Estrogen-related Receptor α (ERRα) Is a Transcriptional Regulator of Apolipoprotein A-IV and Controls Lipid Handling in the Intestine*

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The estrogen-related receptor α (ERRα) is an orphan member of the superfamily of nuclear receptors involved in the control of energy metabolism. In particular, ERRα induces a high energy expenditure in the presence of the coactivator PGC-1α. However, ERRα knockout mice have reduced fat mass and are resistant to diet-induced obesity. ERRα is expressed in epithelial cells of the small intestine, and because the intestine is the first step in the energy chain, we investigated whether ERRα plays a function in dietary energy handling. Gene expression profiling in the intestine identified a subset of genes involved in oxidative phosphorylation that were down-regulated in the absence of ERRα. In support of the physiological role of ERRα in this pathway, isolated enterocytes from ERRα knockout mice display lower capacity for β-oxidation. Microarray results also show altered expression of genes involved in dietary lipid digestion and absorption, such as pancreatic lipase-related protein 2 (PLRP2), fatty acid-binding protein 1 and 2 (L-FABP and I-FABP), and apolipoprotein A-IV (apoA-IV). In agreement, we found that ERRα−/− pups exhibit significant lipid malabsorption. We further show that the apoA-IV promoter is a direct target of ERRα and that its presence is required to maintain basal level but not feeding-induced regulation of the apoA-IV gene in mice. ERRα, in cooperation with PGC-1α, activates the apoA-IV promoter via interaction with the apoC-III enhancer in both human and mouse. Our results demonstrate that apoA-IV is a direct ERRα target gene and suggest a function for ERRα in intestinal fat transport, a crucial step in energy balance.

Nuclear receptors are ligand-inducible transcription factors that control important metabolic pathways needed for development and homeostasis. This superfamily includes classic receptors for ligands such as steroid hormones, vitamin D, and thyroid hormones as well as orphan nuclear receptors for which there are no physiological ligands associated at the time of their discovery (1). Estrogen-related receptor α (ERRα) is an orphan nuclear receptor originally identified on the basis of its homology with the estrogen receptor α (2). The two receptors display transcriptional cross-talk and share some target genes and synthetic ligands (reviewed in Ref. 3). Furthermore, ERRα may have a function in bone remodeling (4, 5) and as a prognostic marker of breast cancer (6, 7), two classical estrogen-responsive tissues.

Apart from its proposed role as a modulator of estrogen receptor-dependent pathways, several lines of evidence indicate that ERRα acts primarily as a regulator of energy metabolism. First, ERRα is known to be expressed in tissues with high β-oxidation activity such as the brown fat, kidney, heart, and intestine (2, 8, 9). Furthermore, ERRα expression is induced during adipocyte differentiation (10) and in response to stimuli that increase energy demand such as in the liver under fasting conditions (11) and in skeletal muscle and brown fat from mice exposed to cold (12). Second, ERRα regulates the gene encoding medium chain acyl-CoA dehydrogenase (MCAD), which catalyzes the initial step in mitochondrial fatty acid β-oxidation (9, 13, 14). Third, the peroxisome proliferator-activated receptor (PPAR)γ coactivator 1α (PGC-1α), a coactivator central to the control of energy expenditure (15), has recently been described as a key partner of ERRα, assuring both its expression and transactivation potential (10–12, 14, 16). In fact, ERRα appears to be an essential transducer of PGC-1α action in mediating mitochondrial biogenesis (17, 18).

We recently observed that the absence of ERRα in knockout mice leads to reduced fat mass and resistance to high fat diet-induced obesity (19). In addition, gene expression profiling experiments in adipose tissue from ERRα knockout mice revealed alterations in the expression of genes implicated in the regulation of adipogenesis and energy metabolism. However, we could not detect significant changes in dietary intake or energy expenditure between the wild-type and ERRα-deficient mice. Knowing that ERRα is expressed in the intestine, defective uptake or utilization of dietary nutrients in ERRα-deficient mice could also contribute to the observed lean phenotype. In fact, the intestine is the site essential for the transport of alimentary fat. After the digestive phase, the lipolytic products are absorbed by the enterocytes, in which a complex series of

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1 The abbreviations used are: ERR, estrogen-related receptor; apo, apolipoprotein; ChIP, chromatin immunoprecipitation; PGC-1α, peroxisome proliferator-activated receptor α coactivator 1α; PPAR, peroxisome proliferator-activated receptor; FABP, fatty acid-binding protein; Ab, antibody.
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Quantitative Reverse Transcription-PCR—2.5 μg of total RNA from each of the 6 pooled RNA samples used for microarray were used to synthesize cDNA in 20 μl samples using Superscript II (Invitrogen) reverse transcriptase and d(T)12–18 random hexamer primers (Amersham Biosciences). cDNA samples were used as templates, and real-time quantitative PCR was performed using LightCycler and Fast Start DNA Master SYBR Green (Roche Applied Science). Intron-spanning specific primers were designed based on sequences from the Celera data base. The specificity of the PCR product was documented by LightCycler melting curve analysis and migration on ethidium bromide-stained agarose gel. Efficiency of the PCR reaction was calculated using the formula $E = 10\left(\frac{-1}{\text{cycle number}}\right)$, the slopes obtained by plotting cycle crossing point values (CP) as a function of cDNA template (22). The relative quantitation for any given gene was calculated after determination of the difference between CP of the target gene and that of the calibrator gene hypoxanthine ribosyl transferase (HPRT) in ERRα and ERRγ mice, and correction for efficiency, using the formula

$$\text{Ratio} = \left(\frac{E_{\text{target}}^{\text{WT}_{-}^{\text{KO}}}}{E_{\text{calibrator}}^{\text{WT}_{-}^{\text{KO}}}}\right)^{\frac{1}{E_{\text{target}}^{\text{WT}_{-}^{\text{KO}}}}}..$$

CP values are the mean of triplicate measurements, and experiments were done in duplicate.

β-Oxidation Activity in Intestinal Epithelium—Intestinal epithelial cells from adult mice fasted for 18 h were isolated as described in the Western blot section and homogenized on ice with 5 vol of 250 mM NaCl, 10 mM Tris-HCl, and 1 mM phenylmethylsulfonyl fluoride, and 0.25 M sucrose. The homogenate was centrifuged at 600 × g for 5 min, and the resulting supernatant was used for β-oxidation assay as reported previously (20). Briefly, the β-oxidation reaction mixture contained 50 mM Tris-HCl (pH 8.0), 40 mM NaCl, 2 mM KCl, 2 mM MgCl₂, 1 mM dithiothreitol, 5 mM ATP, 0.2 mM (S)-carinate, 0.2 mM NAD⁺, (S)-malate 0.5 mM, 0.05 mM flavin adenine dinucleotide (FAD), 0.12 mM CoA, 0.1 μM of [14C]palmitic acid, 10 μM ATP, and protein extract in a final volume of 200 μl. The reaction was started by adding the protein extract and incubating the preparation at 37 °C for 25 min. The reaction was terminated by adding 200 μl of 0.6 N perchloric acid. Negative controls where the reaction was stopped at zero time were carried out in parallel. The reaction mixture was washed three times with 800 μl of hexane to remove residual radiolabeled palmitic acid. The radioactivity retained in the aqueous phase was measured. The associated radiations were determined in duplicate using Bio-Rad protein reagents.

Fecal Fat Analysis—As previously described for stool lipid analysis (24), 100-mg aliquots of droppings collected from ERRα-null and wild-type mice of the indicated ages were mixed with a small amount of [carboxyl-13C]triolein (112 mCi/mmol) and dried for 1 h in a vacuum oven at 70 °C. The solid matter was extracted with 2 ml of chloroform/methanol (2:1, v/v) for 30 min at 60 °C, passed through a Whatman No. 1 filter, and brought to a final volume of 4 ml with chloroform/methanol (2:1). The material was back-extracted with 1 ml of H₂O, and the organic phase was evaporated to dryness. The pellet was resuspended in 2 ml of chloroform/methanol (2:1) and transferred to preweighed vials. The solvent was evaporated, and the vial was taken to a constant weight by evaporation in a vacuum desiccator at 70 °C. The weight difference between the starting empty vial and the vial containing the dried lipid was the fecal lipid amount, which was expressed as a percentage of the weight of the starting fecal sample. To obtain stool from suckling animals, 10-day-old animals were sacrificed, and the entire colon was removed. The feces was removed by extrusion, pooled from several animals, and processed as for adult animals. The percent recovery of radiolaabeled triolein (85–90%) was determined by subjecting the vial to scintillation counting.

ApoA-IV Promoter Cloning—The mouse apoC-III/apoA-IV intergenic region was amplified by PCR using mouse genomic DNA as template and subcloned into the luciferase reporter plasmid pLuc. Deletion mutants were produced with subcloning into pLuc or pHLuc luciferase reporter plasmids. The integrity of each construct was confirmed by sequencing.

Plasmids and Cell Transfections—Plasmid expressing the VP16-ERRα fusion protein was constructed by subcloning PCR-amplified ERRα cDNA into pCMX-VP16 downstream of the transcriptional activation domain of the VP16 protein. The pCDNA3.1HA-hPGC-1α vector was obtained from A. Kralli (La Jolla, CA). Caco-2/15 cells were a gift from J. Beaulieu (Université de Sherbrooke, Canada), and COS-1 cells were obtained from the American Type Culture Collection. Cells were cultured in phenol red-free Dulbecco’s minimal essential medium containing penicillin (25 units/ml), streptomycin (25 units/ml), and 10% heat-inactivated charcoal-treated fetal bovine serum. 18 h prior to transfection, the cells were seeded in 12-well plates. A total of 1–1.2 μg of DNA/well was transfected including 0.5 μg of reporter plasmid and 0.25 μg of internal control pCMVβGal, 0.05–0.2 μg of pCMX-ERRα, 0.1 μg of pCMX-Luc, and 0.05 μg of pCMX-HA-hPGC-1α vector.
1 μg of pCMX-VP16-ERRα, 0.1 μg of pCDNA3.1HA-hPGC-1α as specified in the figure legends. The DNA was introduced into cells using FuGENE 6 (Roche Applied Science). The cells were harvested in a potassium acetate lysate buffer containing 1% Triton X-100. Luciferase assays were performed as previously described (25). The transfections were normalized to the β-galactosidase activity of each sample. All results represent experiment conducted in duplicate at least three times.

Chromatin Immunoprecipitation Assay (ChIP)—ChIP assays were performed as described previously (26). Briefly, 1 × 10^7 Caco-2/15 cells were transfected with 15 μg of pCMX-ERRα 24 h before cross-linking for 10 min at room temperature. Cells were then washed twice with ice-cold phosphate-buffered saline, collected, and centrifuged. Pellets were incubated in lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, supplemetted with protease inhibitor mixture (Roche Diagnostics) for 15 min and sonicated 5 times for 10 s using a Virsonic 100 (Virtis) sonicator. After centrifugation at 12,000 rpm to remove the debris, soluble chromatin was diluted 10-fold in chromatin immunoprecipitation buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl pH 8.1) and immunoprecipitated using either the specific anti-hERRα polyclonal antibody (16) or the preimmune serum. Following immunoprecipitation, 40 μg of salmon sperm DNA/protein A-agarose (Upsate Biotechnologies) was added and incubated for 2 h. The precipitates were washed sequentially for 10 min each with buffer 1 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1) and 1% SDS, 0.1 M NaHCO3 buffer. The eluates were pooled and incubated with 1% SDS, 0.1M NaHCO3 buffer. The eluates were pooled and incubated at 65 °C for at least 4 h. The isolated DNA fragments were purified according to QIAquick Spin Kit (Qiagen). Quantitative PCR was performed using LightCycler and Fast Start DNA Master SYGB buffer and run 3 times.

Free amino acid analysis of the gut tissues from 3–4 mice were pooled before measurements. To examine the function of ERRα, expression of the latter (28). To investigate the physiological effect of ERRα deficiency on intestinal lipid catabolism, we measure the β-oxidation activity in intestinal epithelial cells isolated from ERRα−/− and ERRα+/+ mice. As shown in Fig. 2, deficiency for ERRα is associated with a significant decrease in intestinal palmitic acid oxidation capacity.

ERRα-null Mice Display Alterations in the Expression of Genes Involved in Intestinal Lipid Handling as Well as Fat Malabsorption in Suckling Mice—Indeed, microarray results also revealed differential expression in a group of genes involved in dietary lipid handling (Table I). Among them, the expression of pancreatic lipase-related protein 2 (PLRP2) was found to be expressed at lower levels in the intestine from ERRα-null versus wild-type mice. The expression of fatty acid-binding proteins 1 and 2 (L-FABP and I-FABP, respectively) were also reduced in ERRα-null versus wild-type mice. Of note are the high affinity and binding of these cytoplasmic FABPs for long chain fatty acids (29). L-FABP is expressed in the liver and small intestine but I-FABP is restricted to the intestinal...
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TABLE I

| Function                      | Gene   | Fold change | Unigene no. | Locus link no. | Description            |
|-------------------------------|--------|-------------|-------------|----------------|------------------------|
| Nuclear receptor              | Esrra  | Absent      | Mn 2563     | 26379          | Estrogen-related receptor α |
| Oxidative phosphorylation     | Atp5j2 | -1.7        | Mn 1674     | 57423          | ATP synthase, H+ transporting, mitochondrial |
|                               | Atp5j3 | -1.7        | Mn 353      | 11957          | ATP synthase, mitochondrial F0 complex subunit F1 |
|                               | Cox5a  | -1.9        | Mn 360      | 12858          | Cytochrome c oxidase, subunit Vα |
|                               | Cox7c  | -1.9        | Mn 43786    | 12867          | Cytochrome c oxidase, subunit 7c |
| Fat metabolism                | Pnlprrp2 | -2.3     | Mn 1230     | 18947          | Pancreatic lipase-related protein 2 |
|                               | Fapl1  | -37.8       | Mn 22126    | 14080          | Fatty acid-binding protein 1, liver |
|                               | Fabp2  | -1.4        | Mn 28386    | 14079          | Fatty acid-binding protein 2, intestinal |
|                               | Apoa4  | -10.4       | Mn 4533     | 11808          | Apolipoprotein A-IV |
| Other metabolism              | Cpo    | -1.9        | Mn 35820    | 12892          | Coproporphyrinogen oxidase |
|                               | Ang1   | -2.1        | Mn 2566     | 11727          | Angiogenin, ribonuclease family, member 1 |
|                               | Ang2   | -2.1        | Mn 57055    | 11731          | Angiogenin, ribonuclease family, member 2 |
|                               | Itln   | -1.5        | Mn 140192   | 16429          | Intelectin |
|                               | Spink4 | -2.1        | Mn 25246    | 20731          | Serine protease inhibitor, Kazal type 4 |

Fig. 2. β-Oxidation activity in the small intestine from wild-type and ERRα−/− mice. Intestinal epithelial cells from adult mice fasted for 18 h were isolated using Matrisperse solution and homogenized in 250 mM sucrose containing 1 mM EDTA and 10 mM HEPES (pH 7.2). The capacity of epithelial cell homogenate to oxidize [14C]palmitic acid was measured as described under “Materials and Methods.” The results are expressed as the percentage of β-oxidation activity over control wild-type mice; n = 8 per group (mean ± S.E.). *, p = 0.01.

Genes in this table have been found to be differentially expressed into 4 of 6 microarray pairwise comparisons: replicated microarrays performed with three wild-type versus ERRα−/− female mice of C57BL/6J genetic background; replicated microarrays performed with three wild-type versus ERRα−/− male mice of C57BL/6J genetic background; and replicated microarrays with two male and two female of either wild-type or ERRα−/− knockout mice of the 129/SvJ genetic background. Each gene was tested by quantitative PCR analysis, and the fold change indicates the median Q-PCR change obtained by a pairwise comparison of samples used for the gene profiling experiment. The primers and condition used are available on request.

Epithelium. Last, apoA-IV was also intensely down-regulated in the microarray analysis relative to ERRα KO mice. ApoA-IV is a component of triglyceride-rich lipoproteins such as chylomicrons, very low density lipoproteins and high density lipoproteins secreted by the enterocytes. Intestinal apoA-IV synthesis is stimulated by fat absorption and the assembly and/or transport of chylomicrons (30). To confirm the effects of ERRα deficiency on the in vivo regulation of apoA-IV gene expression, overnight-fasted mice were subjected to a lipid load by gavages to up-regulate intestinal apoA-IV gene expression. The results shown in Fig. 3A demonstrate that ERRα−/− mice display a significantly lower basal and lipid-induced jejunal apoA-IV expression, compared with wild-type mice. Interestingly, the magnitude of apoA-IV induction by lipid was comparable between wild-type and ERRα−/− mice (2.6- and 2.39-fold, respectively).

Neonatal and suckling mice thrived on high fat diet from maternal milk and are known to have immature gut with physiological fat malabsorption and poor adaptation. Although we did not observe dietary fat malabsorption in adult mice, ERRα-deficient pups at 10-days postnatal exhibited a significantly higher magnitude of steatorrhea, indicative of fat malabsorption, than wild-type pups (Fig. 3B).

Apolipoprotein A-IV is a Direct Target of ERRα—The human and mouse apoA-I, apoC-III, and apoA-IV genes are tandemly organized within a 15-kb DNA segment. Within the gene cluster, the apoA-I and -A-IV genes are transcribed in the same direction, whereas the apoC-III gene is transcribed in the opposite direction. Therefore, the apoC-III/A-IV intergenic region constitutes a common 6.6-kb 5′-flanking sequence for these two genes. In human, the expression of the apoA-IV gene in the intestine is under the control of the −700−310 promoter of the apoA-IV gene combined with the −780−580 enhancer region of the apoC-III gene (31). This enhancer region has also been shown to direct the intestinal expression of the three genes of the cluster (32). Although there is some degree of conservation between the human and mouse genomes, the functional significance of regulatory sequences in the apoA-IV promoter are not well described in mice. Sequence analysis of the mouse apoC-III/A-IV intergenic region reveals the existence of several potential ERR responsive elements containing the core AGGTCA sequence (or its reverse complement). As a first step in evaluating the significance of these sites, the mouse apoC-III/A-IV intergenic region was cloned in front of a luciferase reporter gene. The binding capacity of ERRα to this region was characterized by transient cotransfection using the ERRα-VP16 expression vector in Caco-2/15 cells. As shown in Fig. 4, the constitutively active ERRα-VP16 chimera induced a strong apoA-IV promoter-dependent luciferase induction (11.4-fold). This result confirms that ERRα can interact directly with the apoA-IV promoter/enhancer regulatory unit. Furthermore, coexpression of ERRα with the coactivator PGC-1α results in synergistic activation of the apoC-III/A-IV intergenic region. To determine the region required for ERRα activation, 5′-nested deletion constructs of the apoC-III/A-IV intergenic region were fused to luciferase cDNA and cotransfected with ERRα-VP16, ERRα, or both ERRα and PGC-1α. As shown in Fig. 4, truncation of the apoA-IV promoter/enhancer region to −4513 abolishes responsiveness to ERRα-VP16 and to ERRα in the presence of PGC-1α. This domain maps to the well defined apoC-III promoter/enhancer region. The proximal region of the apoA-IV promoter (−558) was unresponsive to ERRα. Since putative ERRα response elements can be found in both the apoC-III promoter and enhancer region, we next sought to more precisely map the region required for ERRα action on apoA-IV expression. Reporter constructs were designed in which vari...
ous segments of the apoA-IV promoter were cloned in front of the minimal thymidine kinase promoter fused to the luciferase reporter gene. Cotransfection experiments with ERRα-VP16 as well as ERRα in the presence of PGC-1α indicate that the apoC-III promoter/enhancer domain (−5846 to −4487) is indeed responsible for the ERRα regulation of the apoA-IV gene expression. Furthermore, we observed that a 229-bp segment corresponding to the apoC-III enhancer (−5355 to −5116) was specifically responsive to ERRα.

Finally, because of the imperfect homology between the human and mice apoC-III/A-IV intergenic region, we tested whether ERRα also interacts with the human apoC-III enhancer, in the native chromatin environment. A ChIP assay was therefore performed in Caco-2/15 cells containing ectopic human ERRα, a human colon cancer cell line frequently used in the investigation of intestinal apolipoprotein metabolism. As shown in the Fig. 5, ChIP with a human ERRα-specific antibody resulted in an enrichment for the genomic fragment corresponding to the human apoC-III enhancer region (−4-fold). A lower degree of enrichment was also observed for the apoC-III promoter. However, the two regions are very close and detection of the apoC-III promoter in long chromatin segment enriched for the immunoprecipitated apoC-III enhancer is plausible. Taken together, these data support that ERRα interacts with the apoC-III enhancer region and modulates the expression of intestinal apoA-IV via this regulatory region.

**DISCUSSION**

In this report, we used genetic deletion of the ERRα gene in mice in combination with the power of microarray genomic technology to explore the function of ERRα in the small intestine. By using this strategy, we characterized *in vivo* the role of ERRα in the regulation of intestinal energy metabolism and dietary lipid absorption. Furthermore, this approach allowed us to identify apoA-IV as a novel ERRα target gene involved in the absorption and transport of triglycerides.

We previously reported that ERRα was expressed early in intestinal mucosa of the developing mice (9). To further explore
the role of ERRα in the intestine, we examined the distribution of ERRα throughout the digestive tract. We noted that ERRα gene expression was restricted to epithelial cells of intestinal mucosa and that the small intestine displayed higher levels of ERRα compared with the stomach and colon. These data suggest a role for ERRα in dietary nutrient absorption. Furthermore, the high levels of ERRα in neonatal and weaning mice, where most of the energy derives from fat, support a function for ERRα in the regulation of intestinal lipid metabolism. This is in agreement with a previous report showing that ERRα regulates the medium chain acyl CoA dehydrogenase gene (MCAD) involved in fatty acid β-oxidation (9, 13, 14).

A role for ERRα in metabolic control was also supported by the observation that ERRα-deficient mice display a lean phenotype associated with reduced lipogenesis and high fat diet-induced obesity (19). However, the fact that ERRα-deficient mice did not display lower food consumption or higher energy expenditure over a 4-day period pointed out to a possible defect in intestinal processing of dietary nutrients, taking into account that the intestine is the first organ in the chain of energy metabolism. We then evaluated the gut morphology in ERRα-null mice and did not find any gross or histological abnormality. However, gene expression profiling revealed a subset of genes consistently down-regulated in the intestine from ERRα-deficient mice, without regard to gender or genetic background. In particular, ERRα modulated the expression of genes involved in oxidative phosphorylation such as cytochrome c oxidase 5a and 7c, NADH dehydrogenase and ATP synthase. This is in agreement with a previous report showing that ERRα regulates the medium chain acyl CoA dehydrogenase gene (MCAD) involved in fatty acid β-oxidation (9, 13, 14).

Given the fact that the gut produces energy from food available to the rest of the body, it was of physiological importance that ERRα-deficient pups malabsorb dietary fats, as evidenced by steatorrhea. This was not observed in adult ERRα/−− mice, possibly because of compensatory mechanisms or to the large absorptive reserves of the adult small intestine. Nevertheless, fat malabsorption could explain the lower gain weight of ERRα-deficient pups compared with wild-type littermates. In addition, subtle defect in dietary lipid absorption, including faulty apoB synthesis, reduced microsomal triglyceride transfer protein activity or alterations in the Sar1 GTPase of COPII (34, 35) could also contribute to resistance to high fat diet-induced obesity in adult ERRα-deficient mice.

Indeed, it is striking that the second group of genes down-regulated in the intestine from adult ERRα/−− mice play a role in dietary fat digestion and absorption. PLRP2 is part of the lipase gene family derived from common ancestral gene and is the major colipase-dependent pancreatic lipase in suckling mice (36). In addition to the pancreas, PLRP2 is well expressed in the small intestine and its role in triglyceride, galactolipids and phospholipids digestion is reflected by the dietary fat malabsorption in suckling PLRP2-deficient mice (24). Furthermore, FABP-L (liver) and FABP-1 (intestine) are intracellular lipid-binding proteins found to be expressed at lower levels in the intestine from ERRα/−− mice compared with wild-type mice. It is generally accepted that FABPs facilitates the intracellular transport and metabolism of fatty acids. In addition, FABPs have been postulated to protect intracellular polyunsaturated fatty acids against peroxidation (37), as well as to regulate gene expression by delivering fatty acids to the nucleus, where they act as ligands for PPARs (38). In turn, L-FABP gene expression appears to be regulated by some fatty acids and PPAR agonists (39, 40). The role of L-FABP in fatty acid uptake and metabolism is unclear as L-FABP overexpression in Caco-2/15 results in inhibition of fatty acid incorporation (41) and L-FABP knockout mice display gender-specific alterations in body weight (42). Finally, apoA-IV, which is also down-expressed in ERRα-null mice, is a major protein component of intestinal triacylglycerol-rich lipoproteins such as chylomicrons and very low density lipoproteins. Although its precise function remains unclear, apoA-IV has been proposed to play a role in various processes: lipid absorption, transport, and metabolism; food consumption, gastric acid secretion and motility; and protection against lipoprotein oxidation and atherosclerosis (43). In accord with our data, apoA-IV acted as a post-prandial satiety signal, which caused anorectic effect mediated via the central nervous system (44). Furthermore, overexpression of apoA-IV enhanced lipid transport in intestinal cells from newborn swine (45). The physiological relevance of this suppression of food intake has yet to be confirmed since apoA-IV knockout mice had normal lipid absorption, weight gain, and food consumption (46). In the ERRα/−− mice, the observed down-regulation of intestinal apoA-IV could contribute to impaired fat absorption or be the consequence of lipid malabsorption. Indeed, the formation and secretion of chylomicrons stimulate the synthesis of apoA-IV and human pathological states of fat malabsorption are associated with lower levels of apoA-IV (reviewed in 43). In the current investigation, we observed that mice deficient for ERRα showed lower intestinal apoA-IV gene expression in the fasting condition, but normal apoA-IV induction by fat feeding. This suggests that ERRα does not mediate apoA-IV response to feeding but is essential for maintenance of basal apoA-IV intestinal expression.

ApoA-IV expression is limited to intestinal and hepatic cells. In the intestine, the transcriptional regulation of the apoA-IV...
gene involves synergism between a proximal promoter region and a distant enhancer located in the upstream promoter region of the apoC-III gene. Our experiments showed that ERRα interacts with the mouse apoC-III enhancer when fused with VP16 or in the presence of the PGC-1α coactivator. Furthermore, we used a ChIP assay in the Caco-2/15 enterocytic cells to demonstrate that ERRα binds in vivo to the corresponding human enhancer region. Molecular interspecies preservation implies a physiological importance of this transcriptional control pathway in the intestine. Many transcription factors (Sp1, ATF-2, NFκB, and nuclear receptors (COUPTF-I, COUPTF-II, RARα, TRα, HNF-4α) have been shown in vitro to interact with the human apoC-III enhancer region (47). Furthermore, the nuclear farnesoid X receptor (FXR) has been found to be a negative regulator of both human and mouse hepatic apoC-III expression, via a FXR response element located in the apoC-III enhancer (48). In addition, a recent study has demonstrated that liver X receptors (LXRs) modulate both human and mouse apo-AIV gene expression in the liver but not in the intestine (49). Interestingly, the direct regulation by LXRs was mediated by different interaction sites in humans and mice, respectively in the apo-AIV promoter and in the apoC-III enhancer.

In conclusion, by using the ERRα knockout mice in combination with intestinal microarray analysis, we identified ERRα-modulated genes involved in two main pathways: oxidative phosphorylation and dietary lipid digestion and absorption. The first is in agreement with the known function of ERRα in energy expenditure when coactivated by PGC-1α, and the second allowed us to reveal a function for ERRα in energy uptake from fat, a crucial first step in energy balance. Furthermore, we characterized the apo-AIV gene as a novel direct ERRα target gene. At the molecular level, ERRα interacts with the apoC-III enhancer region in both mice and human. The role of ERRα in dietary lipid handling should therefore be taken into consideration during the development of ERRα pharmacologic ligands (50).

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