Ambion). The RNA was blotted onto a Bright Star-Plus nylon membrane (Ambion) and covalently linked to the membrane by ultraviolet irradiation (UVC Crosslinker). The membrane was prehybridized at 68°C for 2 h in ULTRAhyb (Ambion). The same solution was used for hybridization for 16 h at 68°C. The final wash of the membranes was carried out in Low Stringency Wash Solution no. 1 (Ambion) for 15 min at room temperature, followed by two washes for 15 min at 68°C in High Stringency Wash Solution no. 2 (Ambion). The detection was carried out using a Bright Star BioDetect Kit, as described by the manufacturer (Ambion). The chemiluminescence was detected and quantified using Fluor-S-Multimager (Bio-Rad). The intensity of the mHMG-CoA synthase mRNA band was divided by the intensity of the 18S band. The amounts of the transcript are expressed as the ratio between these bands.

Other analyses. NEFA concentrations were analyzed with a colorimetric assay according to the manufacturer (NEFA C, ACS-ACOD method; Wako Chemicals, Neuss, Germany). Serum triglyceride levels were determined using a colorimetric enzymatic assay (GPO-PAP-kit; Boehringer Mannheim, Mannheim, Germany). The content of triglyceride and total cholesterol in the livers was determined after homogenization of frozen liver in ice-cold water, followed by extraction of total lipids (7). The chloroform phase containing total lipid was evaporated until dryness under a stream of N2, and the lipid phase was resolved in ethanol. The mass of triglycerides and total cholesterol was measured in duplicate with enzymatic colorimetric assays (triglycerides: GPO-PAP-kit; cholesterol: CHOD-PAP-kit; Boehringer Mannheim).

Statistics. Values are expressed as means ± SE. Comparisons between means were made by analysis of variance (ANOVA), followed by a Student-Newman-Keuls multiple comparison test.
range test (post hoc test) between individual groups. The values were transformed to logarithms when appropriate.

RESULTS

**In vitro effects of GH and OA on LFABP mRNA and PPARα mRNA.** In a previous study, it was observed that GH increased LFABP mRNA in vitro, but this effect was dependent on the presence of insulin (9). The interaction between GH and OA on LFABP mRNA expression was therefore investigated in the presence of insulin (Fig. 1A). GH (100 ng/ml) increased LFABP mRNA levels 3.6-fold and 500 μM OA increased LFABP mRNA levels 5.7-fold. In the presence of GH, incubation with 500 μM OA resulted in a 4.9-fold increase in LFABP mRNA levels. Incubation with both OA and GH resulted in a 17.6-fold increase in LFABP mRNA expression compared with control cell cultures (Fig. 1A). These findings indicate that GH and OA have an additive effect on LFABP mRNA expression and that the responsiveness of the cultured hepatocytes to OA was not markedly affected by GH incubation. In one previous study (51), it was shown that incubation of cultured hepatocytes with GH resulted in a decrease in PPARα mRNA levels. Therefore, we also studied the interaction between GH and OA in the regulation of PPARα mRNA in the cultured hepatocytes (Fig. 1B). GH incubation decreased PPARα mRNA ∼50%, but incubation with OA had no effect (Fig. 1B). Two-way ANOVA (GH and OA incubation as factors) showed that GH had a significant effect ($P < 0.007$), but the effect of OA was not significant ($P = 0.10$; Fig. 1B). To study whether the effect of GH on PPARα expression was dependent on the presence of insulin, hepatocyte cultures were incubated with GH (100 ng/ml) the last 3 days of culture without insulin. The results from three separate experiments showed that the relative PPARα mRNA level (divided by the densitometric value of 18S) was 0.055 ± 0.005 in cell cultures without hormones and 0.028 ± 0.005 ($P < 0.05$) in cell cultures incubated with GH for 3 days. Thus GH decreased PPARα mRNA to a similar degree without concomitant treatment with insulin.

**In vivo effects of hypophysectomy and GH on PPARα mRNA.** Next, the effects of hypophysectomy (Hx) and GH on liver PPARα mRNA were investigated (Fig. 2A). Hx and combined treatment with T4 and cortisol markedly...
increased PPARα mRNA. GH treatment of Hx female rats given T4 and cortisol decreased the levels of PPARα mRNA (Fig. 2A). Because the substitution with T4 (51) and cortisol (29, 42) may have contributed to the high level of PPARα mRNA in Hx rats, we investigated the effects of Hx without any hormone treatment on PPARα mRNA levels in the liver. Hx of female rats increased PPARα mRNA levels four- to fivefold, and treatment with T4 and cortisol had no further effect (Fig. 2B). To investigate whether the effect of GH might be enhanced in the absence of T4 and cortisol, the effect of GH was investigated with and without T4 and cortisol treatment (Fig. 2C). In this experiment, one-half of the rats in each group were killed between 0930 and 1030, and the other rats in each group were killed between 1330 and 1430. We did not observe any consistent trends in PPARα mRNA levels between rats killed in the morning and those killed in the afternoon (data not shown); therefore, these groups were pooled. As seen in Fig. 2C, GH decreased PPARα mRNA levels to a larger degree in the absence of T4 and cortisol treatment; i.e., T4 and cortisol increased PPARα mRNA levels in the presence of GH.

In vivo effects of dietary triglycerides and GH. Next, the effects of a diet enriched in triglycerides were investigated in Hx female rats with and without GH therapy (Table 1 and Fig. 3). All Hx rats were given T4 and cortisol, and the diet and the hormones were both given for 7 days. The weight gain of the rats was increased by GH treatment but was not affected by the triglyceride diet (Table 1). The triglyceride-enriched diet increased PPARα mRNA levels in Hx rats, but the effect was not significant (Fig. 3A). GH decreased PPARα mRNA levels to a similar extent (~50%) in the rats given a triglyceride diet and in rats given ordinary rat chow (Fig. 3A).

In Hx control rats, LFABP mRNA and cytosolic LFABP levels increased 1.9- and 2.3-fold, respectively, by the triglyceride-enriched diet (Fig. 3, B and C). GH treatment of the Hx rats increased LFABP mRNA levels fourfold and LFABP levels 2.7-fold. In the GH-treated rats, the triglyceride diet resulted in a further 50% increase in LFABP levels but had no significant effect on LFABP mRNA levels (Fig. 3, B and C). We also measured mHMG-CoA synthase mRNA, because this gene product has been shown to be regulated by PPARα and to increase by fat feeding (20) (Fig. 3D). A triglyceride-enriched diet had no effect in Hx rats given T4 and cortisol but resulted in higher mHMG-CoA synthase mRNA levels in GH-treated Hx rats. Thus GH decreased mHMG-CoA synthase mRNA levels during the low-fat diet but increased mHMG-CoA synthase mRNA levels when the rats were given the triglyceride-enriched diet (Fig. 3D).

The effects of the triglyceride-enriched diet on serum levels of NEFA and triglycerides were measured as indications of changes in circulating levels of LCFA (Table 1). The triglyceride diet increased serum NEFA levels in the Hx control rats but did not significantly affect the levels of NEFA in the GH-treated Hx rats. The triglyceride diet increased serum triglyceride levels in the Hx control rats but decreased serum triglyceride levels in the GH-treated group of rats (Table 1). Thus serum triglyceride levels were markedly lower after GH therapy when the rats were given the triglyceride diet, but serum triglyceride levels were not affected by GH therapy when the rats were given the ordinary chow.

The triglyceride-enriched diet increased the triglyceride content of the livers in Hx control rats by 109% and in the GH-treated Hx rats by 49% (Table 1). Liver triglyceride content was not affected by GH treatment. The liver content of cholesterol increased ~30% in both groups of rats by the triglyceride diet but was not influenced by GH (Table 1).

In vivo effects of GH and dietary triglycerides and cholesterol. LFABP may be involved in intracellular cholesterol distribution (23, 49), and we could not exclude that the 30% increase in hepatic cholesterol (Table 1) influenced LFABP expression. Therefore, the effects of a diet enriched in both triglycerides and cholesterol on LFABP expression were studied in Hx female rats (Table 2 and Fig. 4). As in the previous experiment, all Hx rats were given T4 and cortisol, and the diet and the hormones were both given for 7 days. The weight gain of the rats was increased by GH treatment but was not affected by the fat diet (Table 2). The diet induced a twofold increase in LFABP but had no significant effect on LFABP mRNA levels in the Hx control rats (Fig. 4). GH treatment resulted in a 3.5-fold increase in LFABP mRNA levels and a 1.7-fold increase in cytosolic LFABP levels (Fig. 4). In the group of rats given GH, the fat diet increased LFABP mRNA 50% and cytosolic LFABP levels 60% (Fig. 4, A and B).

The triglyceride- and cholesterol-enriched diet resulted in increased serum NEFA levels in both groups.

**Table 1. Effects of GH and dietary TG on body weight gain, liver lipid content, and serum levels of NEFA and TG in Hx female rats**

<table>
<thead>
<tr>
<th>Hormone Treatment</th>
<th>Diet</th>
<th>Weight Gain, g/day</th>
<th>Serum TG, mmol/l</th>
<th>Serum NEFA, mmol/l</th>
<th>Liver TG, μmol/g liver</th>
<th>Liver Cholesterol, μmol/g liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hx</td>
<td>TG</td>
<td>0.2 ± 0.1b</td>
<td>0.96 ± 0.04b</td>
<td>0.24 ± 0.02a</td>
<td>6.1 ± 1.1b</td>
<td>9.3 ± 0.3b</td>
</tr>
<tr>
<td>Hx</td>
<td>TG</td>
<td>0.1 ± 0.1b</td>
<td>1.42 ± 0.13a</td>
<td>0.35 ± 0.02a</td>
<td>12.8 ± 0.5a</td>
<td>12.6 ± 0.4a</td>
</tr>
<tr>
<td>Hx + GH</td>
<td>TG</td>
<td>4.0 ± 0.2a</td>
<td>0.81 ± 0.04b</td>
<td>0.24 ± 0.02b</td>
<td>7.8 ± 0.4b</td>
<td>9.6 ± 0.3b</td>
</tr>
<tr>
<td>Hx + GH</td>
<td>TG</td>
<td>3.2 ± 0.3a</td>
<td>0.58 ± 0.07a</td>
<td>0.29 ± 0.02b</td>
<td>11.6 ± 0.7a</td>
<td>12.2 ± 0.5a</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–7 rats/group. GH, growth hormone; TG, triglyceride; NEFA, nonesterified fatty acid; Hx, hypophysectomized. Dietary TG was 10% corn oil (wt/wt). Rats were treated as described in the legend to Fig. 3. Analyses of serum levels of NEFA and TG as well as liver content of TG and total cholesterol were performed as described in MATERIALS AND METHODS. Values with different superscripts are significantly different from each other (P < 0.05, 1-way ANOVA followed by Student-Newman-Keuls test).

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of Hx rats, but GH treatment had no effect (Table 2). Serum triglyceride levels were markedly increased by the diet in Hx control rats, but not in the group of rats given GH. Thus, as also observed in the other experiment (Table 1), GH treatment resulted in lower serum triglyceride levels in the Hx rats given the high-fat diet but not in Hx rats given ordinary chow (Table 2).

In contrast to the triglyceride-enriched diet, the triglyceride- and cholesterol-enriched diet did not affect the triglyceride content of the livers (Table 2). However, the liver content of cholesterol was increased twofold (Table 2).

**DISCUSSION**

This study showed that GH regulates LFABP in a PPARα-independent manner. Furthermore, it was shown that GH and LCFA have additive effects on the

**Table 2. Effects of GH and dietary TG and cholesterol on body weight gain, liver lipid content, and serum levels of NEFA and TG in Hx female rats**

<table>
<thead>
<tr>
<th>Hormone Treatment</th>
<th>Diet</th>
<th>Weight Gain, g/day</th>
<th>Serum TG, mmol/l</th>
<th>Serum NEFA, mmol/l</th>
<th>Liver TG, μmol/g liver</th>
<th>Liver Cholesterol, μmol/g liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hx</td>
<td>CTG</td>
<td>0 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7 ± 1.6</td>
<td>8.5 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hx</td>
<td>CTG</td>
<td>0.6 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.13 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5 ± 0.6</td>
<td>21.3 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hx + GH</td>
<td>CTG</td>
<td>4.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6 ± 1.5</td>
<td>11.1 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hx + GH</td>
<td>CTG</td>
<td>5.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6 ± 0.3</td>
<td>20.0 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–7 rats/group. CTG; dietary cholesterol (2% (wt/wt)) plus TG (10% corn oil). Rats were treated as described in the legend to Fig. 4. Analyses of serum levels of NEFA and TG as well as liver content of TG and total cholesterol were performed as described in MATERIALS AND METHODS. Values with different superscripts are significantly different from each other (P < 0.05, 1-way ANOVA followed by Student-Newman-Keuls test).

Fig. 3. Effects of GH and dietary triglyceride [TG, 10% corn oil (wt/wt)] on PPARα mRNA (A), LFABP mRNA (B), cytosolic LFABP levels (C) and mitochondrial hydroxymethylglutaryl (HMG)-CoA synthase mRNA (D) in Hx female rats. Female rats were hypophysectomized at 50 days of age. After 7–10 days of observation, the rats were given hormones and dietary fat for 7 days. All Hx rats were given T4 (10 μg·kg<sup>-1</sup>·day<sup>-1</sup>) and cortisol phosphate (C; 400 μg·kg<sup>-1</sup>·day<sup>-1</sup>) as a daily sc injection. Bovine GH (1 mg·kg<sup>-1</sup>·day<sup>-1</sup>) was given as a continuous sc infusion by means of osmotic minipumps. PPARα mRNA and LFABP mRNA levels were determined with solution hybridization assays, and HMG-CoA synthase mRNA levels were measured with Northern blot. Cytosolic LFABP levels were determined with ELISA, as described in MATERIALS AND METHODS. There were 4–7 rats in each group. Values are means ± SE. Values with different superscripts are significantly different from each other (P < 0.05, 1-way ANOVA followed by Student-Newman-Keuls test).
expression of LFABP. In contrast, PPARα mRNA expression was decreased by GH and was not affected by LCFA. Thus LCFA can induce LFABP mRNA to the same degree when PPARα mRNA expression is decreased. This finding is in contrast to another study, which indicated that the increased LFABP mRNA expression by dietary triglycerides could be fully explained by increased PPARα mRNA expression (26). Thus the decreased PPARα mRNA expression cannot explain the effects of GH and LCFA on LFABP expression but may contribute to the understanding of the inhibitory effect of GH on peroxisomal β-oxidation (44, 50). Another kind of interaction between GH and the triglyceride content of the diet was observed when we studied the expression of mHMG-CoA synthase mRNA. GH treatment decreased mHMG-CoA synthase mRNA levels when the rats were given ordinary chow. This effect of GH may be explained by a decreased PPARα expression. On the other hand, GH treatment was important for the normal stimulation of mHMG-CoA synthase mRNA by dietary triglycerides. This effect cannot be explained by the inhibitory influence of GH on PPARα mRNA expression or activity (52). There are several reports that GH stimulates ketogenesis and that this effect is largely dependent on an increased supply of fatty acids (25). The regulation of mHMG-CoA synthase mRNA by GH and a triglyceride-enriched diet may therefore contribute to an explanation at the mRNA level of how fatty acids and GH interact in the control of ketogenesis.

The finding that hypophysectomy of female rats markedly enhanced the expression of PPARα mRNA indicates that, at least in female rats, the sum of the influence of the pituitary-dependent hormones is inhibitory on PPARα mRNA expression. In contrast to LFABP expression (1), PPARα expression follows a diurnal rhythm in the intact rat (29). The rats were killed in the morning, when the hepatic PPARα mRNA levels are low (29). From the present results, it may therefore be concluded that the low expression of PPARα in the morning could not be explained solely by low glucocorticoid levels but was also dependent on the inhibitory effect of GH. We observed no effect of combined treatment with T4 and cortisol to Hx rats, but T4 and cortisol increased expression of PPARα mRNA when GH was given. The results, therefore, indicate that the stimulatory effect of T4 (51) and cortisol (29, 42) is dependent on the presence of GH in vivo. The doses of T4 and cortisol have previously been shown to be within the physiological range with respect to longitudinal bone growth (22, 45). Moreover, the serum levels of T4 obtained are about two times the levels in intact female rats (41). Thus the small effect of T4 and cortisol is not due to the doses of the hormones but may be explained by the use of Hx rats as a model.

It must be pointed out that we did not measure PPARα protein levels. It has been shown in some instances that the PPARα protein levels follow the mRNA levels (3, 29), but it remains to show that the effect of GH on PPARα mRNA levels results in changed PPARα protein expression. For example, the half-life of

Fig. 4. Effect of GH and a diet enriched in cholesterol and triglyceride (CTG, 10% corn oil and 2% cholesterol (wt/wt)) on LFABP mRNA (A) and cytosolic LFABP levels (B) in Hx female rats. Female rats were hypophysectomized at 50 days of age. After 7–10 days of observation, the rats were given hormones and dietary fat for 7 days. All Hx rats were given T4 (10 μg·kg⁻¹·day⁻¹) and cortisol phosphate (C; 400 μg·kg⁻¹·day⁻¹) as a daily sc injection. Bovine GH (1 mg·kg⁻¹·day⁻¹) was given as a continuous sc infusion by means of osmotic minipumps. LFABP mRNA was determined with a solution hybridization assay and LFABP in cytosol with an ELISA as described in MATERIALS AND METHODS. There were 4–7 rats in each group. Values are means ± SE. Values with different superscripts are significantly different from each other (P < 0.05, 1-way ANOVA followed by Student-Newman-Keuls test).
the PPARα protein may be too long to decrease significantly during a week of GH therapy.

The diets had to some extent little or no effect on LFABP mRNA levels, whereas the effects on cytosolic LFABP protein levels were more marked. These results indicate that dietary triglycerides also have a posttranscriptional effect on LFABP expression. In fact, it has been shown that LFABP ligand availability is of importance for the protease susceptibility of the protein (21). Thus it may be speculated that increased cytosolic LFABP content may be due to both increased amounts of translatable mRNA and increased binding of LCFA to LFABP, which may stabilize the protein.

The addition of cholesterol to the triglyceride-enriched diet resulted in largely similar effects on the expression of LFABP and its mRNA as the triglyceride-enriched diet, indicating that cholesterol has no major influence on the expression of LFABP mRNA or its protein. Moreover, the addition of cholesterol to the triglyceride-enriched diet counteracted the effect of dietary triglycerides on liver triglyceride content. This result indicates that the increased LFABP expression is not due to increased hepatic triglyceride content per se, but rather to the flux of fatty acids into the liver cells.

The mechanisms behind the effect of dietary cholesterol on the liver triglyceride content can only be speculated on. This effect was surprising and may be due to the fact that we studied Hx rats because treatment of intact rats with cholesterol has been shown to increase hepatic content of triglycerides (14). However, cholesterol feeding of Hx rats may result in another effect on hepatic triglycerides. Thus the hepatic triglyceride content may have been reduced via an effect of cholesterol on the proportion of fatty acids used for very low density lipoprotein (VLDL) assembly. In favor of this hypothesis is the finding that dietary cholesterol increases the expression of microsomal triglyceride transfer protein (4) and VLDL secretion (14). Moreover, the higher serum triglyceride levels when the rats were given a diet enriched in both cholesterol and triglycerides compared with the effect of dietary triglycerides alone indicate a higher VLDL secretion.

In this study, the NEFA levels in serum were increased by the diets but were not affected by GH treatment. In other studies, an increase, a transient increase, and no effect of GH on serum NEFA levels have been observed (16). Most studies that showed elevation of NEFA by GH treatment were performed in fasting animals (16), which was not the case in this study. We have previously shown that GH treatment of Hx rats given ordinary rat chow slightly decreased or had no effect on serum triglyceride levels (38, 39). In this study, GH had no effect on serum triglyceride levels during chow feeding. However, during high-fat feeding, GH treatment resulted in markedly lower serum triglyceride levels. The mechanism behind the different effect of GH on serum triglycerides during chow feeding and fat feeding is unclear but could be due to either a decrease or a prevention of an increase in serum triglyceride levels during fat feeding. Most probably, a decreased production or an increased degradation of triglyceride-rich lipoproteins explains this effect. We have shown, by use of primary hepatocyte cultures, that GH increases VLDL secretion both ex vivo (39) and in vitro (30). Moreover, OA and GH had an additive effect on VLDL secretion (30). Therefore, the lower serum triglyceride levels in GH-treated fat-fed rats are not likely to be due to a decreased VLDL secretion. However, the effect of GH on serum triglycerides may be explained by an increased proportion of VLDL containing apoB-48 (30, 41), because apoB-48 VLDL have a faster turnover than apoB-100 VLDL. Moreover, GH increases the capacity for degradation of triglyceride-rich lipoproteins via increased hepatic lipase activity and increased lipoprotein lipase activity in heart and skeletal muscle (39).

It is unlikely that the effect of GH on LFABP mRNA is mediated via a decreased metabolism of fatty acids and a build up of an intracellular pool of fatty acids. First, we have previously shown that GH increases LFABP mRNA in vitro within 1–3 h (9). Second, GH has been shown to increase mitochondrial β-oxidation (11) and specifically the oxidation of OA (13). Moreover, GH has been shown to increase the incorporation of OA into hepatic triglycerides (13, 30). These findings indicate that, at least, the intracellular amount of OA does not build up as a result of GH treatment. Because the effect of OA on LFABP mRNA was similar in the absence and presence of GH, a more specific transport or activation is needed to explain the effect of OA on LFABP mRNA expression. However, we cannot rule out the possibility that the intracellular amount of very long chain fatty acids is built up as a result of the inhibitory effect of GH on peroxisomal β-oxidation (44, 50). It is tempting to speculate that a fatty acid-activated transcription factor other than PPARα is involved in the regulation of LFABP and HMG-CoA synthase by GH and LCFA. One such candidate is hepatocyte nuclear factor-4α, which has been shown to be involved in the regulation of both LFABP and PPARα expression (19) and to be regulated by GH at the mRNA level (40).

In summary, this study shows different interactions between GH and LCFA in the regulation of the PPARα-responsive gene products PPARα, LFABP, and mHMG-CoA synthase. Moreover, GH has different effects on PPARα-responsive genes and does not counteract the effect of LCFA on the expression of these gene products.

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