Retinoic Acid Receptor β Stimulates Hepatic Induction of Fibroblast Growth Factor 21 to Promote Fatty Acid Oxidation and Control Whole-body Energy Homeostasis in Mice

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Background: All-trans-retinoic acid ameliorates glucose intolerance and insulin resistance in diabetes. The gene transcription of hepatic Fgf21 and its metabolic effects on lipid oxidation, ketogenesis, and whole-body energy expenditure are up-regulated by RARβ activation. Hepatic RARβ stimulates FGF21 production via the RARE.

Conclusion: The hepatic RAR-FGF21 axis is a potential druggable target for treating metabolic syndrome.

Activation of retinoic acid receptor (RAR) with all-trans-retinoic acid (RA) ameliorates glucose intolerance and insulin resistance in obese mice. The recently discovered fibroblast growth factor 21 (FGF21) is a hepatocyte-derived hormone that restores glucose and lipid homeostasis in obesity-induced diabetes. However, whether hepatic RAR is linked to FGF21 in the control of lipid metabolism and energy homeostasis remains elusive. Here we identify FGF21 as a direct target gene of RARβ. The gene transcription of Fgf21 is increased by the RAR agonist RA and by overexpression of RARα and RARβ, but it is unaffected by RARγ in HepG2 cells. Promoter deletion analysis characterizes a putative RA-responsive element (RARE) primarily located in the 5'-flanking region of the Fgf21 gene. Disruption of the RARE sequence abolishes RA responsiveness. In vivo adenaloviral overexpression of RARβ in the liver enhances production and secretion of FGF21, which in turn promotes hepatic fatty acid oxidation and ketogenesis and ultimately leads to increased energy expenditure in mice. The metabolic effects of RA or RARβ are mimicked by FGF21 overexpression and largely abolished by FGF21 knockdown. Moreover, hepatic RARβ is bound to the putative RAREs of the Fgf21 promoter in a fasting-inducible manner in vivo, which contributes to FGF21 induction and the metabolic adaptation to prolonged fasting. In addition to other nuclear receptors, such as peroxisome proliferator-activated receptor α and retinoic acid receptor-related receptor α, RAR may act as a novel component to induce hepatic FGF21 in the regulation of lipid metabolism. The hepatic RAR-FGF21 pathway may represent a potential drug target for treating metabolic disorders.

All-trans-retinoic acid (RA), a major active metabolite of vitamin A, plays essential roles in development, cellular differentiation, and survival through three retinoic acid receptors (RARs) of the nuclear receptor superfamily (1, 2). RA is currently used for clinical cancer therapy (1). RAR is the ligand-dependent nuclear receptor that heterodimerizes with the retinoid X receptor (RXR) to transactivate its target genes by binding to gene promoters harboring retinoic acid response elements (RAREs) (1, 2). RARβ is relatively more abundant in the liver compared with RARα and RARγ (3). Recent studies indicate that hepatic retinoid signaling is impaired in humans with non-alcoholic fatty liver disease (4). Hepatic RAR receptor binding capacity is suppressed in high fat diet rats, accompanied by decreased expression of RARβ and unaltered expression of other RAR isoforms (5). Recently, several independent studies have demonstrated that administration of RA ameliorates obesity and glucose intolerance and suppresses adipose lipid stores in mouse models of obesity and diabetes (6–8). However, the signaling molecules that mediate the metabolic actions of RAR remain largely unknown.

The recently discovered fibroblast growth factor 21 (FGF21) is a member of an atypical subfamily of FGFs, which includes FGF15/19 and FGF23, all of which circulate as hormones (9). FGF21 acts through cell surface receptors composed of classic FGF receptors complexed with Klotho (10). In obese rodents or monkeys, pharmacological administration of FGF21 improves insulin sensitivity, normalizes plasma lipids levels, causes weight loss, and increases whole-body energy expenditure.

The abbreviations used are: RA, all-trans-retinoic acid; FGF21, fibroblast growth factor 21; RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, RA-responsive element; CPT1α, carnitine palmitoyltransferase 1α; PPARα, peroxisome proliferator-activated receptor α; RORα, retinoic acid receptor-related receptor α; CRABP-II, cellular retinoic acid-binding protein II; MCD, medium-chain acyl-CoA dehydrogenase; HMGL, HMG-CoA lyase; HMGCS2, mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2.

*This work was supported, in whole or in part, by National Institutes of Health Grant DK027642. This work was also supported by the Robert Dawson Evans Junior Faculty Merit Award, the Wing Tat Lee Award (to M. Z.), and Boston University CTSI subsidy fund Grant ULTR025771 (to Y. L.).

This article contains supplemental Fig. 1.

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The mouse monoclonal FGF21 antibody was described previously (20). The Quantikine mouse FGF21 ELISA kits (catalog no. MF2100) were from R&D Systems (Minneapolis, MN). The β-hydroxybutyrate LiquiColor Test kit (catalog no. 2440-058) was from Stanbio Laboratory (Boerne, TX). The Dual-Luciferase reporter assay kit was purchased from Promega (Madison, WI).

Generation of Adenoviruses Expressing either FGF21 or RARβ—Recombinant adenoviruses expressing mouse FGF21 and RARβ were generated using an AdEasy system (21). The cDNA clones for mouse FGF21 and RARβ were purchased from OriGene (catalog nos. MC204941 and MC205276). The full-length Fgf21 or RARβ cDNA was amplified by PCR and subcloned into the shuttle vector pShuttle-CMV, as described previously (22–25). The resultant plasmids were linearized by the restriction endonuclease PmeI and purified using phenol/chloroform. The linearized plasmids were transformed into Escherichia coli strain B5183-AD1 competent cells (Stratagene) containing the supercoiled adenoviral vector pAdEasy1 by electroporation (2.5 kV, 200 ohms, 25 microfarads), and recombinant bacteria colonies were selected by kanamycin resistance. Positive recombinant adenovirus colonies were characterized and selected by restriction endonuclease PstI digestion to release a small fragment of either 4.5 or 3 kb and a large fragment of a 35-kb adenovirus vector fragment. The correct recombinant adenoviral constructs were subsequently prepared and linearized with PstI and purified using phenol/chloroform. The linearized adenoviral vectors were transfected into a mammalian HEK293A packaging cell line with Lipofectamine 2000 (Invitrogen) for adenovirus production. Both cells and cultured media were harvested 7 days post-transfection, and viral lysates of transfected HEK293A cells were prepared by three freeze-thaw cycles alternatively using a 37 °C water bath and a liquid nitrogen container. Samples were centrifuged at 500 × g at 4 °C to pellet the cell debris. Recombinant adenoviruses were further propagated in HEK293A cells. Briefly, cells with a confluence of ~60–80% were reinfection by adding the transfected viral supernatants and then cultured in DMEM supplemented with 2% fetal bovine serum. When most of the infected cells were rounded up and approximately half of the cells were detached at 3–4 days following infection, the infected cells and cultured media were collected. Several rounds of amplification in HEK293A cells were performed. For the amplification and purification of high titer recombinant adenoviruses, large numbers of HEK293A cells were infected with viral supernatant at a multiplicity of infection of ~10 pfu/cell. The adenoviruses producing FGF21 or RARβ, respective, were purified using the Adenovirus Purification Kit (Puresyn, Malvern, PA).

Animal Experiments—Male C57BL/6J mice at 8 weeks of age were purchased from Jackson Laboratory. The C57BL/6J mice were divided into two groups: fed and fasted. The fed group was fed ad libitum, and the fasted group of mice was fasted for 24 h. Feeding regimens were carried out in a staggered fashion, so that all mice were euthanized at the same time. All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee at Boston University School of Medicine.
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In Vivo Adenoviral Gene Transfer—Adenovirus-mediated overexpression of either FGF21 or RARβ in the liver of C57BL/6 mice was accomplished via intravenous injection, as described previously (25, 26). One hundred microliters of adenovirus (5 × 10^10–1 × 10^11 pfu) per mouse were delivered into mice via tail vein injection. Two weeks postinjection, each group of mice was sacrificed under isoflurane anesthesia. Tissues were rapidly taken, freshly frozen in liquid nitrogen, and stored at −80 °C until biochemical analysis.

Metabolic Cages—Mice injected with Ad-GFP or Ad-RARβ were housed individually in metabolic cages designed by Comprehensive Laboratory Animal Monitoring Systems (CLAMS) (Columbus Instruments, Columbus, OH). After 1 day of acclimation, metabolic data were collected automatically. On the third day of the experiment, food was removed for a 24-h fast. The rates of VO₂ and VCO₂ were expressed as average values measured every 18 min over a 12-h block of light and dark cycles. The energy expenditure (kcal/kg/h) was calculated with the formula, (3.815 + 1.232 × VCO₂/VO₂) × VO₂ × 0.001, as described previously (27, 28). Physical activity was measured on x and y axes using infrared beams to count the bean breaks in CLAMS cages. The values were then summed (x_sum + y_sum) over 12-h intervals of the light and dark cycles, respectively. These experiments were carried out in the Metabolic Pheno-typing Core at Boston University School of Medicine as described previously (29, 30).

Body Composition Analysis—Body composition was determined by an NMR system with the body composition analyzer Echo 900 (Echo Medical Systems, Houston, TX). Body fat, lean mass, body fluids, and total body water were measured in live conscious mice with ad libitum access to chow.

Generation of Fgf21 Promoter-driven Luciferase Constructs—A series of Fgf21 promoter-driven luciferase plasmids encoding various lengths of 5′-flanking region of the human Fgf21 promoter were constructed as described previously (24). A 2207-bp region of the Fgf21 promoter, including a 2090-bp upstream sequence of the transcriptional start site, was amplified by PCR from a bacterial artificial chromosome with the clone number RP11-110K24 (BACPAC Resources Center, Oakland, CA). The following primers containing restriction endonuclease sites, denoted with underlining, were used: forward, AATGCGCTAGCACACGGTGAAACCCTGTCTC (HindIII); reverse, AATGCGCTAGCACACGGTGGAATGACC (HindIII). The amplified fragment was inserted into the NheI/HindIII sites of the pGL3-Basic luciferase reporter vector (Promega) to generate the full-length Fgf21 promoter luciferase reporter plasmid. This plasmid was used to amplify various shorter fragments of the Fgf21 promoter, which were also inserted into the pGL3-Basic vector. Different promoter regions were amplified using distinct forward primers: AATGCGCTAGCACACGGTGAAACCCTGTCTC for Fgf21 (−1500/+117); AATGCGCTAGCACACGGTGAAACCCTGTCTC for Fgf21 (−1290/+117); AATGCGCTAGCTTCTCAGAAGATGGCTTTGAGTTAGTCAGGCTTCT for Fgf21 (−1040/+117); AATGCGCTAGCTTCTCAGAAGATGGCTTTGAGTTAGTCAGGCTTCT for Fgf21 (−850/+117); AATGCGCTAGCTTCTCAGAAGATGGCTTTGAGTTAGTCAGGCTTCT for Fgf21 (−600/+117); AATGCGCTAGCTTCTCAGAAGATGGCTTTGAGTTAGTCAGGCTTCT for Fgf21 (−200/+117); and AATGCGCTAGCTTCTCAGAAGATGGCTTTGAGTTAGTCAGGCTTCT for Fgf21 (−50/+117). The Fgf21 (−700/+117)–Luc (mut RARE) was created using the QuikChange site-directed mutagenesis kit (Stratagene) as well as the following primers: TGCTAGCGGAGTAGTGGAGGCTTTGACCGATGGCAGAAGCT (forward) and CTGTTGGTTTCTGACACCCAGCTTCAACTCCCGCTAGC (reverse). Mutated bases of the RARE-like sequence were indicated by underlining. All plasmid constructs and mutations were verified by restriction endonuclease mapping and DNA sequencing.

Cell Treatment and Small RNA Interference—Human HepG2 cells were cultured as described previously (23–25, 31). Cells were treated for 24 h with an RAR agonist or PPARα agonist in serum-free DMEM. Knockdown experiments of RARβ, PPARα, and FGF21 were performed using siRNA oligonucleotides from Dharmacon RNAi Technology, Fisher. ON-TARGET plus Non-targeting Pool (catalog no. D-001810-05) was used as the control siRNA. The following siRNA sequences were used: siRARβ#7, CAGCUGAGUUGGAGCUAGUC (catalog no. J-003438-07-0005); siRARβ#8, CGAGAUAGAAGUGUUA (catalog no. J-003438-08-0005); siFGF21#6, GCCUUAGACCGGAGAUUA (catalog no. J-013305-06-0005); and siPPARα#6, GCUCUUGCUUAGCAGGAUA (catalog no. J-003434-06-0005). HepG2 cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen).

Dual Luciferase Activity Assays—Cells were transfected with 0.5 μg of reporter plasmids, including human FGF21-Luc and 3XRARE-Luc, along with 40 ng of Renilla luciferase plasmid pRL-SV40 (Promega) as an internal control in a 12-well plate. Thirty-two hours post-transfection, cells were treated with either agonists or antagonists of RAR or PPARα as described previously (24). Dual luciferase assays for firefly luciferase and Renilla luciferase activities were performed in duplicate according to the manufacturer’s protocols (Promega). Luciferase activity was measured using an Infinite M1000 microplate reader (Tecan Group Ltd.). The firefly luciferase activity was normalized to the Renilla luciferase activity (firefly luciferase/ Renilla luciferase) and presented as relative luciferase activity.

Quantitative Real-time PCR—Total RNA was isolated using TRizol reagent (Invitrogen). cDNA was synthesized from total RNA by SuperScriptII reverse transcriptase (Invitrogen) with oligo(dT). The resulting cDNA was subjected to real-time PCR analysis with gene-specific primers in the presence of SYBR Green PCR master mix with the Applied Biosystems StepOne-Plus as described previously (24, 26). Gene expression levels were normalized to β-actin and presented relative to the control. The real-time quantitative PCR primers were designed as follows: Fgf21 (mouse), CTGGGGGCTTACCAAGCATATA (forward) and CACCCAGGATTTGGAATGACC (reverse); Rara (mouse), AGAGCAGCGTTCGAGAAG (forward) and AGTAGGCGGAGGATTTG (reverse); Rarb (mouse), AATGCACCTTCTTCAAGGGT (forward) and GCTGTCGACACAGGAATG (reverse); Rarb (mouse), CACCCAAATGCATTCATAAG (forward) and ATCCCGAGCAGTATAGGATTC (reverse); Crabp2 (CRABP-II) (mouse), TGGGATGGAGGCCTGTCAAG (forward) and TAGACCGCGTCACACACAA (reverse); Rora (mouse), TCGGCGCAGCAGGAGG (forward) and CCAGGCAGGAGGAGG (reverse); and car-
RESULTS

The Correlated Alterations of Hepatic RAR and FGF21 during the Metabolic Adaptation to Prolonged Fasting in Vivo—In response to nutritional challenges, metabolic gene transcription needs to be precisely controlled to maintain lipid homeostasis, which is associated with a network linking the nuclear receptor and hormonal signaling to the transcriptional regulation of metabolic pathways. Given that the computational screening results with the NUBIScan program (32) predict several possible RARE sites on the Fgf21 promoter, it is postulated that RAR might play a role in regulating hepatic FGF21 in response to physiological stimuli, such as nutrition deprivation. The alterations of RARβ, FGF21, and fasting-related metabolic processes were compared in mice under both fed and fasted conditions. As shown in Fig. 1, hepatic mRNA levels of CRABP-II, the hallmark target gene of RAR (33, 34), were significantly increased in mice after a 24-h fast. No significant changes in RARβ mRNA were noted in the fed and fasted mice. These results suggest that fasting stimulates RAR transcriptional activity, but it is not likely to be due to altered RAR expression. Hepatic and plasma levels of FGF21 were increased ~20-fold and ~7-fold in the fasted mice, compared with the fed mice, consistent with earlier studies (15). The fasting-inducible FGF21 might be predicted to result in enhanced fatty acid oxidation and ketogenesis via its autocrine/paracrine effects (15, 19, 35). In accordance with this notion, mRNA levels of Cpt1α,
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The Gene Transcription of Fgf21 Is Stimulated by the Natural RAR Ligand, RA, in HepG2 Cells—To determine the effect of pharmacological activation of RAR on FGF21 in hepatocytes, an FGF21(−2090/+117) promoter-driven luciferase construct expressing a 2207-bp fragment of the 5′ flanking region of the Fgf21 promoter was generated as described previously (24). Because HepG2 cells have strong endogenous RAR activity (37), this cell line was used to assess the reporter activity of the 3XRARE-driven luciferase reporter gene as well as the Fgf21 promoter-driven reporter gene. Cells were treated with RA at increasing concentrations (0.1–5 μM), dosages that have been previously established in HepG2 cells and primary hepatocytes (8, 38), and dual luciferase reporter assays were conducted. The transcriptional activity of the 3XRARE-driven reporter was robustly stimulated by RA. Consistently, Fgf21 promoter activity was enhanced by RA in a concentration-dependent manner (Fig. 2, A and B). Moreover, the transactivation of the Fgf21 promoter was significantly increased by other retinoids, such as 9-cis-retinoic acid, an RXR ligand (Fig. 2C). These results suggest that the transcription of Fgf21 is up-regulated by RA, the most active biological retinoid, and its 9-cis-isomer.

The Transcription of the Fgf21 Gene Is Up-regulated by RARα and RARβ but Not by RARγ in HepG2 Cells—RAR consists of three isotypes (RARα, β, and γ) (1, 2). To gain direct insight
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FIGURE 3. The effect of siRNA-mediated RARβ knockdown on FGF21 in HepG2 cells treated with RA or under conditions of nutrient deprivation. A, the mRNA amounts of RARβ are increased by RA treatment and decreased by RARβ knockdown. B, activation of RAR by RA stimulates endogenous FGF21 in an RARβ-dependent manner. Cells were transfected overnight with control siRNA, siRNA RARβ#7, or siRNA RARβ#8 and then treated with or without RA (5 μM) for an additional 24 h. C, RARβ is required for RA to stimulate Fgf21 promoter activity in HepG2 cells. D, the knockdown of RARβ by siRNA in HepG2 cells is confirmed by immunoblots with anti-RARβ antibody. E, the ability of nutrient deprivation to increase Fgf21 transactivation is attenuated by RARβ knockdown. HepG2 cells were transfected overnight with the FGF21-Luc reporter plasmid and then incubated in the conditions to mimic different in vivo nutrient states: normal growth medium (Fed) and serum-free medium for 16 or 24 h (Fasted). F, the production of ketone bodies in cultured media of cells in response to nutrient deprivation is suppressed by RARβ knockdown. *, p < 0.05 versus control cells; #, p < 0.05 versus treatment cells. Error bars, S.E.

...whether the transcription of Fgf21 is modulated by different RAR isotypes, the effect of overexpressed FLAG-tagged mouse RARβ on FGF21 was initially examined in human HepG2 cells. As shown in Fig. 2, D–F, the 3XRAR luciferase reporter activity was dose-dependently stimulated by RA treatment and potentiated by overexpressed FLAG-RARβ. Compared with control cells, mRNA expression of endogenous FGF21 was stimulated ~3-fold in cells transfected with FLAG-RARβ. Next, overexpression experiments with isotype-specific mouse RARα, RARβ, and RARγ were performed to assess the specific role of the three isotypes in FGF21 regulation. To this end, three plasmids encoding each of the different mouse RAR isotypes were transfected into HepG2 cells, and they were approximately equally expressed, as confirmed by elevated mRNA levels of each mouse RAR isotype. It was worth noting that RARγ was a more potent inducer of FGF21, although mRNA levels of Fgf21 were significantly increased by both exogenous RARα and RARβ. No significant changes in FGF21 were noted in cells transfected with mouse RARγ (Fig. 2, G and H). These results suggest that RARα and -β, but not RARγ, appear to regulate Fgf21 transcription. To further test a possible role of RARγ in RA-induced FGF21, luciferase reporter assays showed that RA-stimulated FGF21 activity appeared to be unaffected by RARγ-selective antagonist MM11253 (Fig. 2I). Together, these results indicate that RARα and RARβ play a major role in regulating FGF21. Because RARβ, an inducible isotype of RAR (39), more potently stimulates FGF21 expression, RARβ was the main focus of all subsequent studies.

RARβ Plays a Critical Role in Inducing FGF21 in HepG2 Cells in Response to RA and under Conditions of Fasting—To further test the specific role of RARβ, the effect of RARβ knockdown on FGF21 was characterized in HepG2 cells. To rule out possible off-target effects of a small interfering RNA against RARβ, siRNAs that targeted distinct areas of RARβ mRNA were used. As shown in Fig. 3, A–C, the mRNA levels of RARβ were effectively suppressed by siRNA RARβ#7, with the greatest knockdown efficiency being ~60%, and, to a lesser extent, by siRNA RARβ#8. Consistent with early studies showing that RARβ is greatly inducible by RA in both HepG2 cells and human livers with no significant changes in RARα and RARγ (39), RA treatment caused a ~3-fold increase in mRNA amounts of RARβ, the bona fide target gene of RA (40). Knockdown of RARβ by siRNA resulted in a profound reduction in the basal and RA-stimulated RARβ expression, suggesting that RA is a more efficacious RAR ligand in HepG2 cells. Similarly, RA treatment also caused a ~2-fold increase in endogenous FGF21 in cells transfected with control siRNA. The stimulatory effect of RA was almost abolished by RARβ knockdown. Similar results were obtained with Fgf21 promoter activity. Given that the induction of FGF21 by RA is mimicked by overexpression of RARβ and is completely abrogated by knockdown of RARβ, our studies using molecular and biochemical approaches indicate that RARβ is sufficient and necessary for the up-regulation of FGF21 in response to RA.

To understand the functional importance of RARβ on FGF21 and ketone body production under conditions of fasting, an in vitro model of nutrient deprivation that mimics in vivo fasting was generated as described previously (41), and Fgf21 promoter activity and ketone bodies were determined in HepG2 cells transfected with control siRNA or RAR siRNA. As shown in Fig. 3, D and E, Fgf21 luciferase activity was significantly increased ~2-fold in cells incubated in serum-free medium for 16 h and was sustained for 24 h. In concert with the increase in FGF21, β-hydroxybutyrate levels in cultured medium were increased...
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FIGURE 4. The gene transcription of Fgf21 is independently regulated by RAR\(\beta\) and PPAR\(\alpha\) in HepG2 cells. A, the expression vector encoding mouse PPAR\(\alpha\) is transfected into HEK293T cells; B, the mRNA abundance of Fgf21 is stimulated by overexpression of PPAR\(\alpha\) in HepG2 cells. C, the transcriptional activity of the Fgf21 promoter is dose-dependently increased by RA and further enhanced by the PPAR\(\alpha\) ligand. HepG2 cells were transfected overnight with the plasmid encoding FGF21-Luc reporter and then treated with increasing doses of RA (1–5 \(\mu\)M) in the absence or presence of a synthetic PPAR\(\alpha\) agonist, Wy14643 (30 \(\mu\)M), for 24 h. D, the mRNA abundance of PPAR\(\alpha\) is largely suppressed in cells transfected with PPAR\(\alpha\) siRNA. E, the ability of RA (5 \(\mu\)M) to enhance FGF21 expression is abolished by knockdown of RAR\(\beta\) but not by knockdown of PPAR\(\alpha\). F, the ability of synthetic PPAR\(\alpha\) agonist, GW7647 (1 \(\mu\)M), to induce FGF21 is abrogated by knockdown of PPAR\(\alpha\) but not by knockdown of RAR\(\beta\). Cells were treated with either GW7647 or RA in the presence of control siRNA, PPAR\(\alpha\) siRNA, or RAR\(\beta\) siRNA, as indicated. *, \(p < 0.05\) versus untreated cells transfected with control siRNA; **, \(p < 0.05\) versus untreated cells transfected with the corresponding nuclear receptor siRNA; #, \(p < 0.05\) versus treatment cells transfected with control siRNA. Error bars, S.E.

by over 2-fold, consistent with increased ketone body production in primary hepatocytes in a similar starvation state (41). RAR\(\beta\) knockdown by siRNA caused a ~50% reduction of fasting-induced Fgf21 promoter activity. Consequently, fasting-induced ketone body production was largely abolished, suggesting that FGF21 induction by fasting may stimulate the ketogenic process in an RAR\(\beta\)-dependent manner. Taken together with the in vivo findings (Fig. 1), these data suggest that fasting-induced FGF21 expression and ketogenesis are probably mediated through RAR\(\beta\).

Fgf21 Transcription Is Up-regulated by RAR\(\beta\) Independent of PPAR\(\alpha\) in HepG2 Cells—Recent studies have implicated other nuclear receptors, such as PPAR\(\alpha\) and ROR\(\alpha\), as critical regulators of FGF21 in hepatocytes (15, 17). Consistent with previous studies (17), mRNA abundance and promoter activity of Fgf21 were dose-dependently elevated by increased expression of mouse ROR\(\alpha\) in HepG2 cells (supplemental Fig. 1), similar to those of RA treatment and RAR\(\beta\) overexpression (Fig. 2). As shown in Fig. 4, A–C, mRNA levels of Fgf21 were significantly increased by overexpression of PPAR\(\alpha\). In parallel, FGF21 expression was also stimulated in cells treated with the synthetic PPAR\(\alpha\) agonist GW7647. Additional reporter gene experiments showed that the transcriptional activity of Fgf21 was stimulated by a synthetic PPAR\(\alpha\) agonist, Wy14643. Strikingly, the promoter activity of Fgf21 was dose-dependently increased by RA treatment, and this induction was potentiated by GW7647, suggesting that RAR\(\beta\) and PPAR\(\alpha\) may synergize to induce Fgf21 transcription. Together, these findings indicate that RAR\(\beta\) appears to be one of the critical nuclear receptors that result in induction of FGF21 expression in hepatocytes.

Two pieces of data with a small interfering RNA approach further addressed the distinct role of RAR\(\beta\) and PPAR\(\alpha\) in FGF21. First, in cells in which PPAR\(\alpha\) was effectively suppressed by siRNA-mediated knockdown (Fig. 4D), FGF21 expression could be stimulated by RA treatment. In contrast, effective down-regulation of RAR\(\beta\) by siRNA caused a profound reduction in RA induction of FGF21, to the nearly normal levels seen in untreated cells (Fig. 4E). Second, similar experiments were performed to examine the role of PPAR\(\alpha\) in regulating FGF21, and it was found that the induction of FGF21 by GW7647 was completely abolished in cells with effective knockdown of PPAR\(\alpha\), but it was not affected in cells with siRNA-mediated suppression of RAR\(\beta\) (Fig. 4F). These results provide direct evidence that the Fgf21 transcription is independently up-regulated by PPAR\(\alpha\) and RAR\(\beta\).

RAR Acts as a Transcriptional Activator of FGF21 in Vitro—To further delineate a molecular basis for the transcriptional regulation of FGF21 by RAR, experiments for structure-function analysis of the Fgf21 promoter were designed to identify a potential DNA element responsible for RAR using the full-length FGF21 (−2090/+117) promoter as the “wild-type” promoter. A series of 5′-deletion FGF21 promoter vectors was generated by introducing various lengths of the proximal regulatory region of the Fgf21 promoter into the pGL3-Basic reporter vector, each driving production of firefly luciferase. As shown in Fig. 5, the full-length FGF21 (−2090/+117) promoter
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FIGURE 5. The identification and characterization of putative RARE sequences on the Fgf21 promoter. A, schematic representation of a series of pGL3 luciferase reporter plasmids encoding various lengths (−2090/+117 to −70/+117) of the proximal regulatory region of the human Fgf21 promoter. HepG2 cells were transfected with various plasmids encoding 5′-deleted Fgf21 promoter luciferase reporters and then treated with RA (5 μM) for 24 h. The normalized luciferase activity of FGF21 (−2090/+117) promoter in the absence of RA was arbitrarily assigned a value of 100. B, the mutant Fgf21 reporter with a disrupted RARE sequence largely attenuates the stimulatory effect of RA on FGF21. The difference in putative RARE DNA sequence between WT reporter and the mutant RARE reporter (mutRARE) in the proximal region (nucleotides −644 to −632) of the Fgf21 promoter is shown. Cells were transfected with different deletion promoters or Fgf21 promoter (−650/+117) plasmids containing either WT or mutRARE and then treated with RA (5 μM). *, p < 0.05 versus untreated cells expressing the corresponding promoter. C, two RARE-like sequences are located at positions −602 to −586 and at positions −537 to −521 in the 5′-flanking region of the mouse Fgf21 promoter. Primers against the RARE-containing region (−699 to −500) and its upstream region (−5200 to −5000) of the mouse Fgf21 promoter as indicated were designed for ChIP-quantitative PCR. D, the occupancy of endogenous RARβ on the Fgf21 gene in mouse livers is increased by fasting. In vivo quantitative ChIP assays were performed using either RARβ antibody or control IgG. The specificity of the ChIP signal at the Fgf21 locus was confirmed by minimal binding that occurred in the distal upstream region or with IgG immunoprecipitation. The value obtained from fed mice with control IgG was set to 1, and -fold enrichment relative to this value was presented as the mean ± S.E. (error bars) (n = 4–5); *, p < 0.05 versus fed mice.

showed a ~2-fold increase in FGF21 activity in HepG2 cells in response to RA. Progressive 5′-flanking deletion reporters, including FGF21 (−1500/+117), FGF21 (−1290/+117), FGF21 (−1040/+117), and FGF21 (−850/+117), caused a reduction in basal transcriptional activity but permitted a robust response to RA, to an extent similar to that of the “wild type” FGF21 (−2090/+117) reporter. Conversely, each of the 5′-flanking deletion reporters, including FGF21 (−600/+117), FGF21 (−200/+117), and FGF21 (−70/+117), revealed a decrease in basal FGF21 activity and led to an elimination of almost all of the RA responsiveness (Fig. 5A). These promoter mapping studies suggest that a functional RARE is possibly located in the nucleotide sequence between −850 and −600 on the Fgf21 promoter. To precisely define a putative RARE site, further analysis of FGF21 deletion reporters, including FGF21 (−800/+117), FGF21 (−750/+117), FGF21 (−700/+117), and FGF21 (−650/+117), showed a maximal response to RA, comparable with that of the FGF21 (−850/+117) reporter. In contrast, the FGF21 (−600/+117) deletion reporter led to the elimination of nearly all of the RA responsiveness (Fig. 5B), suggesting that the regulatory region located between −650 and −600 is responsible for RA-induced FGF21. The nucleotide sequences in this regulatory region were analyzed to identify one putative RARE sequence separated by one base (DR1) in the human Fgf21 gene as well as two putative RARE sequences spaced by five nucleotides (DR5) in the mouse Fgf21 promoter (Fig. 5C). Furthermore, the functional importance of this RARE-like nucleotide sequence within the FGF21 (−650/+117) promoter was assessed by mutagenesis studies. The ability of RA to stimulate FGF21 transactivation was abolished by the point mutation in the promoter containing a disrupted RARE when compared with that of the same reporter harboring the wild type RARE (Fig. 5B), strongly suggesting that induction of FGF21 by RA is dependent on a proximal RARE. These studies clearly establish FGF21 as a bona fide target gene of RAR.
like DNA sequence of the mouse Fgf21 gene. As shown in Fig. 5D, significantly less association of RARβ with this proximal region of the Fgf21 promoter was noted in livers of the fed mice. Strikingly, hepatic RARβ was recruited and bound to the putative RARE sequence in a fasting-inducible manner, which functionally contributes to the induction of hepatic FGF21 in these mice (Fig. 1A). The specificity of the occupancy of hepatic RARβ on the Fgf21 gene in vivo was evidenced by less binding of RARβ with negative controls using 1) a nonspecific PCR primer that amplified a genomic sequence located at over 5 kb upstream of the mouse Fgf21 promoter from the transcriptional start site and 2) an immunoprecipitation with a nonspecific IgG in lieu of RARβ antibody. These in vivo studies illustrate that the RARβ/RARE is responsible for fasting-induced Fgf21 transcription in mouse livers.

Adenovirus Overexpression of RARβ in the Liver Is Sufficient to Enhance Production and Secretion of Hepatic FGF21 and to Promote Hepatic Fatty Acid Oxidation and Ketogenesis in Mice—To gain important insight into the physiological relevance of hepatic RARβ to FGF21 actions in the regulation of lipid metabolism, overexpression and siRNA-mediated knockdown of RARβ were utilized. Adenovirus-mediated expression of RARβ in the liver was achieved by tail vein injection of adenovirus expressing RARβ (Ad-RARβ) into C57BL/6 mouse livers. As shown in Fig. 6, A–H, expression of recombinant RARβ was confirmed by a 2–3-fold increase in hepatic mRNA and protein levels of FGF21, consistent with the induction of FGF21 by RA treatment and RARβ overexpression in HepG2 cells (Fig. 2). Consequently, hepatic secretion of FGF21 was markedly increased, as reflected by an elevation of circulating FGF21 levels, suggesting that hepatic overexpression of RARβ for 2 weeks persistently increases hepatic and circulating FGF21 levels in mice. To explore the functional consequence of hepatic RARβ-FGF21 axis, mRNA levels of key components of fatty acid oxidation and ketogenesis were determined by quantitative RT-PCR. Hepatic overexpression of RARβ resulted in the stimulation of fatty acid oxidation, as shown by elevations in CPT1α and MCAD, some of which were consistent with adaptive metabolic response to fasting (Fig. 1). Additionally, hepatic overexpression of RARβ significantly enhanced expression of HMGCS2 and HMGCL as well as elevated plasma β-hydroxybutyrate (Fig. 6, F–H). These studies suggest that hepatic RARβ...
positively regulates FGF21 at the transcriptional level and recapitulates some of physiological metabolic adaptation to fasting. To further determine whether RARβ mediates the metabolic effect of RA in vitro, expression of the key enzyme CPT1α was increased over 3-fold in HepG2 cells exposed to RA, comparable with that of FGF21 induction (Fig. 2F). This effect of RA was reduced by ~40% in cells transfected with RARβ siRNA (Fig. 6I). In agreement with increased gene expression of fatty acid oxidation, secreted β-hydroxybutyrate levels in the cultured medium were significantly elevated by RA treatment and reduced by RARβ knockdown (Fig. 6I), which was well correlated with the alterations of FGF21 and CPT1α (Figs. 2F and 6I). These findings indicate that RARβ preferentially transduces the RA signaling to fatty acid oxidation in hepatocytes.

The in Vivo Metabolic Effects of Hepatic RARβ Is Mimicked by Adenoviral Overexpression of FGF21 in the Liver—To better understand the in vivo functions of FGF21 in the regulation of hepatic lipid homeostasis, adenoviral gene delivery was accomplished by tail vein injection of adenoviruses expressing either GFP or FGF21 into C57BL/6 mouse livers via tail vein injection, and the animals were sacrificed in a fed state after 2 weeks of injection. A, an adenovirus vector encoding FGF21 (Ad-FGF21) was generated, and overexpression of FGF21 in the liver was verified by quantitative RT-PCR. B, hepatic production and secretion of FGF21 are evident by increased circulating levels of FGF21 in mice injected with Ad-FGF21. C and D, in vivo adenoviral overexpression of FGF21 in the liver is sufficient to increase hepatic expression of key genes required for fatty acid oxidation. E and F, hepatic overexpression of FGF21 results in increased expression of key ketogenic genes. G, plasma β-hydroxybutyrate concentrations are increased in mice expressing FGF21 in the liver. *, p < 0.05 versus Ad-GFP-injected mice. H–J, the ability of RA to increase expression of CPT1α and HMG2 is inhibited by effective knockdown of FGF21 in HepG2 cells. K, the ability of RA to increase secreted β-hydroxybutyrate levels is suppressed by knockdown of FGF21. Cells were transfected with control siRNA or FGF21 siRNA for 24 h and then treated with RA (5 μM) for another 24 h. *, p < 0.05 versus control cells; #, p < 0.05 versus treatment cells. Error bars, S.E.

FGF21 Is Required for RAR to Stimulate Fatty Acid Oxidation and Ketogenesis in HepG2 Cells—To rigorously define whether FGF21 acts downstream of RAR in the regulation of fatty acid oxidation and ketogenesis, HepG2 cells were transfected with a scrambled siRNA control or an siRNA targeting FGF21 and treated with RA. The ability of RA to up-regulate expression of CPT1α was almost abolished by FGF21 knockdown, suggesting

FIGURE 7. Adenoviral overexpression of FGF21 in the liver is sufficient to induce gene expression of key enzymes of fatty acid oxidation and enhance the ketogenic process in mice. Adenoviruses expressing either GFP or FGF21 were delivered into C57BL/6 mice livers via tail vein injection, and the animals were sacrificed in a fed state after 2 weeks of injection. A, an adenovirus vector encoding FGF21 (Ad-FGF21) was generated, and overexpression of FGF21 in the liver was verified by quantitative RT-PCR. B, hepatic production and secretion of FGF21 are evident by increased circulating levels of FGF21 in mice injected with Ad-FGF21. C and D, in vivo adenoviral overexpression of FGF21 in the liver is sufficient to increase hepatic expression of key genes required for fatty acid oxidation. E and F, hepatic overexpression of FGF21 results in increased expression of key ketogenic genes. G, plasma β-hydroxybutyrate concentrations are increased in mice expressing FGF21 in the liver. *, p < 0.05 versus Ad-GFP-injected mice. H–J, the ability of RA to increase expression of CPT1α and HMG2 is inhibited by effective knockdown of FGF21 in HepG2 cells. K, the ability of RA to increase secreted β-hydroxybutyrate levels is suppressed by knockdown of FGF21. Cells were transfected with control siRNA or FGF21 siRNA for 24 h and then treated with RA (5 μM) for another 24 h. *, p < 0.05 versus control cells; #, p < 0.05 versus treatment cells. Error bars, S.E.
that the stimulatory effect of RAR on fatty acid oxidation is mediated by FGF21 (Fig. 7, H and I). In agreement with altered CPT1α, the effect of RA on the key ketogenic enzyme, HMGCS2, and secreted β-hydroxybutyrate in the cultured medium were significantly diminished by FGF21 knockdown (Fig. 7, J and K), indicating that RA-activated RAR controls fatty acid homeostasis in an FGF21-dependent manner. Together with in vivo overexpression studies, these results suggest that hepatic RARβ augments fatty acid utilization and supplies an energy source in part through the induction of hepatic FGF21.

Adenoviral Overexpression of RARβ in the Liver Is Sufficient to Enhance Energy Expenditure in Mice—Altered rates of hepatic fatty acid oxidation can contribute to enhanced energy expenditure and body weight loss (43). Whereas pharmacological activation of RAR by RA protects against diet-induced obesity and insulin resistance (7), little is known about a role of hepatic RARβ in the control of systemic energy balance. Because FGF21 has emerged as a critical regulator of energy balance in mouse models of obesity and diabetes (12), the direct functional impact of RARβ on systemic energy metabolism in vivo was explored. A cohort of Ad-RARβ-injected mice, along with Ad-GFP-injected mice, was subjected to indirect calorimetry to measure oxygen consumption (VO₂) and carbon dioxide production (VCO₂) as an assessment of energy expenditure. As shown in Fig. 8, A–E, in both fed and fasted states, the rates of VO₂ and VCO₂ were significantly increased during light and dark cycles in mice expressing RARβ in the liver, compared with those of control mice. The calculated energy expenditure was also increased. Due possibly to the increase in both VO₂ and VCO₂, the respiratory quotient (RQ = VCO₂/VO₂) appeared unchanged in mice expressing RARβ, compared with control mice in fed and fasted states (data not shown). The RQ was not affected in mice expressing RARβ, which possibly reflects the relatively equal use of carbohydrates versus lipids as a source of energy in whole body, as was described previously (45). Furthermore, circadian locomotor activity was similar in mice under the fed and fasted conditions because horizontal and vertical movements were comparable in mice expressing either hepatic GFP or FGF21 during light and dark cycles. Moreover, body composition by NMR measurement showed that a small but insignificant reduction in fat mass was noted in mice expressing RARβ, compared with control mice. The lean mass and body weight were indistinguishable between the two groups of mice. These findings illustrate that hepatic induction of FGF21 by RARβ may contribute to whole-body energy homeostasis possibly through increased hepatic fatty acid oxidation or other endocrine effects.

DISCUSSION

The present study characterizes the hepatocyte-derived hormone FGF21 as a novel target gene of RARβ. First, mRNA expression of FGF21 is selectively up-regulated by RARα and -β but not by RARY in HepG2 cells. Second, the gene expression and transcriptional activity of Fgf21 are stimulated by RA at least in part through RARβ. In cells with suppression of PPARα, FGF21 is still inducible by RA. Third, functional dissection of the Fgf21 promoter illustrates that a putative RARE site of the Fgf21 promoter is responsible for RARβ-stimulated Fgf21 transcription. Fourth, hepatic overexpression of RARβ by the adenoviral gene transfer approach persistently simulates expression of FGF21 and recapitulates much of FGF21 metabolic actions, such as enhanced hepatic fatty acid oxidation and ketogenesis, as well as increased whole-body energy expenditure in mice. Finally, the ability of RA or RARβ to stimulate fatty acid oxidation and ketogenesis is mimicked by FGF21 overexpression and diminished by FGF21 knockdown. Although recent studies implicated PPARα and RORα in the regulation of FGF21 in hepatocytes (15, 17), the present study provides an alternative mechanism by which gene transcription of Fgf21 is mediated through hepatic RARβ via the putative RARE sequence in mice under conditions of nutrient deprivation. This additional layer of the transcriptional regulation of FGF21 by RARβ could further enhance the long term adaptive response to metabolic stresses. As depicted in Fig. 8F, hepatic RAR activation, particularly that of RARβ, can up-regulate FGF21, leading to enhanced metabolic adaptation to fasting. This study may provide a rationale for the therapeutic potential of RAR-selective agonists for humans with cancers and metabolic disorders, especially because RA has been clinically used for cancer therapy in humans (1, 2).

The Interplay of RAR and FGF21 in the Liver Plays a Role in Enhancing Hepatic Fatty Acid Oxidation and Ketogenesis—An interesting finding of the present study is that RA/RAR simulates gene expression of lipid oxidation and ketogenesis in an FGF21-dependent manner. Our results also highlight a potential role of FGF21 in stimulating β-oxidation and ketogenesis in hepatocytes in response to nutrient deprivation. This mechanism would allow hepatocytes to increase mitochondrial fatty acid oxidation and ketogenesis and meet the energetic requirement in the circumstance of energy stress. Multiple studies have established that the treatment of RA, the active vitamin A metabolite, induces weight loss and attenuates insulin resistance in obesity and diabetes (6, 7). One critical mechanism of the actions of RA is that activation of RAR may reduce hepatic lipid accumulation and normalize serum triglyceride levels (6, 46). Unfortunately, the currently described RAR isotype knockout models display severe developmental defects; they display congenital malformations with the fetal and postnatal vitamin A deficiency syndrome (47), making it impossible to evaluate the impact of RAR on energy metabolism. Therefore, in vivo adenoviral gene transfer is used to generate mice with a moderate overexpression of RARβ in the liver, which displays an elevation of hepatic and plasma levels of FGF21, induction of fatty acid oxidation, and an increase in energy expenditure. There is a strong overlap between hepatic lipid metabolic genes that are regulated by enforced overexpression of FGF21 and those controlled by overexpression of RARβ in vivo. Because the phenotypic changes seen in mice expressing RARβ or FGF21 in the liver, such as elevation of enzymes for fatty acid oxidation (CPT1α and MCAD) and ketogenesis (HMG2S and HMGCL), recapitulate much of the adaptive response to nutrient deprivation, understanding the hepatic RARβ-mediated production of FGF21 may provide insight into a molecular basis for reprogramming metabolic gene transcription during physiological, nutrient stresses. Supporting this notion, FGF21 induction can be explained by the strong binding of hepatic...
RARβ to the putative RARE sequence on the Fgf21 promoter in the fasted mice. Future studies with generation of tissue-specific knock-out mice of isotype-specific RAR will be important for establishing the relative contribution of individual RAR iso-type to the regulation of FGF21 and lipid homeostasis in physiological and pathological states.

The identification of additional components of the adaptive fasting pathway possesses potentially clinical implications. The fundamental fasting response has recently gained considerable attention as a potential therapeutic avenue for the treatment of metabolic dysfunction. Based on reduced hepatic fat accumulation in RA-treated obese mice (6, 7), the stimulation of fatty

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**FIGURE 8.** Adenoviral overexpression of RARβ in the liver is sufficient to enhance whole-body energy expenditure in mice. A–C, the effect of hepatic RARβ on the metabolic rate in mice. The rates of VO₂ (A), VCO₂ (B), and energy expenditure (C) are measured by comprehensive metabolic monitoring over a 24-h period with food and over a 24-h fast in mice expressing either GFP or RARβ in the liver. The top panels represent circadian changes in energy metabolic parameters in mice in the fed state during the dark cycle, and the bottom panels represent metabolic parameters in mice in fed and fasted states during both light and dark cycles. D, the locomotor activity is expressed as total counts, as measured by summing x and y beam breaks. The top panel represents the circadian locomotor activity of two groups. The bottom panel represents the average locomotor activity over a 12-h block of the light and dark phases. E, body composition analysis of mice expressing either GFP or RARβ in the liver. Bar graphs are presented as the mean ± S.E. (error bars) (n = 4). *, p < 0.05 versus Ad-GFP-injected mice. F, a proposed model for hepatic RAR as a novel regulator of FGF21. Activation of RAR by retinoid acid, the natural ligand, induces expression of FGF21 in hepatocytes, which in turn simulates hepatic fatty acid oxidation and ketogenesis possibly through autocrine/paracrine actions of FGF21. The RAR/RXR heterodimer may serve as the main functional regulator transducing RA signaling to the transcriptional regulation of FGF21 via the putative RARE sequence. Hepatic FGF21 induction by RARβ may result in enhanced systemic energy expenditure, possibly through increased hepatic β-oxidation and/or some of endocrine effects of FGF21. The physiological regulation of FGF21 by RAR probably contributes to the adaptive response to nutrient deprivation.
FGF21 Is a Direct Target Gene of RARβ in the Liver

acid oxidation and energy expenditure by RARβ overexpression may explain most of the beneficial effects of RA and provide a rationale for pharmacological intervention with RAR-selective agonists to counteract the detrimental effects of fatty liver disease and metabolic syndrome.

FGF21 Is a Direct Target Gene of RAR in Hepatocytes—The major finding of the present study is the identification and characterization of RAR as one of the major nuclear receptors that regulate hepatic FGF21 and lipid metabolism. Strikingly, cells with knockdown of PPARα remain responsive to FGF21 induction by RAR agonists, suggesting that additional FGF21 regulators such as RAR may modulate its transcription. We have demonstrated that both RARα and RARβ, but not RARγ, can induce Fgf21 transcription. We notice that the three isoforms contain a highly conserved DNA binding domain that allows them to bind to a RARE site on a target gene promoter. However, the variability of the modulating domains may determine the specificity of the interaction of each RAR isotype with different co-regulators or cofactors on target gene promoters and may result in a different potential for the transcription of various target genes.

Our results indicate that hepatic RARβ is sufficient and necessary for the induction of FGF21 because Fgf21 transcription is stimulated by pharmacological activation of RAR and overexpression of RARβ as well as suppressed by RARβ knockdown. This cross-talk raises the possibility that RARβ may directly regulate FGF21. Functional analysis of deletion promoter reporters indicates that the putative RARE sequence present in the proximal region of the Fgf21 promoter is responsible for RARβ-stimulated FGF21 in hepatocytes. Previous observations that deletion of PPARs in mice cannot abolish the fasting-inducible FGF21 suggest that other regulators may contribute to the induction of hepatic FGF21. One such mechanism described in this study is that FGF21 induction is possibly mediated through the recruitment of RARβ to the putative RARE-containing region of the Fgf21 promoter in mice under the well-established, physiological adaptive response to prolonged fasting. The data presented here show that mRNA levels of CRABP-II, a known target gene of RAR, are increased by fasting, which reflects a net effect of increased activity of RAR isoforms, despite no significant changes in RARβ mRNA levels being seen in the fasted mice. The nutrient sensing and/or hormonal inputs that regulate RAR activity, however, remain largely unknown. Accumulated evidence demonstrates that post-translational modifications of RAR, such as protein kinase A (PKA) phosphorylation of RAR and NAD-dependent deacetylase SIRT1-mediated deacetylation of RAR, may also affect the transcription of RAR or its target genes (48, 49). Given that expression and/or activity of SIRT1 and cAMP/PKA are stimulated by fasting (50), nutrient sensor SIRT1 and other hormonal signaling linked to PKA may possibly be involved in the regulation of RARβ activity during fasting. The cross-talk between nutrient sensing pathways, the nuclear receptor, and FGF21 in the regulation of lipid homeostasis remains to be further investigated.

Hepatic RARβ Is Implicated in the Control of Whole-body Energy Balance—An intriguing observation is that increasing RARβ in the liver causes a moderate increase in whole-body oxygen consumption and energy expenditure, and these changes are probably attributable to the induction of hepatic FGF21 and fatty acid oxidation. Dysregulation of total energy balance/expenditure contributes to the pathogenesis of metabolic disorders, such as obesity and type 2 diabetes (44). Despite extensive studies, the pathogenesis and treatment of human obesity have not been fully elucidated. Obesity results from a positive energy balance, where energy intake exceeds energy expenditure. Manipulation of energy expenditure has long been pursued in the development of new therapies for obesity. For instance, thyroid hormone stimulates energy expenditure and promotes weight loss, but it also produces side effects, such as loss of lean mass and production of cardiac toxicity. Thus, the identification of a novel and druggable signaling pathway that controls energy metabolism could create many pharmacological opportunities. The present study uncovers a previously uncharacterized link between selective activation of RAR, a major development pathway, as well as FGF21, a nutrient sensor, in the regulation of lipid homeostasis and energy balance. Although elevated hepatic FGF21 levels and enhanced energy expenditure have been seen in mice expressing RARβ in the liver, future studies are needed to determine whether selective activation of RARβ in the liver can effectively reduce individuals susceptible to obesity-induced diabetes and energy imbalance.

In conclusion, the present study clearly establishes FGF21 as a novel target gene of RARβ in hepatocytes. Hepatic overexpression of RARβ stimulates FGF21, which in turn increases β-oxidation and ketogenesis and enhances energy expenditure in vivo. During prolonged fasting, hepatic RARβ is recruited to the putative RARE sequences of the Fgf21 promoter, leading to the induction of Fgf21 transcription in vivo. Because of the associated weight loss and systemic insulin sensitivity seen in diabetic mice treated with RA (6, 8), targeting the RAR-FGF21 axis potentially represents a novel therapeutic avenue for treating fatty liver disease and metabolic syndrome.

Acknowledgments—We greatly appreciate Dr. Richard A. Cohen’s strong support. We are also grateful to Drs. Yanqiao Zhang and Grace L. Guo for constructive suggestions in the nuclear receptor studies. We thank Dr. David Pimental for kindly providing an adenovirus backbone plasmid and Dr. Rui Lu for a gift of a FLAG plasmid. We are grateful for Dr. Jianwei Jiang for technical assistance and Drs. Ravi Jasuja and Gianluca Toraldo for great assistance in metabolic cage studies.

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