Evidence against the peroxisome proliferator-activated receptor α (PPARα) as the mediator for polyunsaturated fatty acid suppression of hepatic L-pyruvate kinase gene transcription

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Abstract **The glycolytic enzyme, L-pyruvate kinase (L-PK), plays an important role in hepatic glucose metabolism. Insulin and glucose induce L-PK gene expression, while glucagon and polyunsaturated fatty acids (PUFA) inhibit L-PK gene expression. We have been interested in defining the PUFA regulation of L-PK. The *cis*-regulatory target for PUFA action includes an imperfect direct repeat (DR1) that binds HNF-4. HNF4 plays an ancillary role in the insulin/glucose-mediated transactivation of the L-PK gene. Because the fatty acid-activated nuclear receptor, peroxisome proliferator-activated receptor (PPARα), binds DR1-like elements and has been reported to interfere with HNF4 action, we examined the role PPARα plays in the regulation of L-PK gene transcription. Feeding rats either fish oil or the potent PPARα activator, WY14,643, suppressed rat hepatic L-PK mRNA and gene transcription. The PPARα-null mouse was used to evaluate the role of the PPARα in hepatic transcriptional control of L-PK. While WY14,643 control of L-PK gene expression required the PPARα, PUFA regulation of L-PK gene expression was independent of the PPARα. Transfection studies in cultured primary hepatocytes localized the *cis*-regulatory target for WY14,643/PPARα action to the L-PK HNF4 binding site. However, PPARα/RXRα heterodimers did not bind this region. Although both WY14,643 and PUFA suppress L-PK gene transcription through the same element, PUFA regulation of L-PK does not require the PPARα and PPARα/RXRα does not bind the L-PK promoter. These studies suggest that other intermediary factors are involved in both the PUFA and PPARα regulation of L-PK gene transcription.**—Pan, D. A., M. K. Mater, A. P. Thelen, J. M. Peters, F. J. Gonzalez, and D. B. Jump. **Evidence against the peroxisome proliferator-activated receptor α (PPARα) as the mediator for polyunsaturated fatty acid suppression of hepatic L-pyruvate kinase gene transcription.** ***J. Lipid Res.*** 2000. **41: 742–751.**

Supplementary key words Hepatic nuclear factor-4 (HNF4) • peroxisome proliferators • cytochrome P450 4A • glycolysis • lipogenesis

Liver-type pyruvate kinase (L-PK) plays a central role in glycolysis by regulating the flux through the phosphoenolpyruvate/pyruvate cycle. Acute control of L-PK involves phosphorylation and allosteric factors. Chronic control involves changes in gene expression mediated by both hormones and nutrients (1). Although insulin and glucose augment L-PK gene expression, the role of insulin in this process is to increase glucokinase levels and stimulate glucose metabolism (2, 3). Enhanced glucose metabolism, possibly through the pentose phosphate pathway, may also be important for induction of hepatic L-PK gene expression (2, 3).

Studies in primary hepatocytes have identified a *cis*-regulatory region within the L-PK promoter required for glucose-mediated transactivation. This region is located between −183 and −96 bp upstream from the L-PK transcription start site and is termed glucose (or carbohydrate) response region [GlRR/CHORR] (4–7). The GlRR contains binding sites for 3 classes of transcription factors. Two E-boxes (CANNTG) separated by 5 nucleotides bind USF-related transcription factors. Mutational analyses have shown that the E-boxes are critical for glucose induction of L-PK gene transcription. However, studies using USF1 or USF2-null mice and dominant negative versions of USF1 and USF2 in primary hepatocytes have not clari-

Abbreviations: L-PK, liver type pyruvate kinase; GlRR, glucose response region; CHORR, carbohydrate response region; PUFA, polyunsaturated fatty acids; MLTF, major late transcription factor; HNF4, hepatocyte nuclear factor 4; LF-A1, liver factor activator 1; NF1, nuclear factor 1; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; CYP4A2, cytochrome P450 A2; CAT, chloramphenicol acetyltransferase; AOX, acyl CoA oxidase; AOX-PPRE, acyl-CoA oxidase peroxisome proliferator response element; IVTT, in vitro transcription translation; EMSA, electrophoretic mobility shift assays; DR1, direct-repeat-1.

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fied the role these transcription factors play in the glucose control of this gene (8, 9). The identity of the glucose-regulated transcription factor controlling L-PK gene transcription is unknown.

Mutational analyses have identified a second region that plays a key ancillary role in glucose-mediated transactivation of L-PK (5–7). This region, located between −145 and −120 bp, contains an imperfect direct repeat of TGAACT separated by one nucleotide and is termed a DR1. This region binds HNF4 and NF1 (4, 9, 10). HNF4 has a stimulatory role, while NF1 may be inhibitory to L-PK gene expression (10). NF1 also binds at −115/−97 bp .

L-PK gene expression is suppressed by diabetes, glucagon, and polyunsaturated fatty acids [PUFA] (1–4, 11, 12). Highly unsaturated fatty acids like eicosapentaenoic acid (20:5, n−3) are particularly strong suppressors of L-PK gene expression (11, 12). PUFA-regulated factors interfere with the glucose-mediated transactivation of L-PK by targeting the 3′ region of the GlRR. This PUFA-response region (PUFA-RR) is located between −145 and −120 bp and contains the DR1 binding site for HNF4 (12).

We have been interested in defining the molecular basis of PUFA control of L-PK gene expression. A likely candidate for this control is the peroxisome proliferator-activated receptor (PPAR)α. This view is based on the following evidence. First, the PPARα is the principal PPAR subtype expressed in liver and its activity is controlled by structurally diverse hydrophobic molecules, including fatty acids (13–15). Previous studies in our lab have shown that the PPARα is required for PUFA-mediated induction of the peroxisomal enzyme acyl CoA oxidase and the microsomal enzyme CYP4A2 (16). Second, the PPARα forms heterodimers with RXRα that bind DR1-type elements in promoters of regulated genes (13, 16, 17). The PPARα/RXRα heterodimer can induce the expression of multiple hepatic genes involved in fatty acid transport, binding, and oxidation (13, 18–20). Finally, Hertz et al. (21, 22) have reported that the PPARα competes with HNF4 for binding a DR1 element within the apoC-III and transferrin promoters. Their studies showed that while HNF4 binding supports apoC-III promoter activity, the PPARα suppresses promoter activity.

Based on this evidence and the fact that PUFA regulation of L-PK targets a region containing a DR1 (12) there is the possibility that PPARα/RXRα may compete with HNF4 binding the DR1 to suppress L-PK gene transcription. We have tested this hypothesis by evaluating the effect of PPARα activators, i.e., WY14,643, gemfibrozil, clofibrate, on L-PK gene transcription and mRNA levels in rat and mouse liver and rat primary hepatocytes. These studies will show that while PPARα activators affect L-PK gene expression in vivo and in primary hepatocytes, this effect does not involve direct interaction of PPARα with the L-PK promoter.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats (125–150 g) were obtained from the Charles River Breeding Laboratories (Kalamazoo, MI). Hepatic RNA samples from male C57BL/6N X Sv/129 mice (25–35 g), F6 homozygote wild-type (+/+) or PPARα-null (−/−) mice were obtained from two previous feeding studies (14, 16). Rats were housed in the Michigan State University Animal facility, and all experimental protocols were approved by the All University Animal Use Committee at Michigan State University. With the exception of mice fed clofibrate or WY14,643, the animals were trained to a meal-feeding regimen of a high carbohydrate diet (16, 23). Test diets for rats consisted of a high carbohydrate (58% glucose) diet (ICN, Cleveland, OH) supplemented with either 10% (w/w) of complex fats (triolein, olive oil, fish (menhaden) oil), 0.1% WY-14,643 (ChemSyn Laboratories, Lenexa, KS) or 0.2% gemfibrozil (Sigma, St. Louis, MO) for 5 days. Diets were supplemented with 0.1% butylated hydroxytoluene as an antioxidant. Mice were fed clofibrate or WY14,643 at 0.5% or 0.1% (w/w) in a chow control diet for a 2-week period.

Northern and nuclear run-on analysis

Total RNA from rat and mouse livers and from cultured rat hepatocytes was isolated and used for Northern analysis (12, 23). Hybridization was quantified using a Molecular Dynamics phosphorimager (Sunnyvale, CA). The cDNA probes used for L-PK, microsomal cytochrome P450 4A2 (CYP4A2), fatty acid synthase, and β-actin were described previously (12, 23). PO (Genbank accession # Z29530) encodes a highly conserved rodent ribosomal phosphoprotein. The human version of this sequence is 3B64. A PO cDNA was prepared by RT-PCR of rat liver RNA using the following sense and antisense primers: [sense: ATATATGAATTCC GTGATG CCCAGGGAAGACAGGCGA; antisense:ATATATGGA TCCTCATCGG ATTCCTCCGACTC]. PO was used in place of β-actin in some studies to monitor the specificity of drug effects on hepatic gene expression.

Rat liver nuclei were extracted and used for nuclear transcriptional run-on analysis as previously described (11). cDNAs used in this analysis included L-pyruvate kinase [L-PK] (11, 12), fatty acid synthase [FAS] (11), acyl CoA oxidase [AOX] (16), cytochrome P450 4A [CYP4A2] (16), and β-actin (11). Hybridization signals were quantified using a Molecular Dynamics Phosphorimager.

Preparation and transfection of hepatocytes

Primary rat hepatocytes were isolated by a modified collagenase perfusion method as described by Mater, Thelen, and Jump (23). For RNA studies, primary hepatocytes were plated onto 100-mm Primaria plates at 10^7 cells/plate in Williams E medium containing 10 mm lactate, 10% fetal bovine serum, 200 nm insulin, 10 nm dexamethasone, 100 U penicillin/ml, and 100 μg streptomycin/ml. For transfection studies, hepatocytes were plated on 60-mm Primaria plates at 3×10^6 cells/plate in the same medium. After an attachment period of approximately 6 h, the cells were washed with phosphate-buffered saline (PBS), switched to serum-free Williams E media containing 10 mm lactate, and transfected with cesium chloride-purified reporter plasmids. The construction of the chloramphenicol acetyltransferase (CAT) reporter plasmids used in this report were described previously (12, 16). LPK-CAT-2800 contains the L-PK promoter extending from −2800 to +12 bp; PKCAT-197 contains the L-PK promoter extending from −197 to +12 bp; LPK-CAT-96 contains the L-PK promoter extending from −96 to +12 bp; LPK-CAT-LS4 and LPK-CAT-LS5 contain linker scanning mutations within the −197/+12 region at −143/−135 and −130/−123 bp. The TK223 construct contains the rat AOX-peroxisome proliferator response region fused upstream from the thymidine kinase promoter [TK] (16).

Cells were transfected using Lipofectin (Life Technologies, Inc., Grand Island, NY) at a ratio of 6.6 μg of Lipofectin to 1 μg of reporter plasmid DNA. Two micrograms of reporter construct

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were added per 60-mm plate. After overnight transfection, cells were treated for 48 h with serum-free Williams E containing 50 μm albumin (ALB) and 10 mm lactate or 25 mm glucose. Cells were also treated with either oleic acid (18:1, n−9) or arachidonic acid (20:4, n−6) at 300 μm in 25 mm glucose media (11, 12). Alternatively, the cells were treated with either DMSO as vehicle or 100 μm WY14,463 or 100 μm gemfibrozil in 25 mm glucose media. CAT activity and protein concentration was measured (16, 23) and results are expressed as CAT activity in units [1 unit of CAT activity = 1 CPM [^{14}C]acetylated chloramphenicol/h/100 μg protein].

Electrophoretic mobility shift assays (EMSA)

The PPARα and the RXRα were synthesized in vitro using TNT® T7 coupled reticulocyte lysate (IVTT) system (Promega, Milwaukee WI). HNF4 was synthesized in vitro using a T3 coupled IVTT and the plasmid BL-rHNF4 [obtained from F. M. Sladek] (24). These expression plasmids produce a single protein using [^{35}S]methionine labeling. EMSA was carried out as described (25) using ^{32}P-labeled DNAs (~20,000 cpm) and in vitro synthesized PPARα, RXRα, or HNF4 (2 μl each) in 25 mm Tris-Cl, pH 7.5, 40 mm KCl, 0.1% Nonidet P-40, 1 μg of poly(dI:dC), 10% glycerol. After incubation at room temperature, samples were electrophoretically separated in 10% acrylamide:bisacrylamide (BioRad) with 90 mm Tris borate, pH 8.3, 2.5 mm EDTA as buffer (150 V for 1 h). In binding competition studies, 1 fmol to 1 pmol (in 10-fold increments) of competitor DNA (non-radioactive) was preincubated with IVTT nuclear receptors for 20 min at room temperature prior to the addition of ^{32}P-labeled DNA. After electrophoretic separation, the gels were dried and exposed to X-ray films. The sense oligonucleotides used in the competition studies were:

5′ to 3′ CCGAACGTGACCTTTGTCCT [AOX-PPRE] (24);
5′ to 3′ AGCTTGCAGGTGACCTTTGCCCAGCGCCA [ApoCIII-DR1] (22);
5′ to 3′ CTCCCGTGGTTCCTGGACTCTGGCCCCCAGTG TACAA [L-PK-LFA1] (12);
5′ to 3′ GATCCTCAGGTCAAAGGTCAGAG [DR-1] (26).

Statistical analysis

All data are presented as either mean ± SD or mean ± SE. Statistical comparisons were made by a single-factor factorial analysis of variance (ANOVA; $\alpha = 0.05$) using Microsoft Excel version 7.

RESULTS

Effects of dietary fat and WY14,643 on liver L-PK and CYP4A2 mRNA abundance

The PPARα is activated in vivo by both WY14,643 and fish oil feeding (14, 16). The effects of these compounds on rat liver mRNAs encoding L-PK, FAS, CYP4A2, and β-actin were compared (**Fig. 1**). CYP4A2 was used as a positive control for PPARα activation (16) while β-actin was used to assess the specificity of the treatment effect. Animals were meal-fed high carbohydrate diets supplemented with triolein or fish oil at 10% (w/w) or WY14,643 at 0.1% (w/w) for 5 days. When compared to triolein-fed animals, fish oil and WY14,643 suppressed L-PK by 81 and 83%, respectively. FAS mRNA was suppressed 70% by fish oil, but was not significantly affected by WY14,643. While $mRNA_{CYP4A}$ was induced 3.7- and 6.7-fold, respectively, β-actin was not significantly affected by these treatments.

To determine whether the effects of fish oil and WY14,643 on L-PK mRNA were due to changes in gene transcription, transcriptional run-on analysis of nuclei isolated from livers of the animals described in Fig. 1A was performed. Run-on results for FAS, CYP4A, and β-actin are presented for controls for the specificity of response. Figure 1B is a representative autoradiogram illustrating the run-on data for L-PK, FAS, β-actin, CYP4A, and AOX. L-PK, CYP2A, and β-actin run-on activity in rat liver is low relative to FAS. L-PK run-on activity was suppressed 73% and 84% by fish oil and WY14,643 treatment, respectively. FAS run-on activity was suppressed 85% after fish oil feeding, but was not significantly affected by WY14,643 feeding. CYP4A run-on activity was marginally (20%) induced by fish oil and induced 2-fold after WY14,643 feeding. β-Actin run-on activity was not significantly affected by fish oil or WY14,643.

Comparing the effect of both fish oil and WY14,643 on the suppression of $mRNA_{LPK}$ (Fig. 1A) and run-on activity (Fig. 1C) indicates that the principal mechanism for WY14,643 and fish oil control of L-PK gene expression is at the transcriptional level. While FAS transcription was suppressed by fish oil ingestion, feeding WY14,643 did not significantly affect FAS gene transcription or $mRNA_{FAS}$. The pronounced effect of both fish oil and WY14,643 on $mRNA_{CYP4A}$, with marginal effects on run-on activity, may indicate that these treatments may affect $mRNA_{CYP4A}$ stability, as well as CYP4A gene transcription.

WY14,643 and clofibrate, but not PUFA, require the PPARα to suppress L-PK gene expression

Finding that both fish oil and WY14,643 regulate L-PK gene transcription is consistent with a role for PPARα as a regulator of L-PK gene expression (Fig. 1). To determine whether PPARα is required for the transcriptional effects of fish oil and WY14,643 on L-PK, we took advantage of the PPARα-null mouse (14). In the first study, wild-type (+/+) and PPARα-null (−/−) mice were fed a high carbohydrate diet supplemented with either olive or fish oil at 10% w/w for 5 days (**Fig. 2A**). Interestingly, $mRNA_{LPK}$ levels in livers of olive oil-fed animals were increased 2-fold in the PPARα-null mice when compared to the wild-type animals. When compared to olive oil-fed animals, hepatic $mRNA_{LPK}$ was suppressed 50% in the wild-type animals and 70% in the PPARα-null mice with fish oil feeding. By comparison, fish oil induced $mRNA_{CYP4A2}$ 6.2-fold in the wild-type, but had no significant effect on CYP4A expression in the PPARα-null mice. β-Actin remained unaffected by these treatments. These results indicate that while the PPARα is required for PUFA induction of $mRNA_{CYP4A2}$, it was not required for the PUFA-mediated suppression of $mRNA_{LPK}$.

A similar analysis was performed with two hypolipidemic drugs, i.e., clofibrate and WY14,643. Mice were fed diets supplemented with clofibrate (0.5%, w/w) or WY14,643 (0.1%, w/w) for 2 weeks (Fig. 2B) (14). As before (Fig. 2A), L-PK mRNA was increased 50% in the PPARα-null mice when compared to wild-type mice. In wild-type mice, both clofibrate and WY14,643 suppressed

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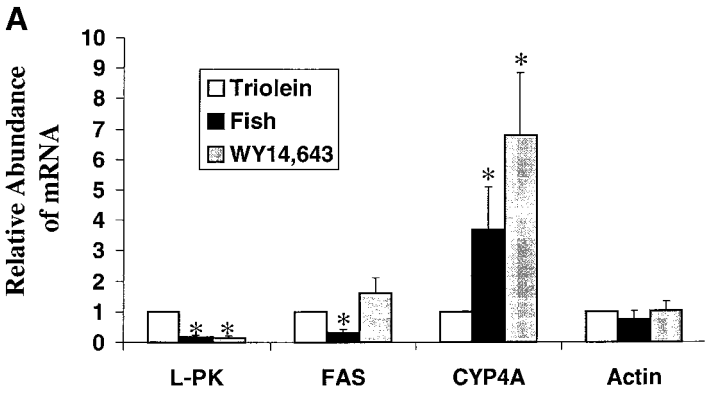

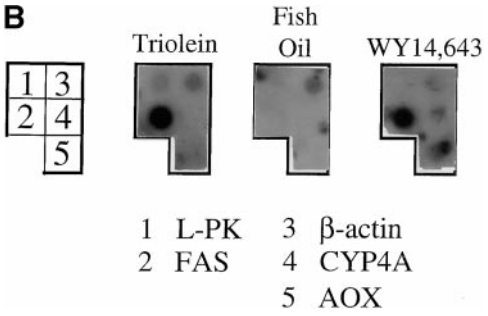

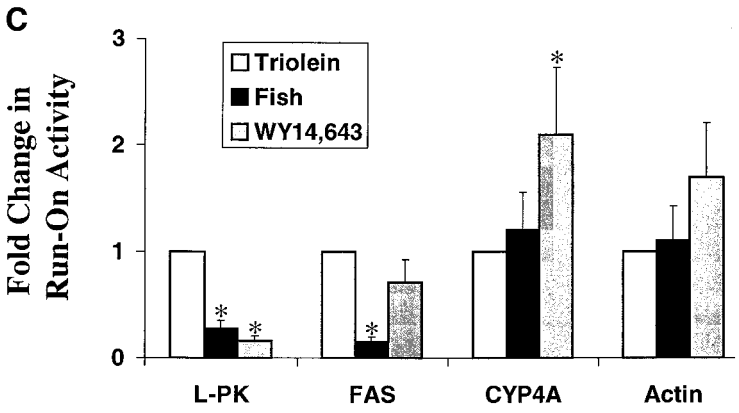

Fig. 1. Effect of dietary fat and WY14,643 on rat hepatic L-PK, FAS, CYP4A, and β-actin gene expression. Rats were meal fed a high carbohydrate (HiCHO) (58% glucose) diet supplemented with either triolein [10%, w/w] (open bars), fish oil [10%, w/w](black), or WY14,643 [0.1% w/w] (gray bars) for 5 days. A: Effect of dietary fat and WY14,643 on L-PK gene expression. Total hepatic RNA was prepared and analyzed by Northern analyses for the effect of feeding on L-PK, FAS, CYP4A, and β-actin mRNA abundance. Hybridization was quantified by phosphorimager analysis and normalized against the level of expression of each mRNA in liver of triolein-fed rats. Results are presented as mean ± SE with 4 animals/group (n = 4). The results of this analysis are representative of 3 separate studies. (*) designates a statistical difference ($P \leq 0.05$) between the triolein and fish oil-fed animals or the triolein and the WY14,643-fed animals. The mean ± SE phosphorimager units from the Northern analysis for the L-PK, FAS, CYP4A, and β-actin in triolein-fed rats are: 36569 ± 8009; 36723 ± 8190; 542311 ± 68589; 36806 ± 5923, respectively. B and C: Transcriptional run-on analysis. Run-on analysis was preformed on nuclei derived from the same animals as described in Fig. 1A (11). Fig. 1B is a representative hybridization dot blot illustrating the level of hybridization for L-PK, FAS, CYP4A2, AOX, and β-actin. Fig. 1C represents the quantified data for L-PK, FAS, CYP4A, and β-actin. AOX is not illustrated because it displays the same control as CYP4A. The level of hybridization was quantified by phosphorimager analysis and normalized against the level of run on activity in the triolein meal-fed rats. The mean ± SD phosphorimager units from the run-on analysis for the L-PK, FAS, CYP4A, and β-actin in triolein-fed rats are: 5042 ± 561; 39705 ± 7153; 2549 ± 956; 5262 ± 411, respectively. Results are presented as mean ± SD with 4 animals/group. (*) designates statistically significant difference ($P \leq 0.05$) between the triolein and fish oil-fed animals or the triolein and the WY14,643-fed animals.

$mRNA_{LPK}$ by ⩾80%. Drug treatment of the PPARα-null mice had no significant effect on hepatic $mRNA_{LPK}$ levels. $mRNA_{CYP4A}$ was induced 28- and 81-fold by clofibrate and WY14,643, respectively, in wild-type mice but was not significantly affected by either compound in livers of the PPARα-null mice. $mRNA_{PO}$, (encodes a ribosomal phosphoprotein) was not significantly affected by these treatments. These results indicate that PPARα is required for the clofibrate and WY14,643 regulation of both L-PK and CYP4A gene transcription.

Localization of the *cis*-regulatory target for the PPARα action on the L-PK promoter

As WY14,643 suppression of L-PK gene transcription requires PPARα (Figs. 1 and 2), the next step was to localize the *cis*-regulatory target for this control. Primary hepatocytes have been used previously to assess the role of PUFA on L-PK gene expression (12). To assess PPARα activator control of L-PK gene expression in primary hepatocytes, cells were treated with gemfibrozil or WY14,643 at 100 μm for 48 h. These treatments suppressed $mRNA_{LPK}$ levels by 45 and 80%, respectively (**Fig. 3**). $mRNA_{CYP4A2}$ was induced 8.4- and 33-fold by these same treatments, while β-actin was unaffected. Clearly, these PPARα activators act directly on primary rat hepatocytes to promote a robust gene-specific response, a response that parallels the one seen in vivo (Fig. 1).

Our next objective was to establish that WY14,643 could activate the endogenous PPARα and control the expression of a transfected reporter gene in primary hepatocytes. Accordingly, primary hepatocytes were transfected with TK223, a reporter plasmid containing the AOX-PPRE linked to the thymidine kinase (TK) promoter (**Fig. 4**). CAT activity in cells transfected with TK223 was induced >3.2-fold by WY14,643. More robust transactivation of TK223 was achieved by co-transfecting a PPARα expression vector (25).

LPK-CAT reporter plasmids were transfected into primary hepatocytes to assess their responsiveness to WY14,643. Our initial test used a deletion analysis to localize the *cis*-regulatory region for WY14,643 control. Because L-PK gene expression and LPK-CAT activity is induced by insulin/glucose (5–9), cells were treated with lactate (10 mm), glucose (25 mm), or glucose (25 mm) plus

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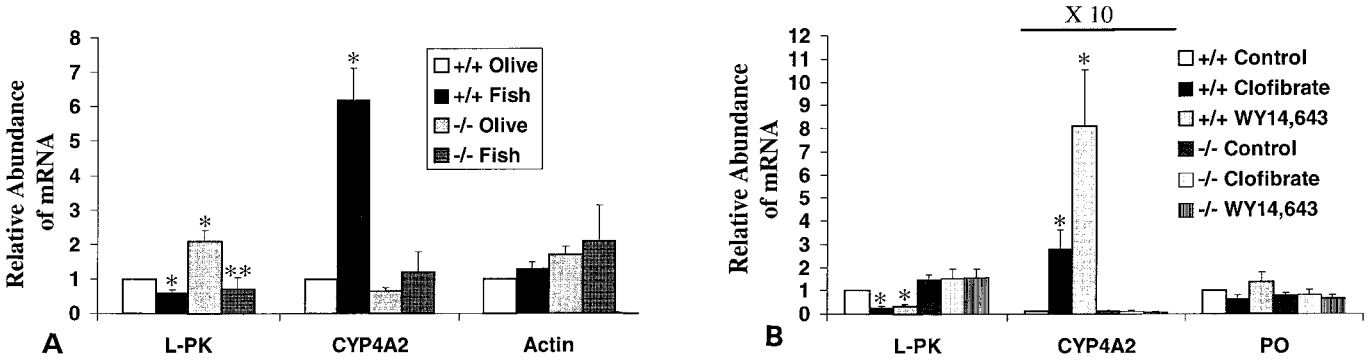

Fig. 2. Effect of dietary fat and hypolipidemic drugs on L-PK and CYP4A2 gene expression in wild-type (+/+) and PPARα null (−/−) mice. A: Effect of fish oil on L-PK and CYP4A expression in wild type and PPARα null mice. Mice from each genotype were meal-fed high carbohydrate (HiCHO) diets supplemented with 10% (w/w) olive or fish oil for 5 days. At the conclusion of the dietary treatment, liver RNA was extracted and assayed for the expression of L-PK and CYP4A2 as described in Fig. 1. The effects of this feeding protocol on mRNAs encoding β-actin, AOX, CYP4A, S14, and FAS from wild-type and PPARα-null mice was reported previously (16). β-Actin expression was not significantly affected by olive or fish oil feeding (16). The CYP4A data is shown here for comparison. The level of expression is normalized against the level of expression in livers of olive oil-fed wild-type mice. The phosphorimager units for L-PK, CYP4A, and β-actin in the olive oil-fed wild-type mice are: 907514 ± 170647, 1025984 ± 112813, and 20407 ± 6854, respectively. Results are expressed as mean ± SE with 4 animals/group. The statistical comparisons (ANOVA) were: (*) olive oil wild-type versus fish oil-fed wild-type mice ($P \leq 0.1$); olive oil wild-type vs. olive oil PPARα-null type ($P \leq 0.1$); (**) olive oil-PPARα null type vs. fish oil-PPARα null type ($P \leq 0.05$). No other comparisons were significant. B: Effect of clofibrate and WY14,643 on L-PK, CYP4A2, and PO gene expression in wild-type (+/+) and the PPARα-null (−/−) mice. PO (a ribosomal phosphoprotein) is used here instead of β-actin, to assess the specificity of the treatment as well as to assess RNA loading of the agarose gels. Mice of each genotype were fed a chow diet [Bioserve, NJ] supplemented with 0.5% (w/w) clofibrate or 0.1% (w/w) WY14,643 for 2 weeks, ad libitum (14). Total liver RNA was prepared and examined by Northern blot analysis for L-PK, CYP4A2, and PO mRNA levels. The effects of this feeding protocol on mRNAs encoding β-actin, AOX, and CYP4A from wild-type and PPARα-null mice was reported previously (14). β-Actin expression was not significantly affected by clofibrate or WY14,643 (14). The results were quantified and normalized against the level of hepatic mRNA expressed in control-fed rats. The phosphorimager units for L-PK, CYP4A, and PO in the control-fed wild type mice are: 7278 ± 621, 90270 ± 42745, and 10925 ± 627, respectively. These results are represented as mean ± SD with 3 animals/group. The statistical comparisons (ANOVA) were: (*) +/+ control versus +/+ clofibrate or +/+ WY14,643-fed mice ($P \leq 0.1$). No other comparisons were significant.

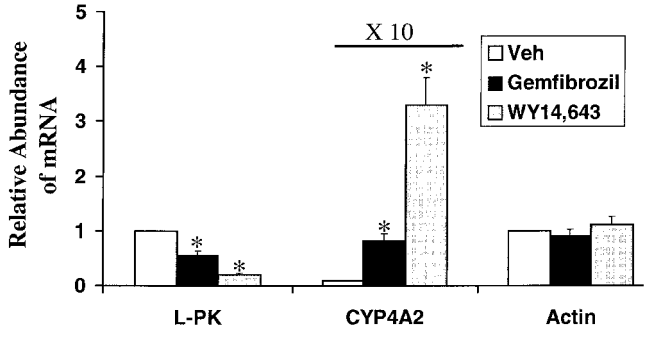

Fig. 3. Effect of gemfibrozil and WY14,643 on L-PK, CYP4A, and β-actin mRNAs in primary rat hepatocytes. Primary rat hepatocyte cultures were fed Williams E media containing 25 mm glucose, 200 nm insulin, 10 nm dexamethasone. Control cultures received DMSO (0.1%), the vehicle for gemfibrozil and WY14,643. Treated cultures received gemfibrozil or WY14,643 at 100 μm for 48 h. All cultures received one media change. At the end of the treatment period, total RNA was isolated and analyzed by Northern blots for L-PK, CYP4A2, and β-actin mRNA. The results were quantified and normalized against the level of expression of each mRNA in vehicle (DMSO) treated cells. The phosphorimager units for L-PK, CYP4A, and β-actin of vehicle-treated cells are: 22808 ± 2671, 12957 ± 745, and 316050 ± 14187, respectively. Results are presented as the mean ± SD, with 3 plates of cells/group. Results are representative of 2 separate experiments. The statistical comparisons (ANOVA) were: (*) vehicle vs. gemfibrozil or WY14,643 ($P \leq 0.02$).

WY14,643 (100 μm). LPK-CAT reporter genes with 5′ end points at −2800, −197, and −96 bp upstream from the L-PK transcription start site were used. Glucose induced LPK2800 and LPK197 4.8- and 6.5-fold, respectively. However, glucose did not significantly induce LPK96 or TK223. The lack of a glucose effect on TK223 indicates the specificity of the glucose effect. The lack of a glucose effect on LPK96 is consistent with the deletion of the glucose response region (GlRR) located between −197 and −96 bp in the LP-K promoter (7).

When compared to the glucose-treated cells, the glucose-WY14,643 treatment suppressed LPK-CAT2800 and LPK197 CAT activity by 40 and 50%, respectively. A similar analysis of cells receiving only lactate showed that WY14,643 had no effect on LPK-CAT activity (not shown). This indicates that WY14,643 interfered with the glucose/insulin-mediated transactivation of L-PK. This notion is reinforced by the absence of a significant WY14,643 effect on LPK-96, a reporter plasmid containing no GlRR. Based on this deletion analysis, the *cis*-regulatory target for WY14,643 action is between −197 and −96 bp, the putative GlRR.

The GlRR contains two functional regions (Fig. 4B), a USF region binding E-box-related proteins like USF1 and USF2 and a direct repeat (DR1) element binding HNF4 (2–8). Previous studies had established that the *cis*-regulatory

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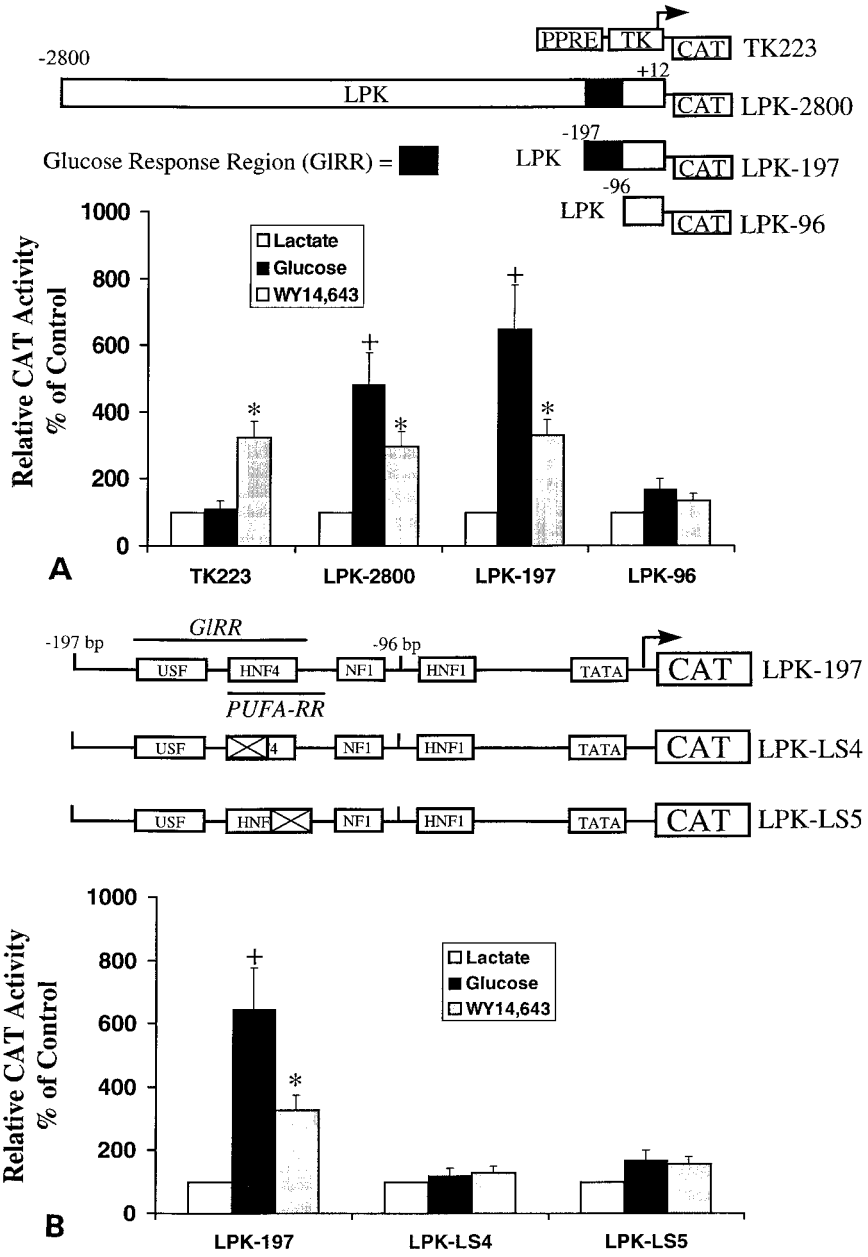

Fig. 4. Localization of the *cis*-regulatory target for WY14,643 regulation of L-PK gene transcription. A: Localization of the WY14,643 *cis*-regulatory region within the L-PK promoter. A schematic illustrates the reporter plasmids. TK223 contains the acyl CoA oxidase PPRE fused upstream of the thymidine kinase (TK) promoter (25). LPK-2800, LPK-197, and LPK-96 are LPK-CAT reporter plasmids with 5′ end points at −2800, −197, and −96 bp. Each has a common 3′ end point at +12 bp. The construction of these plasmids was described previously (7, 12). Cultured primary hepatocytes are treated with medium containing 10 mm lactate, 25 mm glucose, or 25 mm glucose plus 100 μm WY14,643. All cultures received insulin at 200 nm. After 48 h in culture with one media change; cells were harvested for measurement of protein and CAT activity. CAT activity for each plasmid is normalized to the level of expression in the lactate-treated cells. CAT activity (Units, see Materials and Methods) in the lactate-treated hepatocytes transfected with TK223, LPK2800, LPK197, and LPK96 was: 96 ± 20, 49 ± 5, 92 ± 20, 212 ± 26 units, respectively. Results are presented as Relative CAT Activity-% of Control [mean ± SE]. The control is the lactate-treated group. The number of plates of cells/treatment were: 12, 6, 24, 6 for TK223, LPK-2800, LPK-197, and LPK-96, respectively. The data presented is pooled data from 2 or more different studies with 3 plates of cells/group within each study. The statistical comparisons (ANOVA) were: (+; $P < 0.01$) lactate vs. glucose; (*; $P < 0.05$) glucose vs. glucose plus WY14,643. B: Linker scanning analysis of the L-PK promoter. Schematic of the L-PK promoter illustrating the location of the TATA box (at −28/−23 bp) and binding sites for HNF1 (−94/−76), NF1 (−115/−97), HNF4 binding a DR1 at (−145/−120), and USF related factors binding E-boxes (CANNTG). The schematic denotes the location of the glucose response region (GlRR), and the PUFA-response region (PUFA-RR). LPK-LS4 has a linker scanning mutations at −143/−135 bp while LPK-LS5 has a linker scanning mutation at −130/−123 bp (boxed **X**). The construction of these plasmids was described previously (7, 12). Primary rat hepatocytes were transfected with the various reporter plasmids, fed media, harvested, and assayed for protein and CAT activity as described above. Results are presented as Relative CAT Activity, % of the Control [mean ± SE]. The control is the lactate-treated group. CAT activity (Units, see Materials and Methods) in the lactate-treated hepatocytes transfected with LPK-197, LPK-LS4, and LPK-LS5 was: 92 ± 20, 249 ± 28, 530 + 143 units, respectively. Results are presented as mean ± SE. The number of plates of cells/treatment were: 24, 6, and 6 for LPK-197, LPK-LS4, and LPK-LS5, respectively. The data presented are pooled data from 2 or more different studies with 3 plates of cells/group within each study. The statistical comparisons (ANOVA) were: (+; $P < 0.01$) lactate vs. glucose; (*; $P < 0.05$) glucose versus glucose plus WY14,643.

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target for PUFA control of L-PK gene transcription was the 3′-half of the GlRR, i.e., between −145 and −120 bp, a region binding HNF4 (12). Here we asked whether WY14,643 targeted the same regulatory elements as PUFA. The LPK-CAT reporter plasmids containing linker scanning mutations, LPK-LS4 (at −142 to −135 bp) and LS5 (at −130 to −123 bp), span the HNF4 binding site (−144 to −126 bp). CAT activity in cells transfected with LPK-CAT-LS4 did not respond to glucose or WY14,643. CAT activity in cells transfected with LPK-CAT-LS5 was marginally (68%) induced by glucose, but was not affected by WY14,643 treatment. This loss of response cannot be explained by low CAT activity because CAT activity in cells transfected with either LPK-LS4 and LPK-LS5 was higher than LPK197 (Fig. 4 legend). Other linker scanning mutations both upstream and downstream of the LS4 and LS5 mutations did not affect WY14,643-regulated CAT activity (not shown). These studies indicate that activated endogenous PPARα targets the same region within the L-PK promoter as PUFA, i.e., the PUFA-regulatory region.

The PPARα does not bind the L-PK promoter

WY14,643, acting through PPARα (Fig. 2), targets the 3′ end of the L-PK GlRR (Fig. 4), a region binding HNF4 (2–8). The HNF4 binding site is a direct repeat with a single nucleotide spacer, i.e., a DR1. PPARα/RXRα heterodimers bind DR1-like elements in the AOX, apoC-III, and transferrin promoters (21, 22, 25). PPARα/RXR heterodimers compete with HNF4 for the DR1 site in the apoC-III and transferrin promoters (21, 22). To determine whether WY14,643 regulation of L-PK gene transcription was analogous to that reported for apoC-III, electrophoretic mobility shift analysis was used.

PPARα, RXRα, and HNF4 were prepared by in vitro transcription/translations (see Materials and Methods). SG5 was used as a control for an unprogrammed transcription/translation. Direct binding of these receptors to the 3′ end of the L-PK GlRR, i.e., (LFA1), the AOX-peroxisome proliferator response element (AOX), the apoC-III HNF4 binding site (apoC-III) and an artificial direct repeat (DR1) was tested (**Fig. 5A**). HNF4 bound the LFA1, AOX, apoC-III, and the DR1 elements. RXRα alone bound only the artificial DR1 element, while PPARα alone did not bind any element. The combination of PPARα/RXRα bound both the AOX and apoC-III element. However, PPARα/RXRα did not bind the LPK-LFA1 element. The binding activity in the PPAR/RXR for DR1 probably represents the RXR homodimer.

To verify that PPARα/RXRα did not bind the L-PK LFA1, competition gel shift analysis was used (Fig. 5B). In the left panel, PPARα/RXRα heterodimer binding to the ^{32}P-AOX-PPRE was tested with increasing concentration of non-radioactive AOX-PPRE. Quantitation of the inhibition of PPARα/RXRα binding yielded a K_d = ~4 nm. A similar result was obtained with the apoC-III element as competitor. However, the L-PK LFA1 element failed to compete for PPARα/RXRα binding to the AOX element, even at a 1000-fold molar excess. HNF4 bound the LFA1

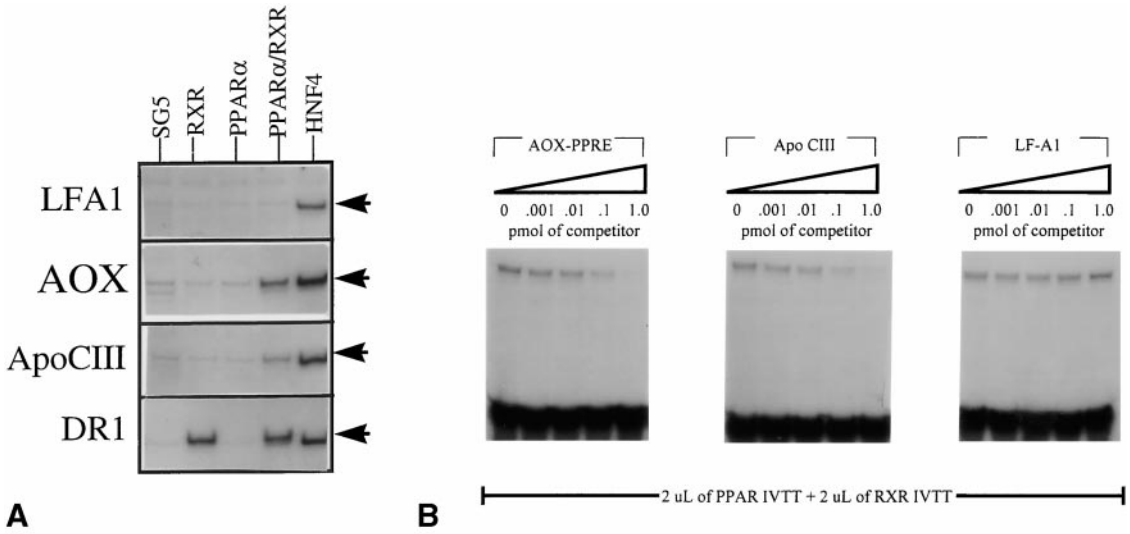

Fig. 5. Electrophoretic mobility shift analysis of PPARα binding to DNA. A: Direct electrophoretic mobility shift assays. Direct binding of in vitro synthesized HNF4, PPARα, RXRα, or PPARα/RXRα heterodimer was assessed using ^{32}P-DNAs: the LPK-LFA1 [LFA1], the AOX-PPRE [AOX], the ApoCIII-DR1 [ApoCIII] or an artificial DR-1, as described in Materials and Methods. SG5 was used to program the in vitro transcription/translation reaction and provides a control for background binding from the reticulolysate. Labeled DNAs were incubated in a 20 μl reaction for 20 min with 2 μl of lysate programmed with SG5, RXRα, PPARα, the PPARα/RXRα heterodimer, or HNF4. The arrow at the right indicates the location of the nuclear receptor-DNA complex. Free probe is not shown in the figure. Faint bands detected in the SG5 lane that correspond to bands seen in other lanes are considered to be derived from the unprogrammed reticulolysate. The results are representative of 4 separate studies. B: Competitive electrophoretic mobility shift assays. Competitive EMSA was performed using a ^{32}P-labeled acyl-CoA oxidase–peroxisomal proliferator response element (AOX-PPRE) oligonucleotide and in vitro transcribed/translated PPARα and RXRα. Competition assays were performed in the presence of 0 to 100-fold molar excess (0–1 pmol) of unlabeled AOX-PPRE, apolipoprotein C-III (ApoCIII), and LPK LF-A1. The LF-A1 element spans nucleotides −156 to −120 bp and contains the PUFA-RR. The sequence of oligonucleotides used in this analysis is shown in Materials and Methods. The results are representative of 3 separate studies.

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with a K_d of ~5 nm (not shown). If these binding relationships prevail in vivo, then it is highly unlikely that PPARα/RXRα binds the L-PK DR1. This finding indicates that WY14,643 suppression of L-PK gene transcription, a PPARα-dependent event, does not involve direct interaction with the L-PK promoter. Thus, WY14,643/PPARα regulation of L-PK gene transcription involves some intermediary factor(s).

DISCUSSION

L-pyruvate kinase (L-PK) is a glycolytic enzyme that plays an important role in glucose metabolism (1–4). In addition to acute regulation by phosphorylation and allosteric factors, L-PK gene expression is regulated by hormones (insulin, glucagon) and nutrients (glucose, alanine, and PUFA). The *cis*-regulatory targets for insulin, glucose, and PUFA regulation have been localized to the GlRR between −183 and −125 bp upstream from the transcription start site (Fig. 4B). Insulin-stimulated glucose metabolism regulates the activity of transcription factors, possibly USF-related factors, that bind E-boxes within the GlRR. HNF4 plays an important ancillary role in this regulatory process.

Our studies show that the PPARα activators, WY14,643, gemfibrozil, and clofibrate, induce hepatic $mRNA_{CYP4A2}$ and suppress $mRNA_{LPK}$ through a transcriptional regulatory mechanism both in vivo and in cultured primary hepatocytes (Figs. 1–4). While PPARα is required for the WY14,643 suppression of L-PK, PPARα is not required for the PUFA suppression of L-PK gene transcription (Fig. 2). Thus, PPARα is not a mediator of PUFA regulation of L-PK gene transcription despite the fact that both WY14,643 and PUFA target the same *cis*-regulatory element within the L-PK promoter (Fig. 4). This *cis*-regulatory target is the 3′ end of the GlRR, a region containing an imperfect direct repeat ($^{-143}$TGGACTCTGGCCC^{-131}) that binds HNF4. Similar DR-like elements are recognized by a number of orphan nuclear receptors including PPAR, RXR, CoupTFI, and CoupTFII (27). Neither PPARα, RXRα or the PPARα/RXRα heterodimers bound the L-PK LFA1 (Fig. 5). In studies not reported here, CoupTF1 or CoupTFII (apoA-I) failed to bind the L-PK LFA1 site.

In addition to the L-PK LFA1 element, HNF4 also bound the AOX and apoC-III DR1-like elements (Fig. 5). While the effect of HNF4 on AOX has not been established, Hertz et al. (21, 22) reported that PPARα/RXR heterodimers competed with HNF4 for binding the apoC-III DR1. HNF4 binding stimulated apoC-III promoter activity, while PPARα/RXRα binding inhibited apoC-III promoter activity. Clearly, WY14,643/PPARα regulation of L-PK through an HNF4 binding site does not parallel the mechanism described for apoC-III.

The fact that PPARα/RXRα heterodimers do not directly interact with the L-PK promoter suggests that WY14,643 regulation of L-PK gene transcription through PPARα involves some intermediary mechanism (**Fig. 6**). Studies will need to establish whether HNF4 binding the L-PK LFA1 is affected after fish oil or WY14,643 feeding of animals. Interestingly, NF-1-related factors also bind the 3′ end of the L-PK GlRR (10). Several NF1 subtypes have been described including NF-1L and NF1/Red1. Yamada et al. (10) have suggested that NF1 binding the L-PK promoter inhibits L-PK activity. Thus, one attractive scenario would involve PUFA and WY14,643 control of hepatic HNF4 abundance, its DNA binding activity or its capacity to transactivate. A decline in HNF4 activity or abundance might increase binding of other factors, like NF1, to the L-PK LFA1 and inhibit L-PK gene transcription. In this regard, Hertz et al. (28) have reported that fatty acyl CoA thioesters affect HNF4 binding and transactivation on the apoC-III promoter. We have been unable to extend this observation to the L-PK promoter.

Interestingly, peroxisome proliferators have been reported to augment rodent hepatic Δ^6-desaturase activity (29). Δ^6-Desaturase is required to convert 18:2, n−6 to 20:4, n−6 and 22:5, n−6. 20:4, n−6 is a potent suppressor of L-PK gene transcription. There are, however, two lines of evidence that argue against this mechanism to explain

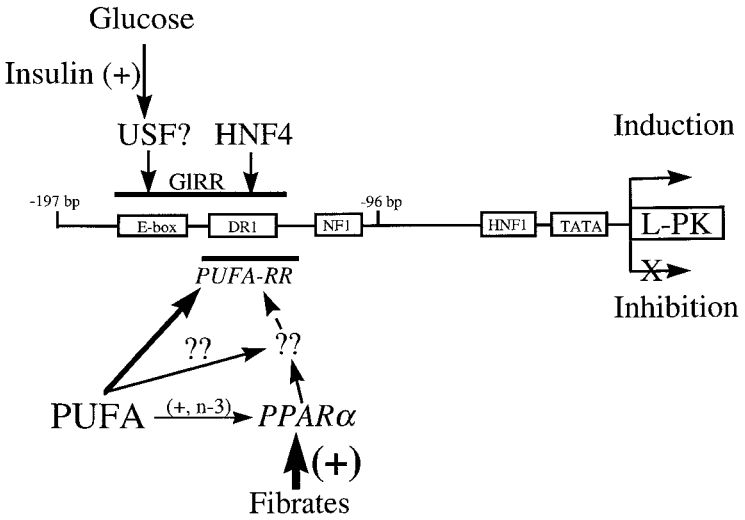

Fig. 6. PUFA and PPARα regulate L-PK gene transcription through separate mechanisms. A schematic of the L-PK promoter is shown illustrating the targets for USF-related proteins and HNF4 within the GlRR. Both PPARα and PUFA-regulated factors act on the L-PK DR1. Fibrates are strong activators of the PPARα, while n−3 PUFA are weak activators (25). N−6 PUFA do not activate PPARα under the experimental conditions used in this study. PPARα does not bind the L-PK DR1 suggesting an intermediary component is involved in the PPARα regulation of L-PK through the PUFA-RR. The intermediary component remains to be identified.

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WY14,643 control of L-PK gene transcription. First, highly unsaturated fatty acids suppress nuclear levels of sterol response element binding protein, SREBP1c (30–32). SREBP1c is involved in the coordinate control of multiple hepatic lipogenic genes (33). While SREBP1c binds E-boxes and SREs in specific promoters of PUFA-regulated genes, such as ACC, FAS, and S14 (32, 34), it does not bind the L-PK promoter at either the E-boxes or the LFA1 region (32). Second, fatty acid synthase expression, a sensitive index for hepatic PUFA suppression through SREBP1c, was not significantly affected by WY14,643 feeding (Fig. 1). Moreover, peroxisome proliferators have no effect on SREBP1 mRNA or its nuclear content (32, 35)

An alternative explanation for the WY14,643 effect on L-PK may rest in the growth-promoting effects of this compound on the liver (14). WY14,643 along with other peroxisome proliferators induce two mitogen-activated protein kinases (MAPK), e.g., ERK1 and ERK2, in immortalized mouse liver cells (36). Inhibition of MAPK signaling pathways increase HNF4 abundance and possibly its activity in HepG2 cells (37). Whether WY14,643 regulation of L-PK involves the suppression of HNF4 abundance/activity will require additional study.

Finally, as an important glycolytic enzyme, suppression of L-PK gene expression by PUFA and hypolipidemic drugs might be expected to affect hepatic glucose metabolism. Indeed, pyruvate kinase activity as well as the ATP:ADP ratio are lower in clofibrate-treated rats (38). However, clinical studies have indicated that glucose tolerance or insulin resistance is unaffected in hypertriglyceridemic patients treated with the hypolipidemic drug, gemfibrozil (39). It is noteworthy that the PPARα-null mice exhibit a phenotype with altered metabolic characteristics. L-PK mRNA is elevated in null mice compared to wild-type mice (Fig. 2). This represents a critical partitioning between gluconeogenesis and fatty acid oxidation and the relationship between fuel oxidation and storage. In contrast to saturated fats, PUFA are readily oxidized (40). Nevertheless, PUFA also constitute structural membrane lipid components whereby increased amounts of PUFA are associated with reduced insulin resistance (41). When the PPARα is eliminated from the system, as is the case in the PPARα-null animals, a major lipid handling system is negated and glycolysis is switched on, a compensatory process in keeping with the Randle cycle (42). Isolating the mechanistic details of dietary fat handling, be it as fuels in oxidation, as structural components, or as regulators of gene expression, is an area that requires elucidation not only for basic understanding but also in the amelioration of numerous metabolic diseases including obesity and Type II diabetes mellitus.

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REFERENCES

1. Granner, D., and S. Pilkis. 1990. The genes of hepatic glucose metabolism. *J. Biol. Chem.* **265:** 10173–10176.
2. Towle, H. C., E. N. Kaytor, and H-M. Shih. 1997. Regulation of the expression of lipogenic enzyme genes by carbohydrate. *Annu. Rev. Nutr.* **17:** 405–433.
3. Yamada, K., and T. Noguchi. 1999. Nutrient and hormonal regulation of pyruvate kinase gene expression. *Biochem. J.* **337:** 1–11.
4. Vaulont, S., and A. Kahn. 1994. Transcriptional control of metabolic regulation genes by carbohydrates. *FASEB J.* **8:** 28–35.
5. Vaulont, S., N. Puzenat, F. Levrat, M. Cognet, A. Kahn, and M. Raymondjean. 1989. Proteins binding to the liver-specific pyruvate kinase gene promoter. A unique combination of known factors. *J. Mol. Biol.* **209:** 205–219.
6. Thompson, K. S., and H. C. Towle. 1991. Localization of the carbohydrate response element of the rat L-type pyruvate kinase gene. *J. Biol. Chem.* **266:** 8679–8682.
7. Liu, Z., K. S. Thompson, and H. C. Towle. 1993. Carbohydrate regulation of the rat L-type pyruvate kinase gene requires two nuclear factors: LF-A1 and a member of the c-myc family. *J. Biol. Chem.* **268:** 12787–12795.
8. Vallet, V. S., M. Casado, A. A. Henrion, D. Bucchini, M. Raymondjean, A. Kahn, and S. Vaulont. 1998. Differential roles of upstream stimulatory factors 1 and 2 in the transcriptional response of liver genes to glucose. *J. Biol. Chem.* **273:** 20175–20179.
9. Kaytor, E. N., H. Shih, and H. C. Towle. 1997. Carbohydrate regulation of hepatic gene expression. Evidence against a role for the upstream stimulatory factor. *J. Biol. Chem.* **272:** 7525–7531.
10. Yamada, K., T. Tanaka, and T. Noguchi. 1997. Members of the nuclear factor 1 family and hepatocyte nuclear factor 4 bind to overlapping sequences of the L-II element on the rat pyruvate kinase L gene promoter and regulate its expression. *Biochem. J.* **324:** 917–925.
11. Jump, D. B., S. D. Clarke, A. Thelen, and M. Liimatta. 1994. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J. Lipid Res.* **35:** 1076–1084.
12. Liimatta, M., H. C. Towle, S. D. Clarke, and D. B. Jump. 1994. Dietary polyunsaturated fatty acids interfere with the insulin/glucose activation of L-type pyruvate kinase gene transcription. *Mol. Endocrinol.* **8:** 1147–1153.
13. Reddy, J. K., and G. P. Mannaerts. 1997. Peroxisomal lipid metabolism. *Annu. Rev. Nutr.* **14:** 343–370.
14. Lee, S. S., T. Pineau, J. Drago, E. J. Lee, J. W. Owens, D. L. Kroetz, P. M. Fernandez-Salguero, H. Westphal, and F. J. Gonzalez. 1995. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell Biol.* **15:** 3012–3022.
15. Kliewer, S. A., S. S. Sundseth, S. A. Jones, P. J. Brown, G. B. Wisely, C. S. Koble, P. Devchand, W. Wahli, T. M. Willson, J. M. Lenhard, and J. M. Lehmann. 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc. Natl. Acad. Sci. USA.* **94:** 4318–4323.
16. Ren, B., A. P. Thelen, J. M. Peters, F. J. Gonzalez, and D. B. Jump. 1997. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alpha. *J. Biol. Chem.* **272:** 26827–26832.
17. Ijpenberg, A., E. Jeannin, W. Wahli, and B. Desvergne. 1997. Polarity and specific sequence requirement of peroxisome proliferator-activated receptor (PPAR)/retinoid X, receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. *J. Biol. Chem.* **272:** 20108–20117.
18. Jump, D. B., and S. D. Clarke. 1999. Regulation of gene expression by dietary fats. *Annu. Rev. Nutr.* **19:** 63–90.
19. Aoyama, T., J. M. Peters, N. Iritani, T. Nakajima, K. Furihata, T. Hashimoto, and F. J. Gonzalez. 1998. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPARα). *J. Biol. Chem.* **273:** 5678–5684.
20. Gonzalez, F. J., J. M. Peters, and R. C. Cattley. 1998. Mechanism of action of the nongenotoxic peroxisome proliferators: role of the peroxisome proliferator-activator receptor alpha. *J. Natl. Cancer Inst.* **90:** 1702–1709.

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21. Hertz, R., M. Seckbach, M. M. Zakin, and J. Bar-Tana. 1996. Transcriptional suppression of the transferrin gene by hypolipidemic peroxisome proliferators. *J. Biol. Chem.* **271:** 218–224.
22. Hertz, R., J. Bishara-Shieban, and J. Bar-Tana. 1995. Mode of action of peroxisome proliferators as hypolipidemic drugs. Suppression of apolipoprotein C-III. *J. Biol. Chem.* **270:** 13470–13475.
23. Mater, M. K., A. P. Thelen, and D. B. Jump. 1999. Arachidonic acid and PGE_2 regulation of hepatic lipogenic gene expression. *J. Lipid Res.* **40:** 1045–1052.
24. Sladek, F. M., W. M. Zhong, E. Lai, and J. E. Darnell, Jr. 1990. Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Gene Dev.* **4:** 2353–2365.
25. Ren, B., A. Thelen, and D. B. Jump. 1996. Peroxisome proliferator-activated receptor alpha inhibits hepatic S14 gene transcription. Evidence against the peroxisome proliferator-activated receptor alpha as the mediator of polyunsaturated fatty acid regulation of S14 gene transcription. *J. Biol. Chem.* **271:** 17167–17173.
26. Umesono, K., K. K. Murakami, C. C. Thompson, and R.M. Evans. 1991. Direct repeats as selective response elements for the thyroid hormone, retinoic acid and vitamin D3 receptors. *Cell.* **65:** 1255–1266.
27. Mietus-Snyder, M., F. M. Sladek, G. S. Ginsburg, C. F. Kuo, J. A. Laidais, J. E. Darnell, Jr., and S. K. Karathanasis. 1992. Antagonism between apolipoprotein A1 regulatory protein 1, Ear3/Coup-TF and hepatocyte nuclear factor 4 modulates apolipoprotein CIII gene expression in liver and intestinal cells. *Mol. Cell Biol.* **12:** 1708–1718.
28. Hertz, R., J. Magenheim, I. Berman, and J. Bar-Tana. 1998. Fatty acid-CoA thioesters are ligands of hepatic nuclear factor-4α. *Nature.* **392:** 512–516.
29. Kawashima, Y., K. Musoh, and H. Kozuka. 1990. Peroxisome proliferators enhance linoleic acid metabolism in rat liver. Increased biosynthesis of omega 6 polyunsaturated fatty acids. *J. Biol. Chem.* **265:** 9170–9175.
30. Worgall, T. S., S. L. Sturley, T. Seo, T. F. Osborne, and R. J. Deckelbaum. 1998. Polyunsaturated fatty acid decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. *J. Biol. Chem.* **273:** 25537–25540.
31. Xu, J., M. T. Nakamura, P. H. Cho, and S. D. Clarke. 1999. SREBP1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic gene by polyunsaturated fats. *J. Biol. Chem.* **274:** 23577–23583.
32. Mater, M. K., A. P. Thelen, D. A. Pan, and D. B. Jump. 1999. Involvement of SREBP1c in the polyunsaturated fatty acid regulation of hepatic S14 gene transcription. *J. Biol. Chem.* **274:** 32725–32732.
33. Shimomura, I., H. Shimano, B. S. Korn, Y. Bashmakov, and J. D. Horton. 1998. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J. Biol. Chem.* **273:** 35299–25306.
34. Lopez, J. M., M. K. Bennett, H. B. Sanchez, J. M. Rosenfeld, and T. F. Osborne. 1996. Sterol regulation of acetyl coenzyme A carboxylase: a mechanism for coordinate control of cellular lipid. *Proc. Natl. Acad. Sci. USA.* **93:** 1049–1053.
35. Yahagi, N., H. Shimano, A. H. Hasty, M. Amemiya-Kudo, H. Okasaki, Y. Tamura, Y. Iizuka, F. Shionoiri, K. Ohashi, J-i. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, and N. Yamada. 1999. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. *J. Biol. Chem.* **274:** 35840–35844.
36. Rokos, C. L., and B. J. Ledwith. 1997. Peroxisome proliferators activate extracellular signal-regulated kinases in immortalized mouse liver cells. *J. Biol. Chem.* **272:** 13452–13457.
37. Reddy, S., W. Yang, D. G. Taylor, X-q. Shen, D. Oxender, G. Kust, and T. Leff. 1999. Mitogen-activated protein kinase regulates transcription of the ApoCIII gene. *J. Biol. Chem.* **274:** 33050–33056.
38. Wilkening, J., and P. Schwandt. 1977. Alteration of the glycolytic pathway and adenine nucleotide state in liver of clofibrate treated rats. *Horm. Metab. Res.* **9:** 132–136.
39. Jeng, C. Y., W. H. Sheu, M. M. Fuh, S. M. Shieh, Y. D. Chen, and G. M. Reaven 1996. Gemfibrozil treatment of endogenous hypertriglyceridemia: effect on insulin-mediated glucose disposal and plasma insulin concentrations. *J. Clin. Endocrinol. Metab.* **81:** 2550–2553.
40. Leyton, J., P. J. Drury, and M. A. Crawford. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Br. J. Nutr.* **57:** 383–393.
41. Pan, D. A., S. Lillioja, M. R. Milner, A. D. Kriketos, L. A. Baur, C. Bogardus, and L. H. Storlien. 1995. Skeletal muscle membrane lipid composition is related to adiposity and insulin action. *J. Clin. Invest.* **96:** 2802–2808.
42. Randle, P. J., P. B. Garland, C. N. Hales, and E. A. Newsholme. 1963. The glucose fatty-acid cycle, its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* **1:** 785–789.

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