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Review

# The multiple universes of estrogen-related receptor α and γ in metabolic control and related diseases

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The identification of the estrogen-related receptors (ERRs) as the first orphan nuclear receptors ignited a new era in molecular endocrinology, which led to the discovery of new ligand-dependent response systems.  Although ERR subfamily members have yet to be associated with a natural ligand, the characterization of these orphan receptors has demonstrated that they occupy a strategic node in the transcriptional control of cellular energy metabolism.  In particular, ERRs are required for the response to various environmental challenges that require high energy levels by the organism.  As central regulators of energy homeostasis, ERRs may also be implicated in the etiology of metabolic disorders, such as type 2 diabetes and metabolic syndrome.  Here, we review the recent evidence that further highlights the role of ERRs in metabolic control, particularly in liver and skeletal muscle, and their likely involvement in metabolic diseases.  Consequently, we also explore the promises and pitfalls of ERRs as potential therapeutic targets.

## Introduction

The term "Big Bang" sometimes refers to the beginning of the universe; however, the "Bang" could perhaps be better referred to as a rapid period of inflation of the universe.  What preceded the "Big Bang", the true beginning of the universe, if indeed there was such a moment, remains a matter of intense debates, hypotheses, experimentations and soul searching.  In the modern era of molecular investigations of nuclear hormone receptor actions and biological functions, of which the 30th anniversary is celebrated in this issue, the identification of retinoid X receptor (RXR) and its ability to heterodimerize with a multitude of nuclear receptors has been referred to as the ''RXR Big Bang'' in a recent review[1].  This is unquestionably a suitable assessment of the importance of this moment in nuclear receptor research because of its substantial impact on the subsequent discovery of entirely new physiological signaling pathways, and the corresponding inflation of the nuclear receptor universe.  However, not only do we not know what preceded the "Big Bang" but the inflationary period that the universe went through immediately after the "Big Bang" may represent only one of many inflationary periods, which have given rise to the concept of multiple parallel universes.  Although the molecular cloning of the classic steroid hormone receptors could be viewed as the pre-"Big Bang" era[2–4], the subsequent identification of distinct classes of nuclear receptors, both RXR-dependent and -independent, could also be perceived as multiple universes; furthermore, each universe could significantly contribute to the inflation of nuclear receptor-regulated developmental, physiological and pathological processes.  In this review, we will describe the parallel universes of the estrogen-related receptors (ERRs), which were identified as the first orphan nuclear receptors and therefore suggested the existence of previously unknown hormone response systems[5].  We will focus on the extensive role of ERRs in the transcriptional control of metabolic genes involved in the generation and utilization of cellular energy, with a particular emphasis on the impact of ERRs on gene regulation in skeletal muscles and the liver in relation to type 2 diabetes, metabolic syndrome and insulin resistance.  Finally, the prospect and potential pitfalls of ERRs as therapeutic targets will be discussed.

## Estrogen-related receptors: not estrogen receptors

The identification and mode of action of the ERR subfamily of nuclear receptors, which comprises three members referred to as ERRα (NR3B1), ERRβ (NR3B2), and ERRγ (NR3B3), has

been extensively reviewed in the past[6–10]; thus, only a few key points will be discussed here. Although their name accurately reflects their molecular origin, the ERRs do not bind natural estrogens and they do not directly participate in classic estrogen signaling pathways or biological processes. In addition, recent functional genomic experiments have clearly demonstrated that ERRs and the estrogen receptor (ERα) display strict binding site specificities[11]; in contrast to ERα, the addition of a hormone/ligand is not required to observe strong and widespread ERR binding to the genome. The ERRs also have a marked preference for the recognition of the sequence TCAAGGTCA, which is referred to as an estrogen-related response element (ERRE). Furthermore, although the ERRE is similar to the half-sites contained within the estrogen response element (ERE), a relatively small overlap exists between the gene set bound by ERα and ERRα[11]. When direct overlap in binding to DNA does occur, it occurs through an ERRE embedded within an ERE[11], and when in place, this functional relationship can be used to fine tune the expression of a small subset of specific genes[11–14]. Another functional distinction between the ERRs and ERα is that, in general, the ERRs exhibit a preference for binding in close proximity to the promoters of genes, whereas ERα typically recognizes enhancers that are often located very distant from transcriptional start sites[8, 15–18]. This functional characteristic of the ERRs provides a vast advantage to the ERRs that facilitates the identification and characterization of their target genes. Finally, the ERRs display significant constitutive transcriptional activity that is dependent on the presence of coactivator proteins, most prominently peroxisome-proliferator activated receptor γ coactivator-1α and peroxisome-proliferator activated receptor γ coactivator-1β (PGC-1α and PGC-1β), which regulate metabolic pathways and exhibit high specificity for the ERRS[19–21]. As such, these coactivators are often considered to function as protein ligands for these receptors. The presence of functional ERRs is required for maximal mitochondrial biogenesis and activity induction by PGC-1α and PGC-1β[22–24].

The identification of ERR binding sites in the genome has demonstrated that they are preferentially associated with metabolic genes involved in processes such as glucose and glutamine metabolism, mitochondrial activity, lipid handling, and energy sensing[8, 25]. Consequently, the ERRs are now considered major transcriptional regulators of energy metabolism, particularly in the context of high-energy demands by cells, tissues or the whole organism in response to physiological and/or environmental challenges[7, 10]. In the next sections, ERR gene regulation will be dissected to understand their roles in energy metabolism pathways. The discussion will be limited herein to ERRα and ERRγ because the expression of ERRβ is higher in embryonic tissues; therefore, less is known regarding its role in adult physiology.

## ERRα, ERRγ, and cellular energy metabolism
### Carbohydrate and energy metabolism
Glycolysis is a process that requires several enzymes to convert glucose into pyruvate, the latter of which is used to feed the mitochondria or is anaerobically converted to lactate. This process produces ATP, but it is also required for the synthesis of several building blocks of the cell, thereby comprising nucleotides, lipids, and amino acids. Most genes involved in glycolysis have been identified as bound and regulated by ERRα and ERRγ, including genes that encode key enzymes, such as phosphofructokinase, hexokinase 2 (*HK2*), glyceraldehyde dehydrogenase (*GAPDH*), and enolase 1 (*ENO1*)[16, 26, 27]. Moreover, ERRα also binds to glucose transporter family members (*Glut1*, *Glut2*, *Glut4*, and *Glut12*)[18, 28], and shRNA-mediated knock down of both ERRα and ERRγ decreases glucose uptake in breast cancer cells[26]. Based on their regulation of most genes in the glycolysis pathway, the ERRs are key components of cellular glucose metabolism.

Another crucial step during glucose utilization is the conversion of pyruvate to acetyl coenzyme A (CoA), which is catalyzed by the pyruvate dehydrogenase complex (PDC). The pyruvate dehydrogenase kinase (PDK) can phosphorylate PDC to inhibit its activity, which results in a switch from glucose oxidation to fatty acid oxidation (FAO). The key role of PDK enzymes, including PDK isoenzyme 4 (PDK4), in energy homeostasis has been demonstrated in PDK4-null mice: these mice exhibit lower blood glucose levels as a result of elevated glucose utilization[29]. Importantly, ERRα and ERRγ have been demonstrated to positively control PDK4 transcription in muscle and hepatic cells[30–33]. Moreover, PDK4 upregulation by the PGC-1α/ERRα axis downregulated glucose oxidation rates in C2C12 myotubes[30]. In addition, ERRα also binds to *Pdk1* and *Pdk2* in mouse livers[18]. Therefore, both ERRs can regulate the switch between glucose and lipid usage by mitochondria.

When oxygen is lacking or when cells are rapidly proliferating, glucose can be converted in lactate (anaerobic) instead of processed by aerobic respiration in the mitochondria. This reversible process is catalyzed by lactate dehydrogenase (LDH), and several genes that encode LDHs are targets of and regulated by ERRα and ERRγ in mouse and human cells, including *LDHB* and *LDHD*[16, 27, 34, 35]. Importantly, ERRγ has been shown to be a negative regulator of anaerobic glycolysis in breast cancer cells, and the inhibition of ERRγ by miR-378* leads to an increase in lactate production and a decrease in aerobic respiration[27]. In addition, inhibition using an siRNA against ERRα and ERRγ leads to a decrease and increase in *LDHA* expression, respectively[27]. The biological impact of LDH regulation by the ERRs can also be supported by the observation that siRNA-mediated knock down of ERRα indicates this nuclear receptor is indispensable for the switch from oxidative to glycolytic metabolism[16]. The recent demonstration that all three ERRs could interact with hypoxia-inducible factor (HIF) and positively coordinate transcriptional activation in hypoxic conditions via the modulation of angiogenic and glycolytic genes is another demonstration of their crucial roles in glucose handling[36–38]. One of the hallmarks of energy metabolism reprogramming following HIF pathway activation is the induction of anaerobic respiration[39]. ERRγ has been shown to be upregulated and to increase several key hypoxic-

response genes, such *PDK4*, *LDHA*, and *VEGF*, under hypoxic conditions[36]. These data demonstrate that ERRs are involved in anaerobic glycolysis and in the response to hypoxic conditions. However, the exact role of each isoform in the differential regulation of genes involved in this process remains to be investigated.

Together, these results demonstrate ERRs can regulate glucose metabolism at several key steps, including glucose uptake, glucose to pyruvate conversion, pyruvate entry in the TCA cycle and anaerobic respiration (Figure 1). For some genes, such as *LDHA*, an opposing effect on transcriptional regulation has been observed, and the global portrait of how each ERR isoform orchestrates glucose metabolism in the cell will require further investigation.

## Lipid synthesis and fatty acid oxidation

*Acadm* encodes the medium-chain acyl-coenzyme A dehydrogenase (MCAD), which is the initial step in mitochondrial fatty acid β-oxidation (FAO), and it is considered one of the gatekeepers that controls the FAO rate in the cell. Therefore, the identification of *Acadm* as the first target gene of ERRα was a good hint to support the role of this nuclear receptor in the modulation of energy metabolism[40, 41]. ERRα is highly expressed in tissues involved in lipid metabolism and energy homeostasis, such as white and brown adipose tissues, and tissues that require high oxidative capacity, such as heart and skeletal muscles[5, 42].

Genetic ablation of ERRα leads to viable and fertile mice with no apparent defects compared with wild-type mice,
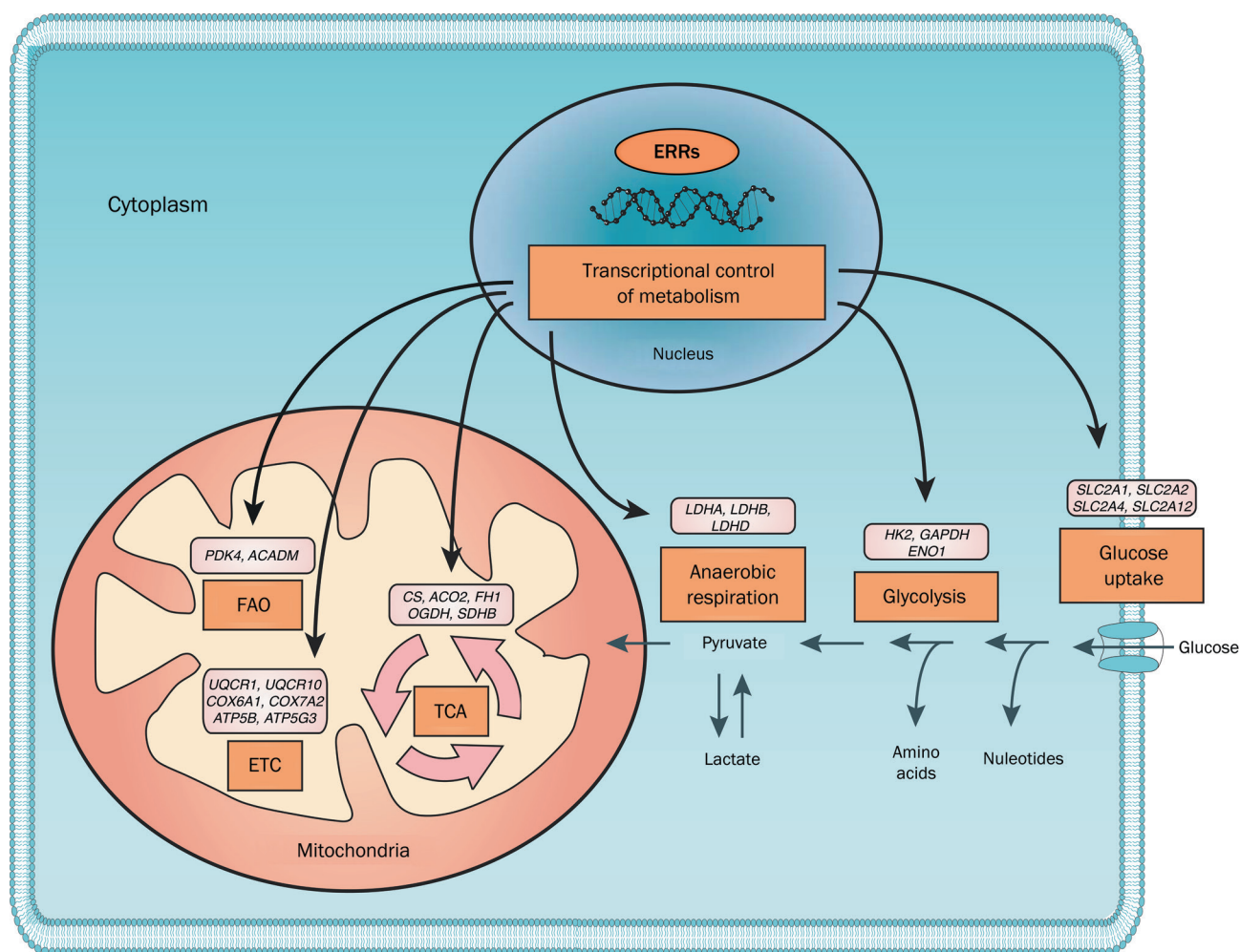


**Figure 1.** ERRs are key transcriptional factors that regulate cellular energy metabolism. Through the control of vast transcriptional networks, the ERRs are master regulators of energy metabolism in the cell. Their target genes include several glucose transporters (*SLC2A1*, *SLC2A2*, *SLC2A4*, and *SLC2A12*, also known as *GLUT1*, *GLUT2*, *GLUT4*, and *GLUT12*, respectively), most genes in the glycolysis pathway, and several genes that encode different LDH isoforms, which indicate the ERRs are key components in cellular glucose metabolism. By regulating the levels of key gatekeepers (such as PDK4), they also control the switch between glucose and fatty acid oxidation (FAO) by the mitochondria. ERRs are also essential for mitochondrial activity by targeting most genes in the TCA cycle and the electron transport chain (ETC). Recent results indicate that they also play a role in other metabolic functions of the cell, including amino acid and nucleotide synthesis and metabolism, which remain to be fully elucidated. Several examples of the regulated genes are provided for each function, which are based on microarray and ChIP-sequencing analysis in human cell lines and mouse models.

with the exception of reduced body weight and peripheral fat deposits, as well as a lower capacity for lipid absorption by the intestine of ERRα-null pups[43, 44]. Unexpectedly, these mice were resistant to high-fat diet-induced obesity as a consequence of the altered expression of genes involved in lipid and energy metabolism in white adipose tissue, such as fatty acid synthase (FAS, encoded by *Fasn*), fatty acid coenzyme A ligase long-chain 5 (*Facl5*), *Acadm*, and several other genes associated with lipid synthesis and mitochondrial activity. These changes in gene expression lead to a decrease in triglyceride synthesis capacity in ERRα-null mice adipocytes. Furthermore, these mice exhibit an important mass decrease in fat deposits, with up to a 50%–60% decrease in inguinal, epididymal and peritoneal fat pads. These observations are consistent with the results obtained in cell culture in which ERRα was up-regulated in adipocytes during differentiation and promoted lipid accumulation under an adipogenic condition[40, 41, 45–47]. Notably, *Acadm* and *Fasn* are directly regulated by ERRα in adipocytes and during differentiation. In differentiated adipocytes, ERRα is a positive regulator of mitochondrial biogenesis and triglyceride production[46, 47].

Less is known regarding ERRγ in lipid handling; however, because it binds to most ERRα target genes, it most likely also regulates the transcription of genes involved in lipid synthesis and metabolism. Using an siRNA mediated knock-down of ERRγ *in vitro* and studying mice treated with a high fat diet, ERRγ was shown to be positively regulated in adipogenic conditions and to act positively for the induction of adipogenic genes and lipid accumulation[48]. Moreover, ERRγ is essential to activate the FAO switch in the neonatal heart, which is discussed in more detail later in this review[49]. Therefore, both ERR isoforms positively regulate lipid synthesis, which can be further used to produce energy through FAO in the mitochondria.

### Mitochondrial activity and oxidative phosphorylation

The ERRα and ERRγ targets include several genes related to mitochondrial activity and biogenesis[16–18, 27, 28, 49–55]. The two receptors bind in the vicinity of the transcriptional start site of most genes that encode the enzymes involved in the tricarboxylic acid (TCA) cycle, including: *Aco2*, *Idh3a*, *Idh3b*, *Sdha*, *Sdhb*, *Sdhc*, Sdhd, *Ogdh*, *Cs*, and *Fh1*. Recent chromatin-immunoprecipitation coupled to a deep sequencing (ChIP-seq) analysis of ERRα binding in mouse liver[17] demonstrated that this nuclear receptor binds the regulatory region of more than one hundred genes involved in the mitochondrial electron transport chain. Some of these genes include members of the NADH dehydrogenase complex (over 30 genes, including several *Ndufa* and *Ndufb* genes), ubiquinol-cytochrome *c* reductases (*Uqcr* genes) and several cytochrome *c* oxidase subunits, and more than 100 peaks were detected in the proximity of 56 genes of the ATPase superfamily. The biological consequences of ERR isoforms binding to these mitochondrial genes are evident because when their activity is impaired, decreases in mitochondrial integrity, activity and ATP production have been demonstrated[16, 17, 26, 27, 50, 53–56]. Conversely, enhanced ERR

expression leads to an increase in mitochondrial biogenesis and activity, which, in turn, stimulates respiration capacity[52, 56].

In addition to ATP synthesis and aerobic respiration, the target genes of ERRs include genes involved in all aspects of mitochondrial functions, such as biosynthetic functions[25]. ERR transcriptional control of these pathways correlates with the finding that ERRα is a positive regulator of glutamine metabolism in breast cancer cells[24]. Overall, ERRα and ERRγ are master regulators of mitochondrial activity, including the positive regulation of aerobic respiration and ATP synthesis. Their roles in other mitochondrial functions, such as amino acid and nucleotide synthesis, remain to be investigated.

### ERRs as key players in adaptive energy metabolism

The phenotypes of the three ERR-null mice are markedly different and range from embryonic lethality for ERRβ-null mice, death shortly after birth for ERRγ-null mice, and viability for ERRα-null mice[43, 49, 57]. The complete knockdown of an individual ERR isoform does not abolish mitochondrial biogenesis or activity. Therefore, each ERR isoform is not *per se* essential for mitochondria functions, which could be explained, in part, by a compensatory mechanism through increased expression of other ERR subfamily members. For example, ERRγ is increased in ERRα-null tissues, such as the liver[17]. The results, primarily obtained from genetically engineered mice, have shown that they are required in a tissue-dependent manner for adaptive energy metabolism. Thus, in response to various environmental insults or stimuli, tissue-specific high-demanding energy processes are activated in an ERR-dependent manner to address a specific physiological challenge.

In various conditions where high energy levels are required, in general, ERRα-null mice display a deficient biological response compared with their wild-type littermates. The complete ablation of ERRα leads to defects in ATP production and transport in the mitochondria of the heart, which develops signatures of heart failure after ventricular pressure overload compared with wild-type mice[28, 58]. In rodents, adaptive thermogenesis is a process that relies on a high mitochondrial activity in brown adipose tissues to produce heat after cold exposure, and this process is defective in ERRα-null mice. These mice are unable to adapt to cold because of dysfunctional mitochondria and decreased mitochondrial mass, which thereby compromises survival[54]. ERRα has also been implicated in muscle cell energy metabolism and is essential in skeletal muscle cells for recovery during regeneration through sustaining mitochondrial size and activity[35, 59, 60]. Interferon-γ-induced activation of ROS production by mitochondria in macrophages for the clearance of *Listeria monocytogenes* was also decreased in ERRα-null mice; this effect was again because of decreased expression of mitochondrial genes in KO mice[53]. ERRα has also been shown to be a metabolic regulator in T-cell activation and differentiation because the inhibition of this nuclear receptor led to decreased T-cell activation and proliferation[51]. Finally, following a xenobiotic insult, ERRα-

null hepatocytes have been shown to have a defective apoptotic program that favored necrosis and inflammation, which thereby promoted hepatocarcinogenesis in diethylnitrosamine-induced hepatocellular carcinoma[55]. ATP production was dependent on the presence of ERRα, and genetic disruption of *Esrra* decreased mitochondrial activity, which blocked the regular stress response-induced apoptotic program. In summary, ERRα is required in a tissue-specific manner to promote the acute energetic response following various metabolic cues and environmental changes, particularly to control mitochondrial biogenesis and activity.

In contrast to ERRα, ERRγ is crucial for mice to survive after birth, which complicates the study of its role in energy metabolism. However, in a manner similar to ERRα, ERRγ is required to reprogram energy producing pathways in response to specific challenges. For example, the transition from cardiac carbohydrate-based fetal metabolism to lipid-predominant adult metabolism is blunted in ERRγ-null mice, which reduces mitochondrial activity and induces lactatemia, thereby contributing to the poor health of newborns and death shortly after birth [49]. ERRγ overexpression in mouse skeletal muscles results in a subsequent coordinate increase in mitochondrial function, in part, through increased TCA cycle activity, larger mitochondria and improved oxidative capacity[52, 56]. In these experiments, a gene set analysis identified an increase in the genes associated with the mitochondrial electron transport chain, TCA cycle, and FAO[56]. What is even more impressive is that ERRγ expression in skeletal muscle was sufficient to increase exercise capacity, activate mitochondrial activity, and force muscle fibers to switch from type II glycolytic to type I oxidative fibers. Conversely, ERRγ$^{+/-}$ mice exhibited lower exercise capacity compared with their wild-type littermates. ERRγ has also been associated with several roles during pregnancy, particularly through trophoblast-related gene regulation. In particular, ERRγ was necessary for the transcription of several voltage-gated K$^+$ channels (*Kcnq1*, *Kcne1*, and *Kcne3*), as well as for the induction of hormone secretion and angiogenesis[61] and the positive regulation of mitochondrial biogenesis[62].

## ERRα, ERRγ, and metabolic disorders
The major metabolic disorders include type 2 diabetes and metabolic syndrome, which are closely linked by their clinical features. Type 2 diabetes is characterized by insulin resistance, hyperglycemia and hyperinsulinemia. Metabolic syndrome includes central obesity, dyslipidemia, hypertension, hyperglycemia and/or type II diabetes[63, 64]. Obesity can also lead to insulin resistance and is recognized as a major risk factor for type 2 diabetes. The transition to insulin-resistance is, in part, a result of an insensitivity to insulin in the skeletal muscle and liver; these two distinct metabolic tissues have been associated with several metabolic alterations identified in type 2 diabetic patients[65]. Skeletal muscle exhibits a defect in mitochondrial activity, whereas the liver exhibits an increase in the gluconeogenesis rate and glucose production, which increases blood glucose levels. As previously discussed, ERRα

and ERRγ are key transcriptional regulators of these processes in these tissues. The next sections will discuss their roles in global energy homeostasis with a special emphasis on the contributions of ERRα and ERRγ in skeletal muscle and liver.

### ERRα, ERRγ, and skeletal muscle
A decrease in the expression of genes linked to oxidative phosphorylation (OXPHOS) occurs early in the development of insulin resistance in human diabetic muscle[66]. ERRα and ERRγ are both expressed in skeletal muscles and positively associated with mitochondrial biogenesis and activity. In addition, ERR activity is associated with increased respiration and mitochondrial activity in skeletal muscles, which therefore suggests the potential for ERR-based interventions to reverse this molecular hallmark of insulin-resistant muscles.

ERRα cooperates with PGC-1α in muscle cells to activate the transcription of mitochondrial genes, such as ATP-synthase 5O (*Atp5o*) and cytochrome c oxidase 5b (*Cox5b*), which are necessary for maximal respiration induction by PGC-1α[67]. The inhibition of ERRα activity induced a type 2 diabetes-like phenotype in skeletal muscle cells, which indicates pharmacological activation of ERRα might have a beneficial effect in humans in this tissue. Interestingly, the expression of transcription factors and coactivators known to positively regulate these genes, such as the coactivators PGC-1α and PGC-1β, is also decreased in insulin-resistant skeletal muscles[66, 68]. In addition to a positive regulation of mitochondrial genes, ERRα binds to several genes associated with the insulin pathway, including several glucose transporters and the insulin receptor (*Insr*)[18, 28]. Moreover, ERRα-null mice exhibit time-dependent hypoglycemia and hypoinsulinemia that appears to be the result of increased glucose uptake because of improved insulin sensitivity[18]. Another way that ERRα may affect the insulin pathway is through the transcriptional regulation of several genes in the AMP-kinase (AMPK) pathway, including some of its subunits (*Prkag2*), its upstream regulator *Lkb1*, and some if its downstream targets (*Acacb*)[28]. AMPK is a master regulator of energy homeostasis in the cell; it is activated in response to a decrease in ATP/AMP ratios and is a major downstream target of the most prescribed drug for patients with type 2 diabetes, metformin[69]. Of note, AMPK pathway activity was decreased in the hearts of ERRα-null compared with wild-type mice based on the Acetyl-Coa-Carboxylase 2 (ACC2, encoded by *Acacb*) phosphorylation status[18]. ACC2 is the predominant ACC form in skeletal muscle and regulates both fatty acid synthesis and oxidation. *Acacb*-null mice exhibit enhanced FAO and enhanced insulin sensitivity[70, 71]. Therefore, ERRα regulation of key genes in the insulin and AMPK pathways and its influence on the activities of these pathways are likely factors that affect glucose handling and insulin sensitivity by ERRα.

In the skeletal muscle, ERRγ is highly expressed in oxidative type 1 fibers, and forced expression of ERRγ in glycolytic type 2 fibers triggers aerobic transformation, vascularization and mitochondrial biogenesis in the absence of exercise[52]. These effects were not dependent on PGC-1α induction, but were linked to AMPK. In these transgenic mice, the AMPK path-

way was constitutively activated, which suggests a convergence between ERRγ and AMPK to direct oxidative metabolism in type 1 fibers in skeletal muscle. Moreover, treatment with the dual ERRβ/γ agonist GSK4716 led to an increase in the expression of genes involved in mitochondrial activity[56]. This finding indicates that pharmacological molecules that target ERRs may help restore insulin sensitivity through the restoration of mitochondrial mass and activity. Although the full relationship between ERRs, the transcriptional modulation of insulin pathway target genes, and the AMPK pathway remain to be defined, it appears clear that there is a convergence between these major pathways of energy metabolism. In summary, both ERRα and ERRγ positively regulate genes associated with mitochondrial functions in skeletal muscle. Moreover, the transcriptional controls of other key pathways that mediate energy homeostasis, including the insulin and AMPK pathways, may also have a beneficial impact on insulin response in this tissue.

### ERRα, ERRγ, and glucose metabolism in liver

Type 2 diabetes is also characterized by the impairment in insulin's ability to inhibit gluconeogenesis in the liver, the main organ in which this pathway is active in vertebrates. The end product of gluconeogenesis is the production of glucose to maintain constant blood levels, particularly during a fasting period. The inability of insulin to shut down this pathway will result in increased glucose production by the liver and higher blood glucose levels. Understanding how gluconeogenesis is controlled in the liver is therefore critical to treat high blood glucose levels and related metabolic disorders.

In contrast to its positive role on OXPHOS, ERRα acts as a repressor of gluconeogenesis[72]. Through the repression of *Pepck* expression, a key enzyme in gluconeogenesis, ERRα activity could contribute to the control of high glucose levels in the fed state. ERRα represses the gluconeogenic program through direct binding to a *Pepck* enhancer and, in contrast to its activity on most of its target genes, antagonizes the stimulatory effect of PGC-1α. Paradoxically, a negative regulation of gluconeogenesis should lead to an increase in blood glucose ERRα-null mice; however, these mice do not exhibit increased plasma glucose levels in the fed state, even though several key gluconeogenic genes are up-regulated in the livers of these mice[18, 72]. In fact, ERRα-null mice exhibit significantly lower glucose levels at Zeitgeber times (ZT) 12 and 0/24, which represent the start and end of the dark cycle, respectively[18]. This observation parallels the observation that hepatic glucose production relies on the metabolic state of an organism in a circadian manner because nutrient blood levels display diurnal variation. Consistent with the results obtained using ERRα-null mice as a model, pharmacological inhibition of ERRα in a diet-induced model of obesity improved insulin sensitivity and glucose tolerance and reduced circulating glucose, free fatty acids and triglyceride levels[73]. Consistent with the circadian cycle role in energy homeostasis, ERRα levels are modulated in a circadian manner in the liver and are also induced by fasting[18, 74].

Unlike ERRα, ERRγ has been shown to activate key gluconeogenic genes, such as *Pepck* and *G6pc*, in response to fasting conditions[75, 76]. ERRγ overexpression in the liver also induced gluconeogenic gene expression, increased blood glucose levels and caused a delayed response to glucose tolerance tests, without significantly changing insulin, triglyceride or fatty acid blood levels[76]. These results indicate that in patients with diabetes, increased hepatic ERRγ activity could play a role in increasing glucose serum levels. Consistent with these observations, the knock down of endogenous ERRγ in the liver decreased hepatic gluconeogenic gene expression and fasting blood glucose levels in *db/db* mice[76]. Moreover, *db/db* diabetic mice treated with GSK5182, a specific inhibitor of ERRγ, exhibited lower hyperglycemia, hepatic fat accumulation, food intake and body weight[75, 76]. Therefore, the regulation of the ERRγ-dependent gluconeogenic program in the liver could be considered a target to control hepatic insulin resistance.

Although ERRα and ERRγ are positive regulators of mitochondrial activity, they appear to antagonize each other in the regulation of gluconeogenesis. Nonetheless, ERRα inhibition or down-regulation is associated with an improvement in glucose handling most likely through the decreased expression of gluconeogenesis, decreased PDK4 expression, transcriptional regulation of genes in the insulin signaling pathway, and control of mitochondrial activity. Moreover, ERRα-null mice exhibit lower blood glucose and increased insulin sensitivity. In a similar manner, ERRγ inhibition in the liver leads to an improvement in diabetic symptoms and a decrease in gluconeogenic gene expression. It is therefore surprising to observe similar beneficial effects on the diabetic condition via the inhibition of ERRα or ERRγ because they act in an antagonistic manner on gluconeogenic genes in the liver. However, their positive regulatory activity on other pathways, including mitochondrial biogenesis, FAO and glucose handling, may be dominant *in vivo*, which could explain why the inhibition of either ERRα or ERRγ has similar global effects. ERR inhibition would act in a manner similar to metformin and reduce mitochondrial activity, which would lead to increased AMPK pathway activity, with all its beneficial effects on metabolic disorders.

### ERRα, mTOR, and lipid metabolism in liver

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder in Western countries and is now regarded as the liver manifestation of the metabolic syndrome[64, 77]. NAFLD is associated with impaired insulin response and type 2 diabetes, which leads to increased gluconeogenesis, and more than 90% of obese patients with type 2 diabetes have NAFLD[77, 78]. As previously discussed, both ERRα and ERRγ are opposite regulators of hepatic gluconeogenesis. However, NAFLD is also a disease associated with increased lipogenesis in the liver and impaired hepatic FAO. The mechanistic target of rapamycin (mTOR), a phosphatidylinositol 3 kinase (PI3K)-related serine/threonine kinase, has been demonstrated to play an important role in the control of energy metabolism, particularly in lipid biosynthesis[79]. mTOR inhibition by pharmacological tools,

such as rapamycin, has been reported to induce side effects, such as hyperlipidemia, hypercholesterolemia, and the induction of hepatic gluconeogenesis, as well as NAFLD in murine models[17, 80–82]. Remarkably, mTOR inhibition in ERRα-null mice or mice exposed to an ERRα inhibitor led to an exacerbated rapamycin-induced NAFDL[17]. This finding might be considered surprising because these mice are resistant to high-fat diet-induced obesity[43]. However, ERRα-null mice have a blunted TCA cycle after rapamycin treatment, with a down-regulation of several genes in this pathway (*Ogdh*, *Aco2*, *Idh3a*, and *Sdhc*), which leads to an accumulation of citrate and other TCA cycle intermediates. The observed rapamycin-induced change in ERRα-dependent activity has been related to a rapid increase in ERRα degradation following mTOR inhibition. The impaired TCA cycle activity in mice with reduced or null ERRα activity while treated with rapamycin suggests that these mice not only have a decreased capacity to oxidize lipids but the accumulation of citrate is reoriented toward lipid biosynthesis. Rapamycin induces a diabetic-like state in which excess energy is converted to fat. A significant number of patients treated with rapamycin and its analogs have suffered from a diabetes-like syndrome, which is associated with insulin resistance and hepatic hyperlipidemia[83]. Therefore, considering the molecular and genetic interactions observed between the ERRα and mTOR regulatory pathways in the liver, this functional crosstalk might play a more global role in the etiology of other metabolic disorders, such as diabetes and metabolic syndrome.

### ERRs as pharmacological targets in metabolic disorders

ERRα is an interesting target for the treatment of type 2 diabetes because it can increase muscle mitochondrial oxidative capacity and suppress hepatic glucose production[50, 72]. Paradoxically, one could have assumed that increased ERRα activity would be associated with the improvement of metabolic disorders; however, the results obtained in murine models indicate the opposite effect. In a polygenic mouse model of diet-induced obesity characterized by hypertriglyceridemia and hyperinsulinemia, treatment with the ERRα inverse agonist C29 decreased body fat without changing lean mass[73]. Similar results were also observed in ERRα-null mice[43]. Treatment with C29 also decreased insulin levels by approximately 66% without changing fed glucose levels, which indicates an improvement in peripheral insulin sensitivity. Circulating triglyceride levels were also decreased by half. In microarrays performed in the skeletal muscles and hepatic tissues of mice treated with C29, the liver was most responsive to ERRα pharmacological inhibition[73]. Despite the phenotypes that affect the physiology of multiple tissues observed ERRα-null mice[11, 18, 28, 51, 53–55, 58, 84], no apparent toxicities or severe adverse effects were reported in mice treated with C29. In various murine models of diet-induced obesity and type 2 diabetes, C29 induced significant improvements in insulin sensitivity, glucose tolerance, and reduced triglyceride levels[73]. Therefore, the pharmacological inhibition of ERRα had a similar beneficial impact on glucose handling, diabetes and obesity

to that of the genetic loss of ERRα[18, 43, 73]. Furthermore, ERRα inhibition may also facilitate the fight against obesity because ERRα-null mice are protected against high-fat diet-induced obesity[43]. Moreover, the observation that the liver was the main target for ERRα inhibition by pharmacological activation indicates that pharmacological compounds may have a tissue-specific modulation of this nuclear receptor's activity. These molecules, if developed, would be similar to the well-known selective estrogen-receptor modulators (SERMs), which display antagonist activity against ERα in the breast, but agonist activity in peripheral tissues, such as the bones and uterus[85].

Inactivation of ERRγ by GSK5182 improves insulin signaling via the inhibition of LIPIN1-induced protein kinase C activation[86]. Moreover, the same inhibitor decreased hyperglycemia in *db/db* mice and gluconeogenic genes, such as *Pck1*, *G6pc* and *Pdk4*, in the liver[75]. GSK5182 completely restored blood glucose levels and gluconeogenic gene expression and also improved liver toxicity caused by diabetic progression. Similar to the effects of C29 on ERRα in a tissue-specific manner, the pharmacological inhibition of ERRγ only decreased the expression of its target genes in the liver without altering gene expression in skeletal muscle[75].

Overall, the hepatic inhibition of both ERRs by pharmacological tools has beneficial effects on insulin sensitivity and glucose handling in mouse models. Moreover, at least in these models, the effects of these therapeutic tools appear to primarily target the liver and not skeletal muscles. This is a crucial requirement for targeted ERR disruption. Thus, considering the beneficial effects of the ERRs in skeletal muscle on glucose uptake and handling, hepatic inhibition of ERRs combined with their activation in skeletal muscle would be an interesting avenue for the design of therapeutic tools (Figure 2).

Hepatic insulin resistance is associated with increased mitochondrial respiration and performance[87, 88]. Patients with type 2 diabetes have an up-regulation of OXPHOS genes in the liver, including a significant positive correlation between ERRα mRNA and OXPHOS[89]. This finding is in contrast to the decreased mitochondrial gene expression in diabetic skeletal muscles[65–67, 90]. It was hypothesized that the up-regulation of OXPHOS genes in type 2 diabetic livers would result in increased gluconeogenesis and therefore contribute to hyperglycemia[89]. This finding is consistent with the reduction in glucose production by the liver following metformin treatment in diabetic patients, a drug that targets mitochondrial activity[69]. It therefore appears that to ameliorate insulin sensitivity and metabolic disorders in the liver, decreased mitochondrial activity is desired as opposed to increased activity in the skeletal muscles. In these conditions, hepatic inhibition of ERR activity would also be beneficial to inhibit hepatic mitochondrial activity. This concept is consistent with the observation that the liver is also the main target for metformin action[91].

The modulation of ERRα and ERRγ activities demonstrates promise for the control and recovery of metabolic disorders, such as type 2 diabetes, obesity and metabolic syndrome. However, the use of pharmacological modulators must be carefully performed because it could also lead to adverse side
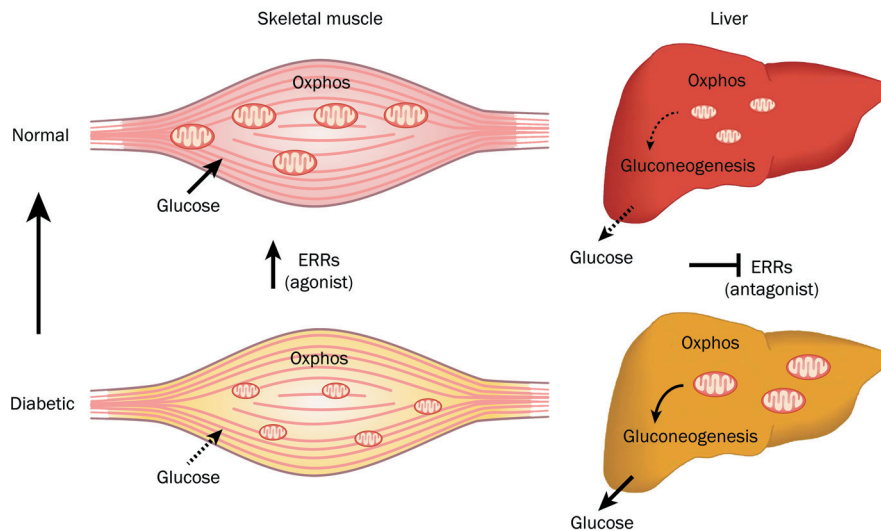
**Figure 2.** ERRs as pharmacological targets to manage type 2 diabetes. In the skeletal muscles of patients with type 2 diabetes, mitochondrial activity is deficient and cells are less responsive to insulin regarding glucose uptake and usage. Increasing ERR activity using a compound with agonist-like activity would result in increased glucose uptake and usage, as well as increased mitochondrial activity, which would therefore ameliorate insulin signaling in skeletal muscle. In the livers of patients with type 2 diabetes, elevated energy production sustains an increased gluconeogenesis that results in higher blood glucose levels, which leads to hyperglycemia. ERR inhibition would have the benefit of blocking mitochondrial activity, which would therefore reduce the ATP production that feeds gluconeogenesis and restore normal blood glucose levels. This mechanism is similar to metformin, the most prescribed drug for the treatment of type 2 diabetes, which inhibits hepatic mitochondrial activity and gluconeogenesis. In addition, the inhibition of ERRγ activity would also have the advantage of directly blocking gluconeogenesis.

effects. Despite the positive results of C29 and GSK5182 in mouse models, ERR inhibition *in vivo* must still be approached with caution. As previously discussed, several potential side effects of ERR downregulation can be thought of, including the induction of NAFLD[17], the sensitivity to hepatic carcinogenesis by xenobiotics[55], and heart failure[58] as a result of FAO blockade in cardiac mitochondria.

## Perspectives and conclusions

ERRα and ERRγ primarily bind to the same target genes as homo- or heterodimers[28, 92]. The functional consequence of this interaction is not yet understood because ERRα is not required for ERRγ activity and vice-versa; however, heterodimers have been reported to have lower activity *in vitro*. It also appears that they are able to counterbalance for the absence of the other because in ERRα-null cells, ERRγ can be up-regulated and vice versa[60, 93]. Nevertheless, it appears that in specific contexts and for specific genes, the two ERR isoforms do not have the same role, such as for the transcriptional control of the key gluconeogenic gene *Pepck*. This duality of the ERRs has also been studied in the context of breast cancer, where ERRα and ERRγ expression is inversely correlated, and the former is associated with a more aggressive disease, whereas the latter is considered a tumor suppressor[14, 94–96]. In the cellular environment, both nuclear receptors appear to act in an opposite manner on cancer cell metabolism because ERRα contributes to the suppression of oxidative metabolism, which is stimulated by ERRγ[27]. Additional work must be completed in this area of research, which will certainly highlight their precise molecular mechanisms of action, as well as the interesting and diverging biological functions of ERRα and ERRγ.

It is clear that the ERRs control vast metabolic gene networks and are key regulators of energy metabolism, particularly in response to various environmental challenges or biological stresses. However, data from multiple genome-wide binding site location analyses, which have yet to be completely investigated, have indicated that the ERRs might also be major orchestrators of other biosynthetic pathways and biological processes; furthermore, this regulation is likely to be cell- and tissue-specific. Therefore, research will continue to explore the multiple parallel ERR universes to fully understand the role of these nuclear receptors in physiology and disease and evaluate their true potential as therapeutic targets.

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