Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 329 (2005) 391–396
BBRC
www.elsevier.com/locate/ybbrc

Regulation of pyruvate dehydrogenase kinase expression by the farnesoid X receptor

Rajesh S. Savkur [a], Kelli S. Bramlett [a,b], Laura F. Michael [a], Thomas P. Burris [a,b,*]

[a] *Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, IN 46285, USA*
[b] *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Received 25 January 2005

Abstract

The pyruvate dehydrogenase complex (PDC) functions as an important junction in intermediary metabolism by influencing the utilization of fat versus carbohydrate as a source of fuel. Activation of PDC is achieved by phosphatases, whereas, inactivation is catalyzed by pyruvate dehydrogenase kinases (PDKs). The expression of PDK4 is highly regulated by the glucocorticoid and peroxisome proliferator-activated receptors. We demonstrate that the farnesoid X receptor (FXR; NR1H4), which regulates a variety of genes involved in lipoprotein metabolism, also regulates the expression of PDK4. Treatment of rat hepatoma cells as well as human primary hepatocytes with FXR agonists stimulates the expression of PDK4 to levels comparable to those obtained with glucocorticoids. In addition, treatment of mice with an FXR agonist significantly increased hepatic PDK4 expression, while concomitantly decreasing plasma triglyceride levels. Thus, activation of FXR may suppress glycolysis and enhance oxidation of fatty acids via inactivation of the PDC by increasing PDK4 expression.

Keywords: PDK4; Glucocorticoids; PPAR; TRB-3; FOXO; Bile acids; Diabetes; Lipid; Glucose

The pyruvate dehydrogenase complex (PDC) plays a pivotal role in intermediary metabolism and is regulated by dietary substrates and hormones. Under conditions of abundant food supply, the PDC is functionally active and promotes the utilization of glucose for the formation of acetyl- and malonyl-CoA and the synthesis of fatty acids. Conversely, in the starved state, the PDC is inactivated thereby limiting glucose oxidation and promoting fatty acid oxidation as the source of fuel. Thus, the activity of PDC serves as an important metabolic switch for fuel selection [1,2]. Activity of the PDC is primarily regulated by reversible phosphorylation of the complex that is mediated by specific kinases and phosphatases. Inactivation of the complex is catalyzed by four pyruvate dehydrogenase kinase (PDK) isozymes (PDK1–PDK4) that are expressed in varying amounts in a tissue-specific manner [3].

Previous studies have demonstrated that an increase in hepatic PDK activity upon acute starvation occurs in conjunction with an increase in expression of both PDK2 and PDK4 [4–6]. However, the mechanisms involved in the regulation of hepatic PDK4 expression differ from those of PDK2. Peroxisome proliferator-activated receptor-α (PPARα) has been implicated to play a critical role in regulating the expression of PDK4 [7–10], but not that of PDK2 [11]. Furthermore, PPARα-null mice exhibit dysregulated hepatic lipid and carbohydrate metabolism [6], consistent with the observation that PPARα agonists increase the expression of PDK4 [11,12]. In addition to the role of PPARα in PDK4 regulation, glucocorticoid receptor (GR) agonists have also been shown to induce the expression of PDK4, but not PDK2 [11]. Since glucocorticoids also induce the expression of PPARα [13], it is conceivable that

* Corresponding author. Fax: +1 317 276 1414.
E-mail address: burris_thomas_p@lilly.com (T.P. Burris).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.01.141

the induction of PDK4 expression by glucocorticoids could involve a pathway mediated by PPARα.

The farnesoid X receptor (FXR; NR1H4) is a member of the superfamily of nuclear hormone receptors [14] that was recently identified as the physiological receptor of bile acids [15,16]. FXR is predominantly expressed in the liver, kidney, and intestine, and plays a critical role in the regulation of bile acid synthesis [17–19]. Furthermore, FXR-null mice display additional deficiencies in lipid metabolism implicating a broader role for the bile acid receptor in the regulation of lipoprotein metabolism [20,21]. We have recently demonstrated that FXR also plays an important role in carbohydrate metabolism [22]. Since the expression of PDK4 is regulated by both PPARα and the FOXO proteins [23], and FXR plays a role in carbohydrate metabolism via PPARα and the FOXO proteins [22], we investigated the ability of FXR agonists to regulate the expression of PDK4.

In this study, we demonstrate that FXR agonists increase the expression of PDK4 in vitro as well as in vivo. Induction of PDK4 via activation of FXR is conserved between human primary hepatocytes, rat hepatoma cultured cells, and mice.

Materials and methods

Cell culture and reagents. H4IIE rat hepatoma cells and cryopreserved human primary hepatocytes (Cambrex, Walkersville, MD) were maintained as previously described [22]. Dexamethasone (DEX), chenodeoxycholic acid (CDCA), and WY14,643 were purchased from Sigma Chemical (St. Louis, MO). FXR agonists, GW4064 and fexaramine (FEX), were synthesized using standard organic chemistry synthetic methods.

mRNA measurements. H4IIE cells were seeded in 6-well plates at 1×10^6 cells per well in DMEM 10% FBS and allowed to attach overnight. The following day cells were washed once in serum free DMEM and serum starved for 24 h prior to treatment with FXR agonists, DEX, or WY14,643. In experiments examining mRNA expression from primary human hepatocytes, cells were treated with FXR and GR ligands, as indicated, for 8 h. Quantitative PCR was performed as previously described using TaqMan reagents and instrumentation [22].

Immunoblot analysis. After treatment of H4IIE cells for 24 h with DEX (1 μM), GW4064 (2.5 μM), CDCA (40 μM) or FEX (20 μM), total cellular protein was isolated and a Western blot was performed as previously described [22]. Relative expression was determined by densitometry. Polyclonal antibodies to the C-terminus of PDK4 (AP7041b; Abgent, San Diego, CA) and GAPDH (ab9485; Abcam, Cambridge, MA) were used for immunoblot analysis.

Analysis of the effects of GW4064 in mice. C57BL/6 mice (7–9 weeks of age) were treated with GW4064 (50 mg/kg BID; IP) or 5% acacia vehicle (4 animals/group). After 7 days of treatment, the mice were sacrificed and total RNA from liver was isolated. The expression of PDK4 was assessed by TaqMan Q-PCR, and plasma triglycerides were measured as previously described [24].

Statistical analysis. Statistical analysis was assessed using the Mann–Whitney test or ANOVA followed by a Tukey test. Values of $p < 0.05$ were considered significant. All values are reported as means ± SEM.

Results and discussion

Previous studies using a rat hepatoma cell line demonstrated that the GR agonist, dexamethasone (DEX), induces the expression of PDK4 [11]. To determine whether FXR ligands affect the expression of PDK4, we treated H4IIE rat hepatoma cultured cells with the potent non-steroidal FXR agonist, GW4064. As illustrated in Fig. 1A, Q-PCR analysis of RNA isolated from H4IIE cells treated with GW4064 significantly increased the expression of PDK4. This increase in PDK4 mRNA expression was comparable to that observed when the cells were treated with DEX (2.0-fold). As illustrated in Fig. 1B, GW4064 dose-dependently increased the expression of PDK4. To ascertain whether the increase in PDK4 mRNA also resulted in an increase in the expression of PDK4 protein, immunoblot analysis was performed using total protein isolated from the H4IIE cells that were treated with three FXR agonists, CDCA, FEX, and GW4064, and the GR agonist, DEX. As shown in Fig. 1C, all the three FXR agonists increased the expression of PDK4 protein in the range of 5- to 9-fold. This increase in PDK4 protein expression was comparable to that observed with cells that were treated with DEX (5-fold).

To investigate whether the increase in PDK4 expression observed with FXR ligands could be detected in cells from human origin, cultured human primary hepatocytes were treated with either GW4064 or DEX for 24 h followed by isolation of total RNA. As illustrated in Fig. 2, both GW4064 and DEX were effective in inducing the expression of PDK4. The induction of PDK4 mRNA was 7.5-fold and 20-fold for 100 nM and 1 μM GW4064, respectively, and 7-fold for 1 μM DEX. The difference in the magnitude of PDK4 induction between human primary hepatocytes and rat hepatoma cells may be due to the differences in sensitivity between the species and the transformed nature of the hepatoma cells, or alternatively, may be a function of the sensitivity of the particular donor of human primary hepatocytes. Nevertheless, these results indicate that the induction of PDK4 expression by FXR agonists is conserved between rat and human.

To determine whether activation of FXR leads to an increase in PDK4 expression in vivo, we treated C57BL/6 mice with the FXR agonist, GW4064. Similar to previous observations [25], activation of FXR by a 7-day treatment with GW4064 resulted in a 52.5 ± 8% reduction in the levels of plasma triglyceride levels (Fig. 3). Consistent with the induction of PDK4 expression in human and rat cultured cells upon treatment with FXR agonists, expression of PDK4 mRNA was increased 340 ± 140% in comparison with the vehicle treated animals (Fig. 3). Thus, activation of FXR in vivo resulted in an increase in PDK4 expression that was associated with a concomitant decrease in plasma

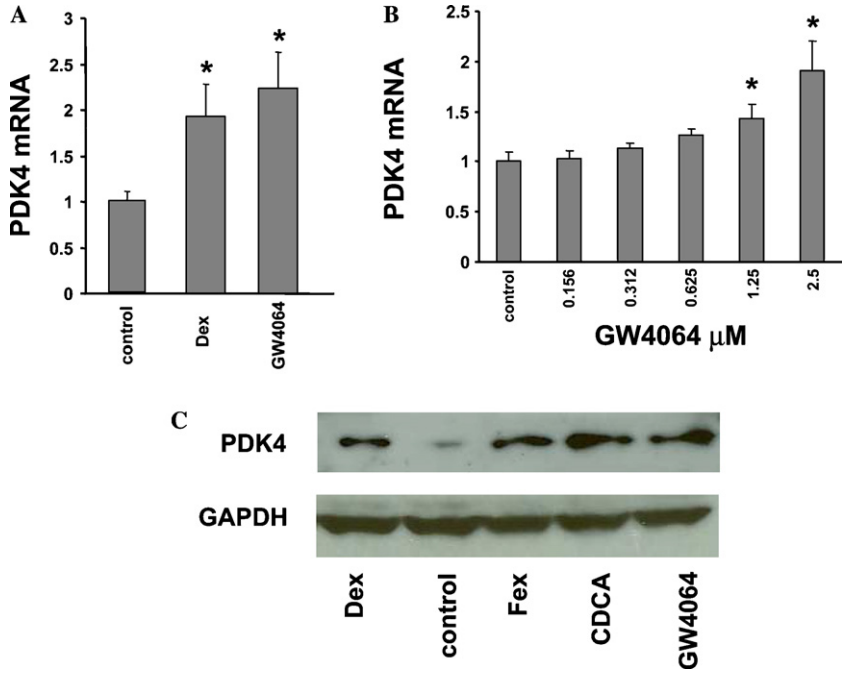

Fig. 1. Stimulation of PDK4 expression by FXR agonists in H4IIE rat hepatoma cells. (A) H4IIE cells were treated with 1 μM Dex or 2.5 μM GW4064 or DMSO (control) for 24 h followed by PDK4 mRNA measurement. (B) Dose-dependent induction of PDK4 expression by the FXR agonist GW4064 in H4IIE rat hepatoma cells. All values were normalized to 36B4 expression. (C) Immunoblot analysis of total protein isolated from H4IIE cells treated for 24 h with Dex (1 μM) or FXR agonists, Fex (20 μM), CDCA (40 μM), and GW4064 (2.5 μM) or DMSO (control). $*p < 0.05$ vs. control.

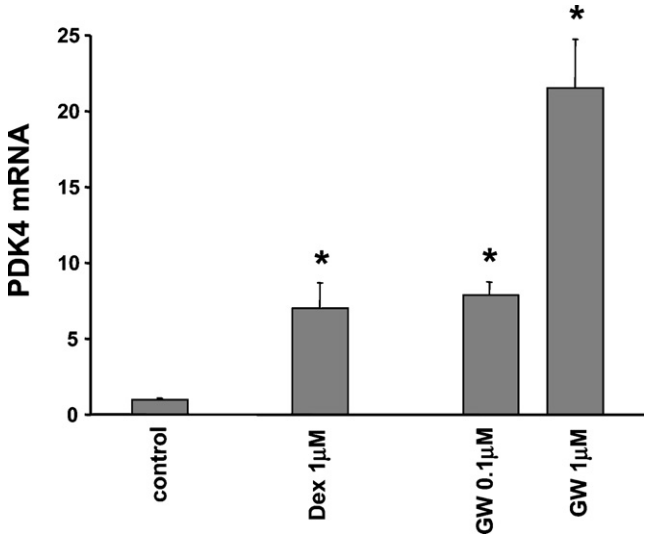

Fig. 2. Induction of human PDK4 mRNA expression by FXR agonists. Human primary hepatocytes were treated with Dex (1 M) or GW4064 (GW; 0.1 and 1 μM) or DMSO (control) for 24 h followed by assessment of PDK4 mRNA expression. All values were normalized to 18S rRNA expression. $*p < 0.05$ vs. control.

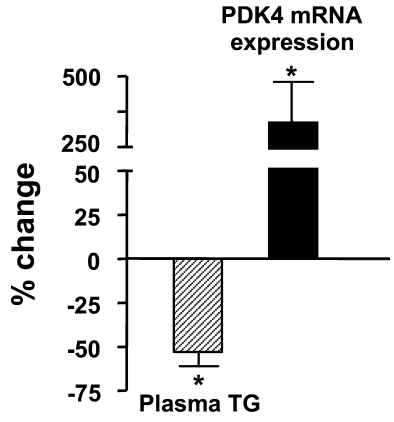

Fig. 3. Effect of FXR agonist, GW4064, in vivo. C57BL/6 mice were treated with GW4064 (50 mg/kg BID; IP) or 5% acacia vehicle. The increase in the expression of PDK4 mRNA (normalized to 18S rRNA) is depicted by a filled box. The decrease in the plasma triglycerides is depicted by a hatched box. $*p < 0.05$ vs. control.

triglyceride levels. These results indicate that activation of FXR results in induction of PDK4 expression, and this effect is conserved between rodents and humans.

Previous studies demonstrated that the expression of PDK4 is induced by both GR agonists and PPARα agonists [11,12]. These observations are consistent with studies indicating that PPARα plays a critical role in regulating the expression of PDK4 [6–10]. Results from our study reveal that the expression of PDK4 is induced by FXR agonists. Interestingly, previous studies, including a study from our laboratory, demonstrated that both GR and FXR agonists also induce the expression of PPARα in both human and rat cultured cells in vitro and in mice in vivo [13,22,26]. Since both GR and FXR agonists induce the expression of PPARα

[13,22,26], we predicted that the addition of a PPARα agonist along with a GR or an FXR agonist would have an additive effect on the expression of PPARα target genes. Furthermore, since PPARα agonists induce the expression of PDK4 [11], we hypothesized that addition of a PPARα agonist with either a GR or an FXR agonist would result in an additive effect on the expression of PDK4. To assess the effects of a PPARα agonist in conjunction with either a GR or an FXR agonist on the expression of PDK4, rat hepatoma H4IIE cells were treated with either a GR agonist, or an FXR agonist alone or in combination with a PPARα agonist—WY14,643. As illustrated in Fig. 4A, either the GR agonist—DEX, or the FXR agonist—GW4064 alone, increased the expression of PDK4 to 2.0-fold. The PPARα agonist alone resulted in a 4.5-fold induction in the levels of PDK4 mRNA. Interestingly, the combination of a GR and a PPARα agonist, or a combination of an FXR and PPAR agonist resulted in a significant additive effect characterized by a 9.5-fold and a 6.9-fold increase in the expression of PDK4 mRNA, respectively. These data suggest that activation of PPARα-mediated induction of PDK4 by its ligand—WY14,643—is further enhanced by DEX-induced (GR-mediated) or GW4064-induced (FXR-mediated) PPARα mRNA expression.

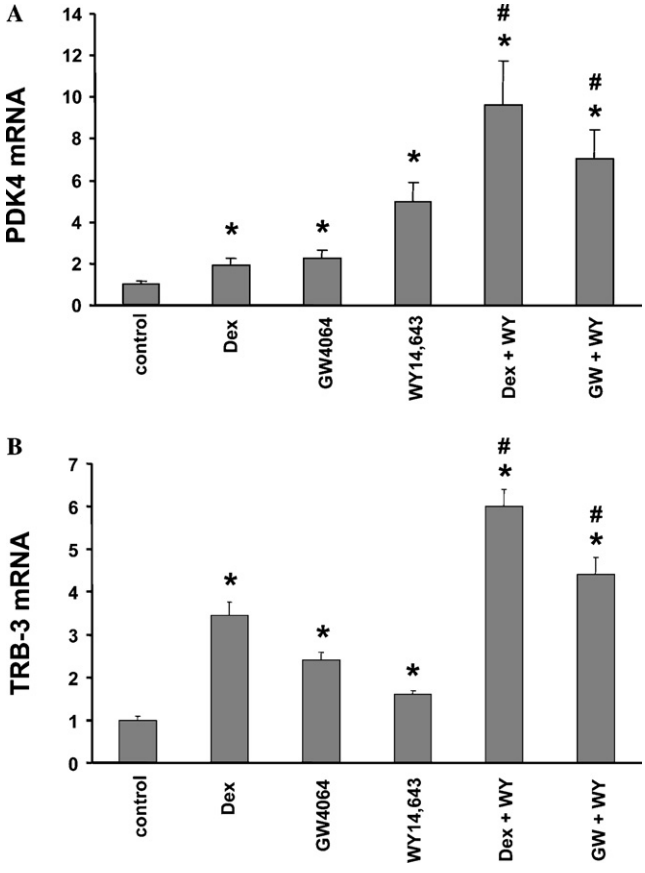

Fig. 4. Additive effect of FXR, GR, and PPARα agonists on PDK4 mRNA expression in H4IIE rat hepatoma cells. (A) H4IIE cells were treated with DMSO (control) or 1 μM Dex or 2.5 μM GW4064 or 25 μM WY14,643 alone or a combination of either Dex + WY14,643 (Dex + WY) or GW4064 + WY14,643 (GW + WY) for 24 h. At the end of the treatment period, PDK4 mRNA quantity was measured. All values were normalized to 36B4 expression. (B) H4IIE cells were treated with DMSO (control) or 1 μM Dex or 2.5 μM GW4064 or 25 μM WY14,643 alone or a combination of either dexamethasone + WY14,643 (Dex + WY) or GW4064 + WY14,643 (GW + WY) for 24 h. At the end of the treatment period, TRB-3 mRNA quantity was measured and normalized to 36B4 expression. Asterisks (*) indicate values significantly different ($p < 0.05$) than control values, and the number signs (#) indicate values that are significantly different ($p < 0.05$) than their respective single treatments.

Recent studies identified PPARα as an FXR responsive target gene [26] and we demonstrated that FXR agonists induce the expression of PPARα and TRB-3 in both human cells and rodents [22]. TRB-3, the mammalian homolog of the *Drosophila tribbles*, promotes hepatic insulin resistance via a pathway involving the FOXO proteins [27]. Interestingly, TRB-3 has been demonstrated to be a target for PPARα, and the expression of TRB-3 is increased upon overexpression of PPARα. This increase in the expression of TRB-3 is further augmented by the addition of a PPARα agonist [28]. Furthermore, the expression of TRB-3 is also induced by glucocorticoids [27]. Thus, with glucocorticoids inducing the expression of PPARα, and PPARα in turn regulating the expression of TRB-3 and PDK4, it is conceivable that the induction of PDK4 expression by glucocorticoids may involve a pathway mediated by PPAR, TRB-3, and the FOXO proteins.

We recently demonstrated that the expression of a key gene involved in carbohydrate metabolism, phosphoenolpyruvate carboxykinase (PEPCK), is also induced by FXR agonists [22]. This study suggested that the increase in PEPCK expression induced by FXR agonists follows a pathway involving PPARα, TRB-3, and the FOXO transcription factors [22]. It has been demonstrated that the expression of PDK4 is also regulated by the FOXO transcription factors [23]. With the activity of FOXO proteins regulated by TRB-3 [27], the expression of TRB-3 regulated by PPARα [28], and the expression of PPARα in turn regulated by both GR and FXR agonists [13,22,26], we predicted that the additive increase in the expression of PDK4 by a combination of either the GR/PPARα or the FXR/PPARα agonists could be due to an additive increase in the expression of TRB-3. As illustrated in Fig. 4B, treatment of rat H4IIE cells with either the GR agonist (DEX) or the FXR agonist (GW4064) alone increased the expression of TRB-3 3.4-fold and 2.4-fold, respectively. The PPARα agonist alone resulted in a 1.7-fold induction of TRB-3 mRNA. As anticipated, the combination of a GR and a PPARα agonist, or the combination of an FXR and PPARα agonist resulted in a significant additive effect characterized by a 6.0-fold and a 4.4-fold increase in the

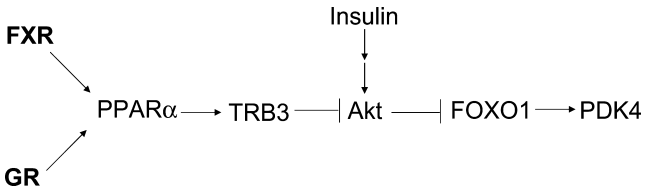

Fig. 5. Proposed mechanism of FXR-mediated regulation of hepatic PDK4 expression. Activation of FXR results in increased expression of PPARα, which leads to increased expression of TRB-3. TRB-3 binds to Akt and inhibits its ability to phosphorylate targets such as FOXO1, leading to an increase in PDK4 expression.

expression of TRB-3, respectively. Thus, our results demonstrating the additive increase in the expression of PDK4 using a combination of GR/PPARα or FXR/PPARα agonists suggest that the combination of the two agonists results in an enhancement in the expression of TRB-3, and this would contribute to the increase in the expression of its downstream target, PDK4.

Although, additional factors and mechanisms could play a role in regulating the expression of PDK4, based on our current study, we propose a mechanism where the FXR-mediated increase in the expression of PDK4 involves the PPARα–TRB-3–FOXO pathway (Fig. 5). It has previously been demonstrated that the increase in the expression of PDK4 upon stimulation with GR or PPARα agonists is inhibited by insulin [11] via a mechanism involving increased phosphorylation of the FOXO transcription factors [23]. It has also been demonstrated that the decrease in Akt/PKB-mediated phosphorylation of the FOXO proteins upon increased expression of TRB-3 is reversed by insulin [27,28]. Consistent with these observations, the increase in expression of PDK4 in H4IIE hepatoma cells we observed upon FXR stimulation is inhibited by insulin (~90% inhibition; data not shown). Furthermore, the insulin inhibition of PDK4 expression in the presence of the FXR agonist, GW4064, is comparable to that observed with GR and PPARα agonists (data not shown).

The hypolipidemic effects of PPARα agonists have been linked to induction of PDK4 expression [10,12]. The ability of FXR agonists to lower triglyceride levels has previously been attributed to inhibition of fatty acid synthesis via a pathway involving SHP and SREBP-1c [29]. Our present study suggests a novel mechanism and an additional pathway for the FXR-mediated reduction in triglyceride levels by increased fatty acid oxidation via a pathway that involves induction in the expression of PPARα and PDK4.

Acknowledgments

We thank Dr. Michael Christe, James Ficorilli, and Todd Cook for their assistance with in vivo techniques.

References

[1] P.J. Randle, Fuel selection in animals, Biochem. Soc. Trans. 14 (1986) 799–806.
[2] M.C. Sugden, M.J. Holness, Interactive regulation of the pyruvate dehydrogenase complex and the carnitine palmitoyltransferase system, Faseb J. 8 (1994) 54–61.
[3] M.M. Bowker-Kinley, W.I. Davis, P. Wu, R.A. Harris, K.M. Popov, Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex, Biochem. J. 329 (1998) 191–196.
[4] M.C. Sugden, L.G. Fryer, K.A. Orfali, D.A. Priestman, E. Donald, M.J. Holness, Studies of the long-term regulation of hepatic pyruvate dehydrogenase kinase, Biochem. J. 329 (1998) 89–94.
[5] P. Wu, P.V. Blair, J. Sato, J. Jaskiewicz, K.M. Popov, R.A. Harris, Starvation increases the amount of pyruvate dehydrogenase kinase in several mammalian tissues, Arch. Biochem. Biophys. 381 (2000) 1–7.
[6] M.C. Sugden, K. Bulmer, G.F. Gibbons, B.L. Knight, M.J. Holness, Peroxisome-proliferator-activated receptor-alpha (PPARalpha) deficiency leads to dysregulation of hepatic lipid and carbohydrate metabolism by fatty acids and insulin, Biochem. J. 364 (2002) 361–368.
[7] M.C. Sugden, K. Bulmer, G.F. Gibbons, M.J. Holness, Role of peroxisome proliferator-activated receptor-alpha in the mechanism underlying changes in renal pyruvate dehydrogenase kinase isoform 4 protein expression in starvation and after refeeding, Arch. Biochem. Biophys. 395 (2001) 246–252.
[8] P. Wu, J.M. Peters, R.A. Harris, Adaptive increase in pyruvate dehydrogenase kinase 4 during starvation is mediated by peroxisome proliferator-activated receptor alpha, Biochem. Biophys. Res. Commun. 287 (2001) 391–396.
[9] M.J. Holness, N.D. Smith, K. Bulmer, T. Hopkins, G.F. Gibbons, M.C. Sugden, Evaluation of the role of peroxisome-proliferator-activated receptor alpha in the regulation of cardiac pyruvate dehydrogenase kinase 4 protein expression in response to starvation, high-fat feeding and hyperthyroidism, Biochem. J. 364 (2002) 687–694.
[10] M.J. Holness, K. Bulmer, N.D. Smith, M.C. Sugden, Investigation of potential mechanisms regulating protein expression of hepatic pyruvate dehydrogenase kinase isoforms 2 and 4 by fatty acids and thyroid hormone, Biochem. J. 369 (2003) 687–695.
[11] B. Huang, P. Wu, M.M. Bowker-Kinley, R.A. Harris, Regulation of pyruvate dehydrogenase kinase expression by peroxisome proliferator-activated receptor-alpha ligands, glucocorticoids, and insulin, Diabetes 51 (2002) 276–283.
[12] K. Motojima, K. Seto, Fibrates and statins rapidly and synergistically induce pyruvate dehydrogenase kinase 4 mRNA in the liver and muscles of mice, Biol. Pharm. Bull 26 (2003) 954–958.
[13] T. Lemberger, B. Staels, R. Saladin, B. Desvergne, J. Auwerx, W. Wahli, Regulation of the peroxisome proliferator-activated receptor-alpha gene by glucocorticoids, J. Biol. Chem. 269 (1994) 24527–24530.
[14] B.M. Forman, E. Goode, J. Chen, A.E. Oro, D.J. Bradley, T. Perlmann, D.J. Noonan, L.T. Burka, T. McMorris, W.W. Lamph, Identification of a nuclear receptor that is activated by farnesol metabolites, Cell 81 (1995) 687–693.
[15] M. Makishima, A.Y. Okamoto, J.J. Repa, H. Tu, R.M. Learned, A. Luk, M.V. Hull, K.D. Lustig, D.J. Mangelsdorf, B. Shan, Identification of a nuclear receptor for bile acids, Science 284 (1999) 1362–1365.
[16] D.J. Parks, S.G. Blanchard, R.K. Bledsoe, G. Chandra, T.G. Consler, S.A. Kliewer, J.B. Stimmel, T.M. Willson, A.M. Zavacki, D.D. Moore, J.M. Lehmann, Bile acids: natural ligands for an orphan nuclear receptor, Science 284 (1999) 1365–1368.

[17] K.S. Bramlett, S.F. Yao, T.P. Burris, Correlation of farnesoid X receptor coactivator recruitment and cholesterol 7 alpha-hydroxylase gene repression by bile acids, Mol. Genet. Metab. 71 (2000) 609–615.
[18] T.T. Lu, M. Makishima, J.J. Repa, K. Schoonjans, T.A. Kerr, J. Auwerx, D.J. Mangelsdorf, Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors, Mol. Cell 6 (2000) 507–515.
[19] B. Goodwin, S.A. Jones, R.R. Price, M.A. Watson, D.D. McKee, L.B. Moore, C. Galardi, J.G. Wilson, M.C. Lewis, M.E. Roth, P.R. Maloney, T.M. Willson, S.A. Kliewer, A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis, Mol. Cell 6 (2000) 517–526.
[20] C.J. Sinal, M. Tohkin, M. Miyata, J.M. Ward, G. Lambert, F.J. Gonzalez, Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis, Cell 102 (2000) 731–744.
[21] H.R. Kast, B. Goodwin, P.T. Tarr, S.A. Jones, A.M. Anisfeld, C.M. Stoltz, P. Tontonoz, S. Kliewer, T.M. Willson, P.A. Edwards, Regulation of multidrug resistance-associated protein 2 (ABCC2) by nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor, J. Biol. Chem. 277 (2002) 2908–2915.
[22] K.R. Stayrook, K.S. Bramlett, R.S. Savkur, J. Ficorilli, T. Cook, M.E. Christe, L.F. Michael, T.P. Burris, Regulation of carbohydrate metabolism by the farnesoid X receptor, Endocrinology. 10.1210/en.2004-0965.
[23] H.S. Kwon, B. Huang, T.G. Unterman, R.A. Harris, Protein kinase B-alpha inhibits human pyruvate dehydrogenase kinase-4 gene induction by dexamethasone through inactivation of FOXO transcription factors, Diabetes 53 (2004) 899–910.
[24] G.Q. Cao, Y. Liang, C.L. Broderick, B.A. Oldham, T.P. Beyer, R.J. Schmidt, Y.Y. Zhang, K.R. Stayrook, C. Suen, K.A. Otto, A.R. Miller, J.N. Dai, P. Foxworthy, H. Gao, T.P. Ryan, X.C. Jiang, T.P. Burris, P.I. Eacho, G.J. Etgen, Antidiabetic action of a liver X receptor agonist mediated by inhibition of hepatic gluconeogenesis, J. Biol. Chem. 278 (2003) 1131–1136.
[25] P.R. Maloney, D.J. Parks, C.D. Haffner, A.M. Fivush, G. Chandra, K.D. Plunket, K.L. Creech, L.B. Moore, J.G. Wilson, M.C. Lewis, S.A. Jones, T.M. Willson, Identification of a chemical tool for the orphan nuclear receptor FXR, J. Med. Chem. 43 (2000) 2971–2974.
[26] I.P. Torra, T. Claudel, C. Duval, V. Kosykh, J.C. Fruchart, B. Staels, Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor, Mol. Endocrinol. 17 (2003) 259–272.
[27] K.Y. Du, S. Herzig, R.N. Kulkarni, M. Montminy, TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver, Science 300 (2003) 1574–1577.
[28] S.H. Koo, H. Satoh, S. Herzig, C.H. Lee, S. Hedrick, R. Kulkarni, R.M. Evans, J. Olefsky, M. Montminy, PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3, Nat. Med. 10 (2004) 530–534.
[29] M. Watanabe, S.M. Houten, L. Wang, A. Moschetta, D.J. Mangelsdorf, R.A. Heyman, D.D. Moore, J. Auwerx, Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c, J. Clin. Invest. 113 (2004) 1408–1418.