Orphan nuclear receptors: therapeutic opportunities in skeletal muscle

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Orphan nuclear receptors: therapeutic opportunities in skeletal muscle. Am J Physiol Cell Physiol 291: C203–C217, 2006; doi:10.1152/ajpcell.00476.2005.—Nuclear hormone receptors (NRs) are ligand-dependent transcription factors that bind DNA and translate physiological signals into gene regulation. The therapeutic utility of NRs is underscored by the diversity of drugs created to manage dysfunctional hormone signaling in the context of reproductive biology, inflammation, dermatology, cancer, and metabolic disease. For example, drugs that target nuclear receptors generate over $10 billion in annual sales. Almost two decades ago, gene products that modulate their action were not known, and they were denoted orphan NRs. Many of the remaining orphan NRs are highly enriched in energy-demanding major mass tissues, including skeletal muscle, brown and white adipose, brain, liver, and kidney. This review focuses on recently adopted and orphan NR function in skeletal muscle, a tissue that accounts for ~35% of the total body mass and energy expenditure, and is a major site of fatty acid and glucose utilization. Moreover, this lean tissue is involved in cholesterol efflux and secretes that control energy expenditure and adiposity. Consequently, muscle has a significant role in insulin sensitivity, the blood lipid profile, and energy balance. Accordingly, skeletal muscle plays a considerable role in the progression of dyslipidemia, diabetes, and obesity. These are risk factors for cardiovascular disease, which is the foremost cause of global mortality (>16.7 million deaths in 2003). Therefore, it is not surprising that orphan NRs and skeletal muscle are emerging as therapeutic candidates in the battle against dyslipidemia, diabetes, obesity, and cardiovascular disease.

Cardiovascular disease (CVD) represents one of the most critical global health threats, and accounts for >16.7 million deaths every year. A cluster of pathologies that constitute CVD include heart disease, vascular disease, atherosclerosis, myocardial infarction, stroke, and hypertension. Furthermore, the links between CVD and the ever-increasing obesity epidemic suggests a grave prognosis for human health in the early decades of the 21st century. Such grim predictions are supported by the realization that CVD contributed to one-third of global morbidity in 2001 (3, 4, 140).

Dyslipidemia associated with anomalous levels of the lipid triad [i.e., elevated levels of triglycerides (TG) and low-density lipoprotein (LDL) cholesterol, low levels of high-density lipoprotein (HDL) cholesterol], hypertension, obesity, sedentary lifestyle, diabetes, chronic inflammation, and smoking all constitute important independent risk factors for cardiovascular disease. Importantly, diet, metabolism (genetic predisposition), and physical activity directly influence these risk factors (3, 4, 140). Research indicates that ~62% of strokes and 49% of heart attacks are caused by hypertension. High blood cholesterol (>5 mmol/l) accounts for ~18% of strokes and 50% of heart disease. Aside from dietary and genetic factors, the prevalence of cardiovascular disease is exacerbated by modern sedentary lifestyles; current data suggest that ~60% of the world’s population do not meet current physical activity guidelines (3, 4, 140).

Approximately 20% of the global population (up to 1 billion adults) are deemed overweight and at least 300 million are clinically obese. Obesity rates have tripled in North America, Eastern Europe, the Middle East, Australia, and China. About 21% of coronary heart disease globally is attributable to an elevated body mass index (>25) (4, 185). Obesity is the trigger for the development of hypertension, dyslipidemia, and diabetes, which ultimately leads to cardiovascular disease. Furthermore, the increasing prevalence of diabetes in adults (estimated at ~4.5% globally) leads to a 3- and 8-fold higher coronary heart disease risk in men and women respectively (185). Currently, 180 million people worldwide suffer from diabetes, and at least 1 in 10 deaths among adults between 35 and 64 years old is attributable to diabetes, primarily through the increased risk of cardiovascular disease (122, 140, 185).

Metabolic syndrome

Metabolic syndrome, or Syndrome X, represents a cluster of metabolic abnormalities that all constitute known risk factors for cardiovascular disease. The National Cholesterol Education Program: Adult Treatment Panel III guidelines define metabolic syndrome as the presence of any three features listed in Table 1 (1). The increased prevalence of metabolic syndrome

Table 1 (1). The increased prevalence of metabolic syndrome

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has arisen primarily as a result of the global obesity epidemic with peripheral insulin resistance providing the most widely accepted and unifying feature of the syndrome (40, 162). While great inroads have been made in the identification of the relationship between the various clinical features of metabolic syndrome, the underlying pathological mechanisms are not well understood.

Excessive circulating levels of saturated fatty acids is known to be a major contributing factor to the development of insulin resistance (40, 162). The development of insulin resistance in response to increased dietary fatty acid intake is likely to be exacerbated further by the ensuing inhibition of the antilipolytic effect of insulin action resulting in an increased release of stored triglycerides into the circulation (84, 157). Increased circulating fatty acids promotes insulin resistance by inhibiting various components of insulin signaling pathways such as protein kinase C in skeletal muscle (84) and insulin receptor substrate phosphorylation in the liver (155). Increased fatty acid delivery to the liver increases hepatic triglyceride synthesis and promotes the production and secretion of ApoB containing very low-density lipoprotein (VLDL) into the circulation contributing to the dyslipidemic features of Metabolic Syndrome (97, 98). Defective glucose metabolism arises primarily from reduced ability of insulin to suppress hepatic glucose production and promote glucose uptake by adipose and muscle tissue. Subsequent compensatory mechanisms to maintain euglycemia result in the characteristic hyperinsulinemia associated with metabolic syndrome (40, 157, 162). While hyperinsulinemia will ultimately have effect on insulin secretion, prolonged exposure to circulating free fatty acids (FFAs) are also implicated in perturbations in glucose stimulated insulin secretion by pancreatic β-cells (92). Several mechanisms have been proposed to underlie pancreatic lipotoxicity in metabolic syndrome (17, 69, 188). More recent studies (93, 186) have suggested a relationship between elevated proinflammatory cytokines, such as TNF-α and the pathogenesis of metabolic syndrome.

SKELETAL MUSCLE CARBOHYDRATE AND LIPID METABOLISM

Skeletal Muscle Metabolism

The three types of muscle tissues are skeletal, cardiac, and smooth. Skeletal muscle is involved in movement, posture, stability, metabolism, heat production, and cold tolerance. Skeletal muscle fibers are classified by two major functional characteristics: speed of contraction and the aerobic (oxidative)/anaerobic (glycolytic) production of ATP. The speed of the fiber reflects how fast the fiber metabolizes ATP. Fast fibers (also known as type IIb or white fibers) utilize anaerobic glycolysis, are low in mitochondria and myoglobin, and are suited to short-term intense activity. Slow fibers (also known as type I or red fibers) utilize aerobic metabolism, are rich in mitochondria and myoglobin, and are suited to endurance activity. There is an intermediate fast fiber type IIa, which combines fast twitch capacity with aerobic fatigue-resistant metabolism and intermediate glycogen levels (107).

Skeletal muscle is a “metabolically flexible” tissue and has a paramount role in energy balance; for instance, it is the primary tissue of insulin-stimulated glucose uptake (disposal), and storage (4-fold the glycogen content of liver), regulates ApoA1-dependent cholesterol efflux and strongly influences metabolism via modulation of circulating and stored lipid flux (57, 74, 78, 136). In fact, lipid catabolism supplies up to 70% of the energy requirements in a fasted individual. During the initial phase of aerobic exercise, skeletal muscle utilizes stored glycogen; however, as exercise continues, glucose and stored muscle triglycerides become important energy substrates (57, 148, 149). Prolonged exercise increasingly depends on fatty acids and lipid mobilization from other tissues. Given the fact that skeletal muscle can utilize both carbohydrate and lipid energy substrates after appropriate mechanical and hormonal cues and considering the relative mass of the tissue it is hardly surprising that normal (and abnormal) skeletal muscle metabolism has a significant impact on insulin sensitivity, the blood lipid profile and the pathogenesis of metabolic disease.

Skeletal Muscle Dysfunction in Metabolic Disease

Skeletal muscle insulin resistance comprises perturbations in both glucose and fatty acid metabolism. It is well established in the literature that pathophysiological conditions that are associated with impaired insulin resistance, such as type 2 diabetes and obesity, correlate with elevations in circulating FFAs (15, 147). Considerable evidence obtained in both animal and human studies have linked accumulation of intra-myocellular lipids with insulin resistance in obesity and type 2 diabetes (49, 75, 109, 133). Investigations aimed at identifying the mechanisms underlying this association have implied an imbalance between fatty acid uptake and the rate of fatty acid oxidation. The direct link between intra-myocellular lipid accumulation and insulin resistance is supported by the observation that insulin sensitivity can be restored by treatments that promote depletion of accumulated triglycerides, such as low-fat diets, fasting, exercise, and leptin administration (125, 163). The significance of this is exemplified by NMR spectroscopy studies (86, 157) that measured intramyocellular lipids and found that levels correlated more closely with insulin resistance than other commonly used indexes, such as body mass index and waist-to-hip ratios.

Impaired mitochondrial function that directs fatty acids toward storage, as opposed to oxidation, and reduced oxidative enzyme activities may in large part contribute to muscle lipid accumulation in obesity and type 2 diabetes mellitus (76, 77, 165, 166). Moreover, the activity of carnitine palmitoyl transferase 1 (CPT1), a key step in the regulation of muscle fatty acid oxidation, has been shown to be reduced in insulin resistant muscle from obese subjects (165). In addition to this, shunting of fatty acids toward storage in skeletal muscle as opposed to oxidation triglyceride accumulation in skeletal muscle of obese and type 2 diabetic subjects was shown to be...
linked to elevated rates of fatty acid transport via increased sarcolemmma fat/cd36 (16). Another hypothesis contends that hyperinsulinemia may increase skeletal muscle and liver lipid content due to increased lipogenesis via elevated expression of SREBP-1C (164). This hypothesis is further supported by the recent observation that SCD-1 expression and activity is elevated in the skeletal muscle of obese humans contributing to the abnormal skeletal muscle lipid partitioning seen in these individuals (63).

Impaired glucose utilization by skeletal muscle in metabolic disease is also linked to elevated circulating FFAs. As early as the 1960s, it was noted that FFA oxidation disrupts glucose catabolism via the inhibition of phosphofructokinase and pyruvate dehydrogenase. In this context, the Randle hypothesis proposed that FFAs compete with glucose for mitochondrial oxidation (144). Similarly, studies support the concept that FFAs inhibit glucose transport and key phosphorylation events (38, 147). Alternatively, mounting evidence points to direct effects of increase circulating and intracellular lipid content to perturbations in insulin signaling pathways (38, 147, 175). Excess triglyceride in insulin resistant muscle has been shown to alter insulin signaling via consequent increases in lipid intermediates, such as fatty-acyl CoA, diacylglycerol, and ceramides. Activation of protein kinase C by diacylglycerol inhibits the tyrosine kinase activity of the insulin receptor leading to reduced insulin action (159). Ceramides activate a protein phosphatase that dephosphorylates AKT/protein kinase B, subsequently inhibiting glut4 translocation and glycogen synthesis (28, 100). Lipid accumulation in skeletal muscle has also been shown to inhibit phosphorylation of Irs-1, thus inhibiting the binding and activation of phosphatidylinositol 3-kinase, a pivotal step in the normal insulin signaling cascade (38, 190).

The metabolic flexibility of skeletal muscle allows it to utilize both carbohydrate and lipid energy sources given the appropriate hormonal and mechanical signals (74, 76, 77). While the precise mechanistic basis underlying abnormal skeletal muscle function in obesity and diabetes remains unclear, it is obvious that biochemical perturbations in this tissue have a profound effect on systemic metabolic homeostasis. Therapeutic targeting of the metabolic derangements in skeletal muscle will undoubtedly promote improvement in systemic metabolic homeostasis and ultimately attenuate the pathogenesis associated with the metabolic syndrome.

SKELETAL MUSCLE AS AN ENDOCRINE ORGAN: MYOKINES

Obesity-linked insulin resistance is known to be associated with a chronic systemic inflammatory response (61, 183). In recent years, it has become apparent that adipose tissue functions as more than just a reservoir for energy storage in the form of triglycerides but rather functions as a vital endocrine organ regulating systemic metabolic homeostasis via controlled storage/release of fatty acids and the secretion of biologically active “adipokines,” such as adiponectin (70), resistin (169), leptin (106), adipin (184), and TNF-α (62). Similarly, the potential endocrine function of skeletal muscle is being recognized with the release of bioactive molecules now termed “myokines” (42, 132). Identification of these factors offers support for a skeletal muscle endocrine axis that is potentially pivotal to metabolic homeostasis.

Muscle tissue expresses and releases several cytokines into the circulation, for example, IL-6, -8 and -15. The autocrine actions of these factors are well documented; however, there is accumulating evidence that these cytokines exert their effect in other parts of the body. IL-15 is abundantly expressed in skeletal muscle, is induced by acute exercise, and has anabolic effects on skeletal muscle protein dynamics (141). Interestingly, administration of IL-15 in rats demonstrated a significant reduction in adipose deposition (23). Studies (142) in adipogenic 3T3 cells revealed that despite little or no expression of IL-15 mRNA, administration of IL-15 inhibited lipogenesis and stimulated the release of adiponectin. Because skeletal muscle expresses and secretes high levels of IL-15, such an observation provides strong support for the hypothesis that a skeletal muscle-adipose endocrine axis exists that modulates lean body/adipose composition and insulin sensitivity.

Another cytokine released by skeletal muscle is IL-6 and has been referred to as the “exercise factor” because its expression and release was found to increase up to 100-fold during muscle contraction and is postulated to underlie many of the metabolically beneficial effects of exercise (43). Circulating IL-6 stimulates AMPK activity, a fuel-sensing enzyme that is activated by changes in the energy state of the cell and in response to adipokines, in adipose and lean tissue (80). IL-6 transcriptional regulation in skeletal muscle is influenced by glycerol content via the p38 MAPK pathway that results in upregulated expression in response to exercise-induced glycogen depletion (26, 27). During exercise, IL-6 appears to play a glucoregulatory role via augmented hepatic glucose production and muscle glucose uptake (43). IL-6 has been shown to be a potent modulator of fatty acid metabolism via increased rates of fatty acid oxidation and reesterification and attenuation of insulin-mediated lipogenesis in both human subjects and isolated rodent muscle samples (19, 173). More recently, IL-6-driven increases in lipolysis were demonstrated in type 2 diabetic patients without the induction of adverse side effects, such as hypertriglyceridemia, suggesting a potential therapeutic utility of this cytokine in the treatment of metabolic syndrome (135). Paradoxically, increased circulating IL-6 has been linked to type 2 diabetes, leading to speculation that IL-6 may contribute to the induction or pathogenesis of type 2 diabetes (137, 139, 152). However, firm evidence of a causative link remains to be demonstrated. Furthermore, IL-6-deficient transgenic mice are glucose intolerant, have reduced endurance and energy expenditure, and develop late-onset obesity (180).

Finally, myostatin, a member of the TGF-β superfamily, is specifically expressed in vertebrate skeletal muscle. Mice lacking myostatin have a dramatic increase in skeletal muscle growth and significantly reduced fat deposition with age (110, 111). Several mechanisms may account for the effect of myostatin on lipid metabolism, including a direct effect on adipocytes (81) or alternatively, anabolic effects in skeletal muscle may produce a metabolic shift in energy consumption resulting in a net reduction in adipose lipid storage. The latter hypothesis is plausible, given the accumulating evidence that muscle fiber composition has a favorable effect on the LDL/HDL cholesterol ratio (55) and the accepted tenet that muscle tissue has a significantly higher metabolic rate than other major tissues.
NUCLEAR RECEPTOR REGULATION OF CARBOHYDRATE AND LIPID METABOLISM

The links between diets rich in free fatty acids and sugars and the increasing prevalence of dyslipidemia, type II diabetes, obesity, and cardiovascular disease are well established. Furthermore, it is becoming increasingly clear that the metabolic regulation of both lipid and carbohydrate homeostasis are intrinsically connected. A pertinent example of this metabolic interconnection is the regulation of triglyceride catabolism by insulin, a signaling molecule known primarily as an essential regulator of glucose disposal. Insulin regulates triglyceride catabolism via inhibition of hormone sensitive lipase. The significance of this regulatory mechanism is highlighted by the demonstration that lipid accumulation and/or elevated lipoprotein lipase expression in nonadipose tissue (e.g., liver and muscle) results in insulin resistance (82, 86). Perhaps paradoxically, it has become evident that diets rich in unsaturated fatty acids protect against metabolic disease prompting speculation that dietary lipids function as signaling molecules that play vital roles in controlling lipid homeostasis. The search for the mechanisms by which various lipid molecules regulate lipid homeostasis and the subsequent targets of such signaling have become an intense area of investigation in recent years in an effort to understand and control the increasing incidence of metabolic disease in modern society.

Insights into the mechanisms regulating such processes have significantly matured since the discovery that metabolism is, in part, regulated by the nuclear hormone receptors (NRs) which function as ligand (hormone)-regulated transcription factors that bind DNA and control gene expression (14, 45). A ligand or hormone can be considered any small molecule that can bind to the receptor and affect its function. For example, steroids (estrogen, testosterone, etc.), thyroid hormones, retinoids, dietary lipids, oxysterols, fatty and bile acids are signaling molecules that bind to nuclear receptors and regulate gene expression thereby controlling endocrine pathways directly relevant to disease (14, 45). Essentially, NRs function as the conduit between diet, metabolism (and environmental stimuli) and gene expression mediating a unique physiological response in a nutritionally dependent manner.

NUCLEAR RECEPTOR FUNCTION: GENERAL CONCEPTS

A decade ago, gene products were identified that belonged to the NR superfamily on the basis of nucleic acid sequence identity. However, the endogenous molecules which regulate their activity were unknown and thus they were called orphan NRs. On the basis of members of the NR superfamily that have been characterized, the orphans forecast an enormous, yet unexploited opportunity for the discovery of novel signaling pathways and therapeutic agents. The potential impact of such a discovery cannot be overstated, because every known NR has been implicated in disease (14, 51, 172). All members of the NR superfamily display a highly conserved structural organization, an amino terminal region AB (that encodes activation function 1; AF-1), followed by the COOH region, which encodes the DNA binding domain; a linker region D and the COOH-terminal E region. The DE region encodes the ligand binding domain (LBD), and a transcriptional domain, denoted as AF-2 (Fig. 1) (4, 105). Nuclear receptors trans-activate the expression of target genes via the binding of DNA consensus elements within regulatory regions as either monomers, homodimers, or heterodimers with the retinoid X receptors. DNA binding is mediated by a highly conserved zinc finger DNA binding domain and in the case of heterodimerization consensus elements have been found as direct repeats, inverted repeats, or palindromic repeats (4, 105). A subgroup of the NR superfamily controls metabolism in an organ-specific manner (45).

The transcriptional activity of nuclear receptors is regulated by coordinated interaction with nuclear cofactors. As a general rule, the interaction of receptors with ligands promotes a

Fig. 1. A: schematic representation of nuclear receptor functional domains. The variable NH2-terminal domain possesses AF-1 ligand independent activity (A/B), the conserved DNA binding domain (C), variable linker region (D), ligand binding and dimerization domain (E) and the AF-2 ligand-dependent transactivation domain (F). B: co-regulator binding of nuclear receptors. Many nuclear receptors are associated with co-repressor proteins in the absence of ligand. Upon ligand binding, co-repressors are displaced in favor of co-activator proteins. CoR, coenzyme receptor; CoA, coenzyme A.
dissociation of co-repressor proteins and a recruitment of coactivator proteins and associated complexes, due primarily to structural changes in the LBD, eliciting a transcriptional response and target gene expression (Fig. 1B). In addition to ligand-induced activation, the function many nuclear receptors is modulated by signaling cascades that phosphorylate the receptor, further controlling activity in a ligand-independent or ligand-dependent manner (34, 72, 161). In the case of true orphan receptors, this level of regulation may prove to be pivotal to the design of small molecules that target the function of these receptors.

Genetic and pharmacological studies have demonstrated that the liver X receptors (LXR)-α and -β, farnesoid X receptor, peroxisome proliferator-activated receptors (PPAR)-α, -β/δ, and -γ, liver receptor homolog-1 and the small heterodimeric partner regulate lipid, glucose, and energy homeostasis (45). Concordantly, dysfunctional NR signaling results in dyslipidemia, diabetes, obesity, and inflammation. Recently, additional orphan NRs have been implicated in the regulation of lipid and carbohydrate metabolism, for example, Nur77, Rev-erb-α and -β, that control lipolysis, and lipid absorption. Moreover, evidence for cross-talk between the β-adrenergic and the Nur77 signaling pathways has appeared in the literature.

**ORPHAN NRs AS THERAPEUTIC TARGETS**

The fundamental importance of NRs in human health is underscored by the gamut of drugs created to treat disorders associated with dysfunctional hormone signaling ranging from cancer and diabetes to reproductive, cardiovascular and inflammatory diseases (28a, 51, 52, 172).

The plethora of orphan NRs has been the catalyst for the development of a new paradigm, called “reverse endocrinology” mediated by functional genomics, high throughput screening and combinatorial chemistry. Reverse endocrinology is the process whereby the orphan NR is used to search for a screening and combinatorial chemistry. Reverse endocrinology” mediated by functional genomics, high throughput screening and combinatorial chemistry. Reverse endocrinology is the process whereby the orphan NR is used to search for a ligand/hormone and signaling pathway is the process whereby the orphan NR is used to search for a ligand/hormone and signaling pathway is the process whereby the orphan NR is used to search for a ligand/hormone and signaling pathway is the process whereby the orphan NR is used to search for a ligand/hormone and signaling pathway. In particular, recent studies on previously denoted orphans [e.g., PPAR-α/γ/δ, LXR, FXR, CAR, SXR] have uncovered a myriad of new signaling pathways that regulate metabolism, and drug tolerance, and provided new insights/treatments into many human diseases (i.e., diabetes, dyslipidemia, obesity, and metabolic syndrome) (45, 50, 90). The identification of low-affinity endogenous and/or high affinity synthetic ligands for orphan receptors has created an orphan receptor subclass often referred to as “adopted orphan” receptors (28a). Low-affinity natural agonists for this class of receptor are commonly dietary lipids and their derivatives.

Drugs targeting nuclear receptors, including the estrogen, thyroid, and glucocorticoid receptors, and PPAR-α and PPAR-γ are among the most commonly prescribed drugs on the market and generate annual revenues in excess of $10 billion (131). Despite the proven therapeutic effectiveness of these pharmaceuticals many are accompanied by undesirable side effects prompting intense efforts to identify and develop new agents that retain the positive attributes of current drugs while avoiding unwanted side effects. Development of tissue and gene specific nuclear receptor ligands (selective modulators) is a key focus of such efforts with this concept best exemplified by the selective estrogen receptor modulators, such as tamoxifen and raloxifene (50, 68). The identification of endogenous and synthetic ligands to orphan NRs has generated considerable excitement in recent years and highlights the potential therapeutic opportunities given the wide range of biological functions of these proteins. A pertinent example of such promise was realized with the identification that the anti-diabetic/insulin sensitizing thiazolidinediones (TZDs) function largely as high-affinity ligands for PPAR-γ, whereas the lipid lowering fibrate class of drugs signal via PPAR-α (91, 94). To date, several synthetic ligands have been identified for other orphan nuclear receptors and are being intensively studied in laboratories, and in many cases, animal and clinical trials are underway. While crystal structure analysis of orphan nuclear receptors suggest many will function as true orphan receptors unable to accommodate molecules within their LBDs, the identification of small molecules that bind to other regions of the receptor and modulate coregulator interactions and receptor function remain attractive avenues to explore (50, 114, 131, 160). In summary, the orphan receptors are key functional regulators of metabolic homeostasis and provide a scaffold with enormous scope and opportunity for the discovery of important new therapeutic agents.

**REGULATION OF SKELETAL MUSCLE METABOLISM BY ADOPTED AND ORPHAN NUCLEAR RECEPTORS**

As discussed, skeletal muscle is a major mass peripheral tissue accounts that for >35% of the total body mass and energy expenditure, and is the major site of fatty acid and glucose oxidation. Moreover, this lean tissue mediates physiological activity and cholesterol efflux, and secretes myokines (myostatin, IL-6, -8, and -15) that control inflammation, energy expenditure, and adiposity. Consequently, muscle has a significant role in insulin sensitivity, the blood lipid profile, inflammation, and energy balance, conferring a significant role in the progression of dyslipidemia, diabetes, and obesity. These are risk factors for cardiovascular disease, the foremost cause of global mortality. These risk factors are recognized by the World Health Organization as being in the top 10 global health problems and are directly influenced by diet, metabolism, and lifestyle.

Many of the recently adopted orphan NRs and skeletal muscle are rapidly emerging as critical therapeutic targets in the battle against dyslipidemia, diabetes, obesity, and cardiovascular disease. Along these lines it has been demonstrated that “ligand-dependent” NRs in skeletal muscle enhance insulin-stimulated glucose disposal, lipid catabolism, energy expenditure, cholesterol efflux, and decrease triglycerides (see below).

Surprisingly, the function of the remaining orphan NRs in skeletal muscle metabolism has not been examined. Nevertheless, given the data on the orphan NR subgroup, abundant expression in skeletal muscle, and their potential utility as therapeutic targets for the treatment of disease, the contribution of this tissue to orphan NR action requires further investigation. Along these lines, emerging stories from cell culture and animal models illustrate that the orphan NRs, including estrogen-related receptor (ERR)-α, retinoic acid-related orphan receptor (ROR)-α, Rev-erb (α and β), and Nur77 regulate lipid absorption, lipolysis, inflammation (IkBα), and myokine expression (71, 88, 102, 104, 108).
Clearly, improvements in skeletal muscle lipid and carbohydrate metabolism mediated by nuclear receptor function and pharmacological activation will yield concomitant improvements in systemic metabolic homeostasis. Numerous metabolic genes regulated by nuclear receptors have been identified ranging from transport molecules, enzymes involved in lipid and carbohydrate anabolism/catabolism, lipid and carbohydrate storage, thermogenesis, and signaling pathways. For brevity, the key genes presented and discussed in this review are outlined in Table 2 with a brief explanation of their respective functional roles. This review will focus on the orphan and adopted nuclear receptors in the regulation of skeletal muscle lipid and carbohydrate metabolism and the implications for the treatment of metabolic disease (Fig. 2).

**PPARs**

PPAR functions in skeletal muscle are emerging as critical targets in the battle against obesity (39), metabolic syndrome X (171), type II diabetes (58), and dyslipidemia (39, 54, 58). For example PPAR-α (116), PPAR-δ (171) (112, 134, 181), and PPAR-γ (58), have been shown to be involved in enhancing insulin-stimulated glucose disposal rate, decreasing triglycerides, and increasing lipid catabolism, energy expenditure, cholesterol efflux and plasma HDL-C levels. Recently, two studies that focused on PPAR-δ action in skeletal muscle cells (39) and tissue (171) demonstrated that this NR induces genes involved in cholesterol efflux, lipid catabolism, and energy expenditure. Moreover, PPARδ activation leads to a predominant type I/slow/oxidative fiber phenotype, and dramatically increased endurance, increased insulin sensitivity, and resistance to obesity (103, 181). PPARs primarily function as systemic lipid sensors that regulate a variety of homeostatic functions, including metabolism, inflammation and development. Three mammalian PPAR subtypes, designated PPAR-α (NR1C1), PPAR-γ (NR1C3), and PPAR-δ (NR1C2) have been identified (12, 41, 90). PPAR activity is regulated by an array of dietary lipids including saturated and unsaturated fatty acids and their respective metabolites derived through the lipoxygenase and cyclooxygenase pathways. Furthermore, PPAR activity is targeted by selective synthetic compounds including hypolipidemic fibrates (PPAR-α), the insulin-sensitizing TZDs (PPAR-γ) (12, 41, 90), and the antisynrome X phenoxy-acetic acid compound GW-501516 currently in clinical trials (PPAR-δ). Cell culture and animal studies over the last decade have revealed discrete functions for the various PPAR subtypes in the regulation of genes that control lipid and carbohydrate metabolism in a range of tissues. PPAR-α and PPAR-γ are predominantly, though not exclusively, expressed in liver and adipose tissue, respectively, whereas PPAR-δ expression is relatively ubiquitous, although it is abundantly expressed in brain, intestine, skeletal muscle, spleen, macrophages, lung, fat, and adrenals (12, 41, 90). Until recently, relatively little was known about the specific function of PPAR-δ. Prostanoids, produced by the conversion of polyunsaturated fatty acids, have been proposed as potential endogenous ligands for PPAR-δ. The development and analysis of a potent and selective PPAR-δ agonist (GW-501516), a phenoxyacetic acid derivative, has generated intense interest by provided evidence for a role of this PPAR subtype in the coordination of glucose metabolism.

### Table 2. Key metabolic genes discussed in this review as targets of nuclear hormone receptor signaling in skeletal muscle

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>ATP binding cassette: transporters that transfer cholesterol to the HDL acceptors, i.e., reverse cholesterol efflux.</td>
</tr>
<tr>
<td>ACS4</td>
<td>Acyl-CoA synthetase-4. Enhances uptake of fatty acids by catalyzing their activation to acyl-CoA esters for subsequent use in catabolic fatty acid oxidation pathways.</td>
</tr>
<tr>
<td>Adiponectin receptors</td>
<td>Adiponectin receptors 1 and 2. Cell surface membrane receptors for adiponectin that regulate glucose uptake and fatty acid oxidation.</td>
</tr>
<tr>
<td>AMPK</td>
<td>5′-AMP-activated protein kinase. A fuel-sensing enzyme that responds to cellular stress by regulating carbohydrate and fat metabolism.</td>
</tr>
<tr>
<td>ApoA1</td>
<td>The major apolipoprotein of HDL.</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E: facilitates cholesterol and lipid efflux.</td>
</tr>
<tr>
<td>CD36</td>
<td>Scavenger receptor involved in the uptake of oxidized LDL.</td>
</tr>
<tr>
<td>CEBPα</td>
<td>CCAAT/enhancer-binding protein transcription factor known to be important for adipogenesis.</td>
</tr>
<tr>
<td>CPT-1</td>
<td>Carnitine palmitoyl transferase-1. Transfers the long-chain fatty acyl group from CoA to carnitine, the initial reaction of mitochondrial import of LCFA's and their subsequent oxidation.</td>
</tr>
<tr>
<td>Δ-6-Desaturase</td>
<td>Involved in desaturation of fatty acids via introduction of double bonds between defined carbons of the fatty acid chain.</td>
</tr>
<tr>
<td>FAS</td>
<td>Fatty acid synthase. Involved in de novo fatty acid production.</td>
</tr>
<tr>
<td>FABP3</td>
<td>Fatty acid translocase, and fatty acid binding protein. Facilitate uptake of LCFA's and LDL's.</td>
</tr>
<tr>
<td>GLUT-4 and -5</td>
<td>Glucose transporters. GLUT4 facilitates glucose uptake in response to insulin stimulation. GLUT5 catalyzes uptake of fructose.</td>
</tr>
<tr>
<td>Glycogenin</td>
<td>Initiates the synthesis of glycogen, the principal storage form of glucose in skeletal muscle.</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Docking protein involved in binding and activating other signal transduction molecules after being phosphorylated by the insulin receptor kinase.</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase. Hydrolysis of lipoprotein triglycerides into free fatty acids and responsible for the uptake of free fatty acids.</td>
</tr>
<tr>
<td>MCAD</td>
<td>Medium chain acyl CoA dehydrogenase, a key enzyme involved in fatty acid oxidation.</td>
</tr>
<tr>
<td>PDK-2 and -4</td>
<td>Pyruvate dehydrogenase kinases: inhibiting the pyruvate dehydrogenase complex, thereby controlling glucose oxidation and maintaining pyruvate for gluconeogenesis.</td>
</tr>
<tr>
<td>PGC1</td>
<td>Co-activator of a number of nuclear receptors involved in adaptive thermogenesis.</td>
</tr>
<tr>
<td>SCD-1 and -2</td>
<td>Stearoyl CoA desaturase-1 and -2. Enzymes associated with adiposity, i.e., storage and esterification of cholesterol, and responsible for cis saturation of stearoyl and palmitoyl-CoA, converting them to oleate and palmitoleate (monounsaturated fatty acids of triglycerides).</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>Sterol regulatory element binding protein-1c, the hierarchical transcriptional activator of lipogenesis.</td>
</tr>
<tr>
<td>UCP1, -2 and -3</td>
<td>Uncoupling proteins. Mitochondrial proteins that uncouple metabolic fuel oxidation from ATP synthesis, regulating energy expenditure.</td>
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LDL, low-density lipoprotein; CoA, coenzyme A; LCFA, long-chain fatty acid.
tolerance, fatty acid oxidation, and energy expenditure in adipose and skeletal muscle (128).

**PPAR-α**

PPAR-α is known to regulate fatty acid oxidation gene expression in the liver, a function which correlates with the lipid lowering effect of the fibrate class of drugs (12, 41, 90). PPAR-α-specific agonists stimulate mitochondrial β-oxidation in vivo in both liver and muscle via the upregulation of genes such as mCPT1, medium chain acyl CoA dehydrogenase, and MTE1 (113, 116, 168). Furthermore, PPAR-α activation promotes thermogenesis in skeletal muscle via the induction of uncoupling proteins-1, -2, and -3 (20, 79). PPAR-α-null mice exhibit low rates of β-oxidation and lipid accumulation in hepatic and cardiac tissues (36, 96). However, these animals exhibited minimal alteration in the expression of PPAR-α target genes UCP-3, CPT-1, and PDK4 after a period of starvation or exertion primarily due to a functional redundancy with PPAR-δ (116). Furthermore, while metabolism was significantly impaired in liver and cardiac muscle, in contrast, skeletal muscle appeared refractory to the deleterious effects of PPAR-α ablation. Myocardial lipid accumulation in PPAR-α null mice highlights the importance of PPAR-α in mitochondrial fatty acid uptake and oxidation in this tissue (36, 176). PPAR-α plays a critical role in normal cardiac lipid homeostasis and its activity is downregulated during cardiac hypertrophy, resulting in diminished fatty acid oxidation and a return to glucose and lactate as primary energy sources (32, 153). Ultimately, such this metabolic shift results in lipid accumulation and associated lipotoxicity of the myocardium (11).

**PPAR-γ**

PPAR-γ is most abundantly expressed in adipose tissue, intestine and macrophages and studies using rodent cell lines demonstrate its essential role in normal fat cell development (150, 151, 154). Mouse PPAR-γ nulls die in utero due to cardiac and placental defects; however, embryonic transfer of mutant embryos onto wild-type placentas allowed the generation of a single live null animal, which exhibited no detectable adipose tissue (9). Human patients with loss of function mutations in the PPAR-γ gene present with a novel syndrome, including lipodystrophy, insulin resistance, type 2 diabetes, and hypertension (2, 13, 52, 156, 158). PPAR-γ has generated intense interest with the identification that the insulin sensitizing TZDs are high-affinity PPAR-γ ligands (94). Subsequently studies aimed at elucidating the functional mechanisms underlying the PPAR-γ function have demonstrated a pivotal role for PPAR-γ in the regulation of systemic lipid and glucose (52, 145, 150, 151). While many studies have focused on the role of PPAR-γ in adipogenesis and the regulation of adipocyte metabolic function accumulating evidence suggests PPAR-γ plays important roles in the regulation of systemic homeostasis via activities in other key tissues such as liver and skeletal muscle (187). While low levels of detectable PPAR-γ expression in skeletal muscle had led to the long-held assumption that the augmentation of insulin sensitivity by TZDs was facilitated primarily via PPAR-γ activity in adipose tissue, TZDs have been shown to exert their insulin-sensitizing effects in skeletal muscle independently of their activity in adipose tissue (21). While the activity of PPAR-γ in adipose tissue undoubtedly contributes to systemic, and specifically skeletal muscle, metabolic homeostasis via storage of circulating fatty acids and the release of adipokines (many of which act directly on muscle cells), numerous studies offer support for a direct role for PPAR-γ in muscle cells. Studies to date support the contention that augmentation of insulin sensitivity in skeletal muscle by PPAR-γ arises primarily via activation of FFA oxidation (25, 30). Furthermore, PPAR-γ can directly activate components of the insulin signaling pathway in skeletal muscle (3, 73, 83, 174). PPAR-γ signaling activates expression of a number of metabolic genes in isolated muscle tissue and muscle cell lines.
including UCP-2, UCP-3, IRS-1, Δ-6-desaturase, and C/EBPα (53, 101, 167, 179) and promotes GLUT4 translocation (189).

More recently, muscle-specific disruption of PPAR-γ expression in mice resulted in severe insulin resistance with skeletal muscle remaining refractory to the insulin-sensitizing effects of TZDs, despite normal TZD responses in liver and adipose tissue and decreased plasma FFA levels (58). Paradoxically another muscle specific mouse knockout model was similarly shown to be insulin resistant, despite apparently normal skeletal muscle insulin sensitivity. In this study, it was proposed that insulin resistance and increased adiposity arose from a failure of insulin to suppress hepatic glucose production (124).

PPAR-δ

Loss-of-function studies in the mouse have revealed a multitude of biological processes in which PPAR-δ is active during development. PPAR-δ-null embryos die at an early stage due to a placental defect, with the few that survive exhibiting a lower body weight than wild-type littermates, reduced body fat mass, skin defects, and abnormal myelination (112, 134). Paradoxically, this phenotype was absent in an adipocyte specific PPAR-δ null model, suggesting a complex regulation of systemic lipid metabolism, as opposed to specific adipocyte functions (8).

PPAR-δ has generated a great deal of interest as a potential therapeutic target in obesity after the identification of potent synthetic phenoxy-acetic acid ligands. Treatment of insulin-resistant and obese rhesus monkeys and Db/Db mice with PPAR-δ agonists lowered fasting insulin levels, raised serum HDL cholesterol, and lowered fasting triglycerides. Although it was unclear which tissue was the major target for this activity, the classification of PPAR-δ as sensor of dietary triglyceride in native VLDLs released by lipoprotein lipase activity suggested skeletal muscle was a potential target tissue (29, 95, 128). Elevation of HDL cholesterol involved increases in the expression of ABCA1 in macrophages, and skeletal muscle and was accompanied by an induction of ApoA1-specific cholesterol efflux (to an even greater extent than agonists for PPAR-α or PPAR-γ). Given the relative mass of skeletal muscle this observation is entirely consistent with the profound effects of PPAR-δ (103). Muscle-specific expression of activated PPAR-δ in mice leads to an increase in type I muscle fibers. Concordantly, increased activity of enzymes involved in oxidative (not glycolytic) metabolism has been reported. Expression analysis also revealed an induction of the genes involved in increased oxidative metabolism, glucose tolerance, preferential lipid utilization, energy expenditure (i.e., troponin-I slow, cytochrome c, UCP2, UCP3, and CPT1) (103), and increased endurance (181).

Numerous studies demonstrated that GW-501516 treatment and skeletal muscle-specific PPAR-δ overexpression in mice ameliorated diet induced obesity, enhanced metabolic rate, lipid oxidation, reduced intramuscular triglycerides, and increased mitochondria in skeletal muscle. Moreover, anatomical analysis revealed the resistance to increased body weight was largely due to reduced mass of visceral and epidermal fat depots. The drug treatment ameliorated the diet induced 1) hypertrophy in epidermal white adipose, and brown fat, 2) hepatic steatosis, and 3) accumulation of intramuscular lipid droplets (103, 171, 181). Interestingly, these mice have profoundly increased endurance capabilities, and resistance to fatigue relative to their wild-type littermates. Selective PPAR-δ agonists (i.e., GW-501516) are currently in clinical trials for the treatment of dyslipidemia, insulin resistance, and obesity.

LXRs

Recent evidence suggests the Liver X Receptors (LXRα and LXRβ) regulate lipid metabolism in skeletal muscle. Analysis of gene expression changes in both rodent quadriceps muscle and cultured skeletal muscle myotubes treated with the synthetic LXR agonist T0901317 revealed an upregulation of key genes involved in lipid and cholesterol efflux and lipogenesis such as ABCA1, ApoE and SREBP-1c (119). While LXR agonists have proven utility in the treatment of hypercholesterolemia their development into an efficacious treatment for this condition has been impaired by the deleterious side effects consequential to the activation of lipogenesis. Significantly, the induction of the SREBP-1c target genes FAS, and SCD-1 involved in lipogenesis was observed in livers but not skeletal muscle of rodents treated with LXR agonists. This observation is believed to stem from the fact the LXRβ is the predominant isoform expressed in skeletal muscle as opposed to LXRα in liver and prompted speculation that LXRβ specific agonists may retain the desired cholesterol lowering activity while avoiding the undesirable hypertriglyceridaemia. The observation that synthetic LXR agonists increase ABCA1 mediated cholesterol efflux in skeletal muscle cells underscores the potential of this tissue as a target of reverse cholesterol transport and HDL cholesterol regulation (119).
ESTROGEN-RELATED RECEPTOR

Estrogen-related receptors (α, β, and γ) are orphan receptors with close homology to the estrogen receptor that have implicated roles in the regulation of cellular energy metabolism. Accordingly ERRs are abundantly expressed in tissues with a high capacity for fatty acid β-oxidation (47, 65). Homozygous knock-out ERRα−/− mice were found to have a significant reduction in fat mass and a commensurate resistance to high fat induced obesity (102). This phenotype was attributable to the altered expression of genes involved in lipid metabolism and adipocyte development. Somewhat paradoxically, a synthetic ERRα agonist was found to increase energy expenditure and thus antagonize the development of obesity (71).

ERR regulation of cellular metabolism appears to involve interactions with the PGC1 co-regulator. PGC1 has been shown to be a potent co-activator of ERRα with a synthetic inhibitor of ERRα significantly attenuating PGC1α-mediated regulation of oxidative phosphorylation and mitochondrial respiration in skeletal and cardiac muscle cells (115). ERRα has also been found to activate PPAR-α in skeletal muscle cells subsequently regulating an array of genes involved in fatty acid uptake, fatty acid oxidation, and oxidative phosphorylation (64). Recent evidence that an ERRα-dependent PGC1α program mediates the induction of mitofusin-2 helps explain the link between exercise and PGC1α/ERRα-mediated modulation of mitochondrial function (24). Mitofusin-2 is a key regulator of mitochondrial biogenesis and is pivotal in the increase in mitochondrial size, content, and oxidative capacity in response to exercise. In light of established links between insulin resistance and impaired mitochondrial function synthetic modulation of ERR function may prove to be an effective therapeutic target in the treatment of metabolic disease.

RORS AND REV-ERBS

RORs and Rev-erbs belong to the NRIF, and NRID subgroups of nuclear hormone receptors, respectively. These are closely related families of orphan nuclear receptors that operate in opposing manners. Three ROR genes have been identified; RORα encodes four alternatively spliced isoforms (RORα1, RORα2, RORα3, and RORαx), which are predominantly expressed in blood, brain, skeletal muscle, and fat cells. RORβ is expressed specifically in the brain and RORγ is found at high levels in skeletal muscle and thymocytes (67). Two Rev-erb genes have been identified: Rev-erbα and Rev-erbβ. The Rev-erbβ gene encodes two alternatively spliced isoforms, Rev-erbβ1 and Rev-erbβ2. The mRNAs are expressed in most tissues, although very abundant expression is seen in energy-demanding tissues, including skeletal and cardiac muscle, liver, kidney, and brown fat.

Analysis of the spontaneously generated “staggerer” (sg/sg) mutant mouse, which carries a loss of function mutation in the ROR-α gene, has been extremely informative on the role of ROR-α in lipid homeostasis and skeletal muscle function. The most obvious staggerer phenotype is ataxia associated with a cerebellar atrophy, aberrant vascular physiology, hyperresponsive inflammatory response, dyslipidemia, and enhanced susceptibility to atherosclerosis (104). In the context of lipid homeostasis, the sg/sg mice exhibit a dyslipidemia, characterized by lower circulating plasma levels of HDL-C, ApoAI, AII, and CIII and plasma triglycerides. Notably, ApoAI is a major constituent of HDL, whereas ApoCIII is a component of HDL and VLDL that plays a role in regulation of triglyceride levels and lipoprotein lipase activity (146, 178). This observation is supported by cell culture studies demonstrating that ROR-α, and Rev-erb-α activate and repress the expression of ApoAI and CIII in cell culture, respectively (18, 66).

Futhermore, sg/sg mice have been shown to have a reduced muscular strength, a finding supported by earlier work conducted in our laboratory, demonstrating that ROR-α potentiates skeletal muscle myogenesis, whereas the Rev-erbs antagonize muscle cell differentiation in culture (22, 37, 87). Ectopic expression of a dominant negative form of ROR-α in skeletal muscle cells attenuated ROR-dependent gene expression, repressed the endogenous levels of ROR-α and ROR-γ mRNA, and also resulted in attenuated expression of many genes involved in lipogenesis and energy homeostasis, including SREBP-1c, SCID1/2, and FAS, a finding consistent with the lowered TG levels observed in sg/sg mice (88). Furthermore, previous studies have demonstrated that MCPT-1 an established target for PPAR-α in cardiac muscle and PPAR-δ in skeletal muscle cells, is also directly regulated by ROR-α raising the suggesting a potential tissue-specific cross-talk between PPAR-α and ROR-α. Cell culture studies and analysis of the sg/sg mouse highlights the importance of ROR-α in the regulation of lipid homeostasis, and we hypothesize that ROR-α in skeletal muscle programs a cascade of gene expression designed to regulate lipid and energy homeostasis in this tissue (88).

Similarly, mice homozygous (−/−) for the Rev-erb-α null allele have a dyslipidemic phenotype and a 20% lower body weight. Male Rev-erb-α-deficient mice show elevated serum VLDL triglyceride levels and increased serum and liver expression of apocIII mRNA (146). Elevated ApoCIII and VLDL triglycerides in Rev-erb-α−/− is in contrast to reduced ApoCIII and triglycerides observed in ROR-α mutant sg/sg mice and concurs with the negative regulation of ApoCIII by Rev-erb-α. Interestingly, homozygous Rev-erb-α-null mice display a significant shift from fast (type IIA) to slow (type I) myosin heavy chain expression in the slow twitch, mitochondrial rich oxidative fibres which encodes the thick filament of the sarcomere. This is in concordance with the selective expression of Rev-erb-α in type IIB glycolytic and intermediate type IIA oxidative fibers (138). This suggests that this orphan NR regulates muscle fiber type, which will have concomitant effects on insulin sensitivity and aerobic/anaerobic metabolism.

Ectopic expression of a dominant negative mutant Rev-erb-βΔE (lacking the LBD) in skeletal muscle cells attenuates a range of genes involved in fatty acid/lipid absorption including CD36, FABP3, and FABP4 (143). Increased expression of SREBP-1c and SCID1 was also observed and is consistent with elevated TG levels in Rev-erb-α−/− mice. Moreover, a dramatic decrease in the myostatin expression and elevated IL-6 expression further highlights a potential role of this orphan receptor in the regulation of muscle mass, adiposity, and inflammation (143). Modulation of these myokines coupled with observed changes in genes controlling muscle lipid absorption and metabolism suggest that Rev-erbβ activity plays important role in muscle growth and lipid homeostasis (143).
These studies underscore the potential therapeutic utility of ROR-\(\alpha_1\), and Rev-erb agonists, that is further highlighted by genetic and biochemical studies demonstrating the anti-atherogenic and anti-inflammatory properties of ROR-\(\alpha_1\), and proinflammatory properties of Rev-erb\(\alpha\). The mechanism involves the modulation of the NF-\(\kappa B\) signaling pathway and regulation of p65 translocation, and of IkB\(\alpha\) activity. Moreover, we propose that the atherogenic and dyslipidemic phenotype of the \(sg/sg\) mouse is related to aberrant ROR\(\alpha_1\) function in skeletal muscle and surmise that ROR\(\alpha_1\) agonists may have therapeutic utility in the treatment of hypercholesterolemia and atherosclerosis.

**NR4A FAMILY**

The NR4A subgroup of orphan nuclear receptors comprises three members Nur1, Nur77, and NOR-1, which largely function as immediate-early genes, whose expression and activation is regulated in a cell-type specific manner in response to a range of signals, such as mitogenic and apoptotic stimuli. Nur77 is expressed in a range of tissues, including the thymus, muscle, lung, liver, testis, ovary, and prostate, and adrenal, thyroid and pituitary glands (33, 120, 126). NOR-1 is expressed in the pituitary and adrenal glands, cardiac and skeletal muscle, thymus, and kidney (44, 127, 191), whereas Nurr1 is expressed in the central nervous system, thymus, liver, and pituitary gland (89, 117, 118). Crystallographic studies suggest the NR4A subgroup are perhaps true orphan receptors that have little space available for ligand interaction within their respective LBDs, but when expressed, are constitutively active and highly responsive to various signaling pathways. Despite the fact that these genes may not afford agonist (or antagonist) modulation of activity in the classic sense, recent evidence has demonstrated the potential for the identification of small molecule regulators of these orphan nuclear receptors. The finding that the purine anti-metabolite 6-mercaptopurine modulated the activity of NR4A family members in an AF1 dependent manner suggests such molecules may provide a platform for the pharmacological and ultimately therapeutic exploitation of these genes (129, 182).

The Nur77 orphan nuclear receptor has recently been linked to adrenergic control of energy balance/homeostasis. In cultured muscle cells \(\beta\)-adrenergic receptor activation by isoprenaline elicited a rapid, striking, and transient upregulation of Nur77 expression (99, 108). Concordantly, it was found that small interfering RNA-mediated knockdown of Nur77 expression in this cell model resulted in attenuation of gene expression pathways associated with preferential lipid utilization and energy balance, such as UCP-3 and AMPK\(\gamma_3\) (108). Similarly, UCP-3 was found to be downregulated in mouse tibialis muscle after injection and electro-transfer of siNur77 in concordance with decreased expression of UCP-3 in the C2C12 culture model (108). In addition, attenuation of Nur77 expression resulted in decreased expression of genes involved in carbohydrate and lipid homeostasis, such as adiponectin receptor 2, CD36 and GLUT4, which correlated with decreased lipolysis in these cells (108). These data are concordant with the known effects of adrenergic agonists on glucose uptake (123) and the links between UCP3 and AMPK in skeletal muscle cells (170). Increased lipolysis and energy expenditure upon Nur77 activation makes Nur77 a highly attractive potential therapeutic target in the treatment of obesity.

**CHICKEN OVALBUMIN UPSTREAM PROMOTOR-TRANSCRIPTION FACTOR**

The chicken ovalbumin upstream promoter (COUP)-transcription factors (TFs) are most related to the retinoid X receptor/retinoid acid receptor, and are generally considered to be repressors of other nuclear receptors, such as PPARs, retinoic acid, thyroid hormone, and vitamin D receptors (130). COUP-TFs are involved in the regulation of several fundamental biological processes, such as neurogenesis, organogenesis, and metabolic homeostasis. COUP-TFs can modulate the activity of genes that are fundamental in the orchestration of glucose and lipid metabolism, such as bile acid synthesis (CYP7A1-cholesterol 7a-hydroxylase) (31), ketogenesis (mitochondrial HMG-CoA synthase) (56), cholesterol transport (cholesterol ester transfer gene and apolipoprotein AI) (46, 121), fatty-acid \(\beta\)-oxidation (medium-chain acyl CoA dehydrogenase) (35), and glucose homeostasis and insulin sensitivity (10). Moreover, COUP-TF can inhibit preadipocyte differentiation, and its ortholog in *Drosophila* (svp) is required for fat cell differentiation (60). Previous studies (7) in muscle cell cultures have demonstrated a role for COUP-TFs in muscle development showing COUP-TFII inhibits the expression and function of MyoD. Despite COUP-TFII involvement in muscle differentiation and its implication in the regulation of carbohydrate and lipid metabolism in other tissues the specific contribution of COUP-TFs in skeletal muscle metabolic homeostasis remains unexplored.

In conclusion, it is increasingly obvious that orphan and adopted nuclear receptors are key regulators of lipid and carbohydrate homeostasis in skeletal muscle. The efficacy of a number of pharmacological agents that target a number of these orphan receptors such as PPAR-\(\gamma\) (TZD) and PPAR-\(\alpha\) (fibrates) underscores the immense potential of orphan nuclear receptors as therapeutic targets in the treatment of metabolic and cardiovascular disease. The relative mass and metabolic flexibility of skeletal muscle, and the observed metabolic and biochemical perturbations in diabetic and obese individuals, attests to the importance of this tissue in systemic metabolic homeostasis. For this reason, NRs and skeletal muscle are valid targets in the battle to ameliorate metabolic perturbations associated with metabolic syndrome, obesity, and cardiovascular disease. Despite recent advances in our understanding of NR function in metabolic homeostasis, considerable future effort is required to realise the potential of NR agonists in the battle against cardiovascular disease.

Dyslipidemia, insulin resistance, blood glucose levels (and glucose tolerance), diabetes, inflammation, and obesity are all serious risk factors for cardiovascular and metabolic disease. NRs and skeletal muscle are validated targets in the battle to ameliorate metabolic perturbations; however, the real promise and potential of NR agonists in the battle against cardiovascular disease has not yet been realized.

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