PGC-1α regulation by exercise training and its influences on muscle function and insulin sensitivity

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PGC-1α regulation by exercise training and its influences on muscle function and insulin sensitivity. Am J Physiol Endocrinol Metab 299: E145–E161, 2010. First published April 6, 2010; doi:10.1152/ajpendo.00755.2009.—The peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) is a major regulator of exercise-induced phenotypic adaptation and substrate utilization. We provide an overview of 1) the role of PGC-1α in exercise-mediated muscle adaptation and 2) the possible insulin-sensitizing role of PGC-1α. To these ends, the following questions are addressed. 1) How is PGC-1α regulated, 2) what adaptations are indeed dependent on PGC-1α action, 3) is PGC-1α altered in insulin resistance, and 4) are PGC-1α-knockout and -transgenic mice suitable models for examining therapeutic potential of this coactivator? In skeletal muscle, an orchestrated signaling network, including Ca2+-dependent pathways, reactive oxygen species (ROS), nitric oxide (NO), AMP-dependent protein kinase (AMPK), and p38 MAPK, is involved in the control of contractile protein expression, angiogenesis, mitochondrial biogenesis, and other adaptations. However, the p38γ MAPK/PGC-1α regulatory axis has been confirmed to be required for exercise-induced angiogenesis and mitochondrial biogenesis but not for fiber type transformation. With respect to a potential insulin-sensitizing role of PGC-1α, human studies on type 2 diabetes suggest that PGC-1α and its target genes are only modestly downregulated (≤34%). However, studies in PGC-1α-knockout or PGC-1α-transgenic mice have provided unexpected anomalies, which appear to suggest that PGC-1α does not have an insulin-sensitizing role. In contrast, a modest (∼25%) upregulation of PGC-1α, within physiological limits, does improve mitochondrial biogenesis, fatty acid oxidation, and insulin sensitivity in healthy and insulin-resistant skeletal muscle. Taken altogether, there is substantial evidence that the p38γ MAPK-PGC-1α regulatory axis is critical for exercise-induced metabolic adaptations in skeletal muscle, and strategies that upregulate PGC-1α, within physiological limits, have revealed its insulin-sensitizing effects.

SKELETAL MUSCLE IS HIGHLY ADAPTABLE to changes in contractile activity and to changes in the substrate/endocrine milieu. Although the molecular bases for these adaptive responses had remained uncertain for many years, the peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) has revealed itself to be a major regulator of exercise-induced phenotypic adaptation and substrate utilization. In the present paper, we provide an overview and perspective on the mechanisms by which PGC-1α is regulated by endurance exercise training as well as on the influence of PGC-1α on fiber type transformation, angiogenesis, mitochondrial biogenesis, lipid metabolism, and insulin sensitivity.

The first part of this review examines 1) the pathways involved in PGC-1α regulation in response to endurance exercise or increased contractile activity and 2) the role of PGC-1α in endurance exercise-induced adaptations in skeletal muscle. The second half of this review examines whether PGC-1α acts as an insulin-sensitizing agent, since one of the most beneficial and clinically relevant effects of exercise is the improvement in insulin sensitivity in skeletal muscle. To this end, the impact of type 2 diabetes on PGC-1α expression and function as well as the use of in vivo models to examine the role of this coactivator as a therapeutic target are discussed.

PGC-1α Function in Skeletal Muscle

PGC-1α, a transcriptional coactivator identified through its interaction with PPARγ in brown fat cells, was found to be greatly upregulated in brown fat and skeletal muscle in response to cold exposure (159). PGC-1α is now known to be involved in the regulation of thermogenesis, energy metabolism, and other biological processes that are critical in controlling phenotypic characteristics of various organ systems (112, 158, 159, 213). PGC-1α interacts with and coactivates a
PGC-1α, EXERCISE TRAINING, AND INSULIN SENSITIVITY

Review

PGC-1α Regulation in Response to Exercise

Multiple signaling pathways act to regulate PGC-1α in skeletal muscle. Table 1 summarizes the main findings of studies investigating pathways potentially involved in PGC-1α regulation in vivo. These and other reports are discussed in the following paragraphs. Current evidence supports that p38 MAPK signaling is functionally required for endurance exercise-induced PGC-1α regulation. This signaling-transcription regulatory axis may present a node of cross-talk with other signaling pathways reviewed here.

Ca2+/calmodulin-dependent signaling. Rhythmic skeletal muscle contractions activate the Ca2+/calmodulin-dependent serine/threonine protein phosphatase calcineurin (CaN) and Ca2+/calmodulin-dependent protein kinases (CaMKs). CaN has been implicated in the expression of slow-twitch muscle genes through dephosphorylation and activation of the nuclear factor of activated T cells (NFAT) (37, 183). Transgenic mice overexpressing constitutively active CaN in skeletal muscle present an increased number of slow-twitch myofibers and elevated expression of slow-twitch troponin I, myoglobin, GLUT4, pyruvate dehydrogenase kinase-4 (PDK4), mitochondrial enzymes, and PGC-1α (142, 174). These mice have low levels of carbohydrate oxidation rates along with high lipid oxidation rates in the glycolytic extensor digitorum longus (EDL) muscle (123, 174) and present enhanced endurance exercise performance (97). The phenotypic changes observed are similar to the ones reported in muscle-specific PGC-1α-transgenic mice (28, 116, 215) and consistent with the notion that CaN activates MEF2 and induces PGC-1α transcription (72, 227). Genetic and pharmacological interventions that inhibit or abolish CaN/NFAT activity have consistently reduced slow-twitch muscle gene expression (129) and blocked mechanical overload-induced transformation of fibers to type IIa and type I (134). However, animals treated with cyclosporine A, a pharmacological inhibitor of CaN, presented normal upregulation of PGC-1α and mitochondrial enzymes in response to endurance exercise training (57). Therefore, there is strong evidence for an essential functional role of the CaN-NFAT regulatory axis in the maintenance of slow-twitch myofibers and in the promotion of IIb/IIId/x-to-IIa fiber type transformation in response to increased contractile activity. A direct role of this signaling pathway in the upregulation of PGC-1α and metabolic adaptations in skeletal muscle, especially in the context of exercise, is not currently supported.

CaMKs have been shown to act synergistically with CaN to activate slow and oxidative fiber-specific gene expression in cultured myocytes (226). Skeletal muscle expression of CaMKIV has also been suggested to link energy deprivation to increased expression of PGC-1α and mitochondrial biogenesis in skeletal muscle (230). The forced expression of a constitutively active CaMKIV in skeletal muscle in transgenic mice increases expression of the PGC-1α gene and enhances mitochondrial biogenesis along with reduced fatigability of EDL muscle (225). However, genetic disruption of the CaMKIV gene did not prevent exercise-induced upregulation of PGC-1α and IIb-to-IIa fiber type transformation (4). Actually, current evidence does not support the expression of CaMKIV in skeletal muscle (171). However, it is possible that a different CaMK, such as CaMKII or CaMKK or other protein kinases, may act upon substrates targeted by the

growing list of transcription factors and nuclear receptors, including estrogen-related receptor-α (88, 89, 159), thyroid receptor (159), nuclear respiratory factor-1 (NRF-1) (227), NRF-2 (136), and myocyte enhancer factor 2 (MEF2) (72, 132). As a consequence, PGC-1α is involved in the coordinated regulation of nuclear- and mitochondrial-encoded genes required for contractile and metabolic adaptations in skeletal muscle (158, 177, 215). Importantly, PGC-1α expression in skeletal muscles along with improved insulin sensitivity and will lead to enhanced expression of target genes as well as PGC-1α itself.

Skeletal muscle is essential for locomotive and other daily activities, and under insulin-stimulated conditions this tissue removes 70–90% of a glucose load (48, 49, 102). In mammals, skeletal muscles present a mosaic of different fiber types. Based on the expression of the predominant isoform of myosin heavy-chain proteins, myofibers can be classified as type I, IId/x, and IIb (54, 201). Type I fibers are slow-twitch fibers (slow contractile force development) (219) and have an oxidative profile (high mitochondrial content and capillary density) (156, 187). These myofibers also actively express glucose transporter 4 (GLUT4) (60) and present great sensitivity to insulin (79). Type IId/x fibers are fast-twitch fibers (fast contractile force development) (53) but have similar oxidative profiles to the type I fibers, whereas type IIb fibers are fast-twitch fibers with a glycolytic metabolic profile (low mitochondrial content and capillary density) (156). Rodents also have type IIB fibers with an even more fast-twitch, glycolytic phenotype than type IId/x fibers (166, 167). It is important to note that insulin sensitivity can be affected within a given fiber type by several mechanisms. These will be discussed in more detail in subsequent sections.

It is well known that increased contractile activity, such as endurance exercise training, promotes phenotypic adaptations in skeletal muscle toward a more oxidative phenotype. Specifically, endurance exercise training leads to fiber type transformation, mitochondrial biogenesis, angiogenesis, and other adaptive changes in skeletal muscles along with improved insulin sensitivity and metabolic flexibility in both rodents and humans (38, 43, 65, 82, 105, 179, 184, 203). The orchestrated regulation of these processes is of extreme elegance, and current findings suggest that PGC-1α is a key player for several of these adaptations.

Animal and cellular genetic models with altered expression of the PGC-1α gene have provided much of the evidence for the role of PGC-1α in fiber type specificity (116), mitochondrial biogenesis (70), angiogenesis (5), GLUT4 expression (72, 132), and improved exercise performance (28). In addition, PGC-1α mRNA and protein expressions are very responsive to endurance exercise (11, 67, 90, 153, 172, 199). In the face of the growing body of knowledge underscoring the importance of PGC-1α in skeletal muscle phenotype determination, the two critical questions to be addressed in the context of exercise are 1) how PGC-1α is regulated and 2) what adaptations are indeed dependent on PGC-1α action.
Table 1. Evidence for basal and exercise-induced PGC-1α regulation in adult skeletal muscle of rodents in vivo

<table>
<thead>
<tr>
<th>Signaling Pathway/Model</th>
<th>Basal PGC-1α Level*4</th>
<th>Exercise-Induced PGC-1α Upregulation*5</th>
<th>Skeletal Muscle Phenotype*6</th>
<th>In Response to Exercise*6</th>
<th>References</th>
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<tr>
<td>Calcium/Calcineurin</td>
<td>Constitutively active</td>
<td>‡Protein Not tested</td>
<td>‡Slow-twitch fibers, ‡troponin I, ‡myoglobin, ‡GLUT4, ‡PDK4, ‡mitochondrial enzymes, ‡lipid oxidation rates, ‡CHO oxidation rates, ‡exercise performance</td>
<td>Not tested</td>
<td>97, 123, 142, 174</td>
</tr>
<tr>
<td>Pharmacological inhibition (cyclosporine A)</td>
<td>Not tested</td>
<td>Normal induction (mRNA and protein) [5-days of exercise]</td>
<td>‡COX I protein, ‡COX IV protein</td>
<td>‡ERRα, ‡PPARδ, ‡NRF-2, ‡TFAM mRNA and several mitochondrial proteins (except COX I and COX IV; compared with sedentary vehicle-treated controls)</td>
<td>57</td>
</tr>
<tr>
<td>CaMKIV</td>
<td>Constitutively active</td>
<td>‡mRNA Not tested</td>
<td>‡Mitochondrial DNA, ‡slow-twitch fibers, ‡myoglobin protein, ‡CPT I protein</td>
<td>Not tested</td>
<td>225</td>
</tr>
<tr>
<td>CaMKIV-KO</td>
<td>‡Protein Normal induction (mRNA and protein) [nerve stimulation and 4 wk of training]</td>
<td>‡Type I fibers, ‡exercise performance</td>
<td>‡myoglobin, ‡MHCIIa and ‡COXIV proteins, ‡exercise performance</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Reactive nitrogen species</td>
<td>Pharmacological inhibition of NOS (L-NAME)*8</td>
<td>‡Protein Higher induction (mRNA) [~53 min of treadmill running]</td>
<td>‡COX-III and ‡COX IV mRNA, ‡COX activity, ‡COX IV protein, ‡citrate synthase and β-HAD activities</td>
<td>‡TFAM mRNA in EDL (compared with sedentary L-NAME-treated controls)</td>
<td>211</td>
</tr>
<tr>
<td>eNOS and nNOS-KO*8</td>
<td>cDNA and mRNA cNOS-KO, nNOS-KO Normal induction (mRNA) [1 and 9 days, 60 min of treadmill running]</td>
<td>‡NRF-1, ‡NRF-2, ‡TFAM mRNA (all in nNOS-KO), no changes in cNOS-KO</td>
<td>‡NRF2a mRNA, ‡cytochrome c protein (both eNOS and nNOS-KO)</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Reactive oxygen species</td>
<td>Pharmacological inhibition of xanthine oxidase (allopurinol)</td>
<td>Not tested</td>
<td>Reduced induction (protein) [~88 min of treadmill running]</td>
<td>Not tested</td>
<td>101</td>
</tr>
<tr>
<td>AMPK</td>
<td>Pharmacological activation (AICAR, metformin)</td>
<td>‡mRNA and protein Not tested</td>
<td>‡Cytochrome c, ‡GLUT4, ‡activity of citrate synthase, malate dehydrogenase, hexokinase II, and β-HAD, ‡exercise performance</td>
<td>Not tested</td>
<td>29<em>9, 93</em>10, 141, 195, 218</td>
</tr>
<tr>
<td>Mutated AMPKγ3*11 (genetic activation)</td>
<td>‡mRNA Not tested</td>
<td>‡NRF-1, ‡NRF-2, ‡TFAM, ‡cytochrome c, ‡COX IV and ‡citrate synthase mRNAs, ‡mitochondrial area and protein expression of respiratory complexes</td>
<td>Not tested</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Dominant-negative AMPKα2</td>
<td>‡mRNA*12 Not tested</td>
<td>‡Cytochrome c protein, ‡ALAS mRNA</td>
<td>Not tested</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Global AMPKα2 and global AMPKα1-KO*13</td>
<td>‡mRNA Normal induction (mRNA) [90-min run test]</td>
<td>‡FOXO1, ‡hexokinase II, ‡PDK4 mRNA (both α1-KO and α2-KO), ‡GLUT4, ‡CPT1, ‡UCP3 mRNA (α2-KO)</td>
<td>‡FOXO1, ‡hexokinase II, ‡PDK4 mRNA (both α1-KO and α2-KO)</td>
<td>98</td>
<td></td>
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<table>
<thead>
<tr>
<th>Signaling Pathway/Model</th>
<th>Basal PGC-1α Level*</th>
<th>Exercise-Induced PGC-1α Upregulation*</th>
<th>Skeletal Muscle Phenotype*</th>
<th>References</th>
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<tr>
<td>AMPKα2 muscle-specific KO</td>
<td>‡Protein</td>
<td>Normal induction (protein) [6 wk of voluntary wheel running]</td>
<td>‡Citrate synthase activity, ‡%type IIa/IIx fibers, ‡hexokinase II protein</td>
<td>168</td>
</tr>
<tr>
<td>Mutated AMPKγ (muscle-specific activation)</td>
<td>‡Protein</td>
<td>Normal induction (protein) [6 wk of voluntary wheel running]</td>
<td>‡Citrate synthase activity, ‡% type IIa/IIx fibers, ‡hexokinase II protein</td>
<td>168</td>
</tr>
<tr>
<td>p38 MAPK MKK6 muscle-specific overexpression (upstream kinase of p38 MAPKs)</td>
<td>‡mRNA</td>
<td>Reduced induction (mRNA) [nerve stimulation]</td>
<td>‡Cytochrome c, ‡COX IV, ‡capillary density</td>
<td>3</td>
</tr>
<tr>
<td>p38γ MAPK muscle-specific KO</td>
<td></td>
<td></td>
<td>‡Type Ib to IIa/IIx fiber type shift, ‡cytochrome c and COX IV (to 4 wk of running), ‡VEGF (nerve stimulation)</td>
<td>154</td>
</tr>
</tbody>
</table>

PGC-1α, peroxisome proliferator-activated receptor (PPARγ coactivator-1α; GLUT4, glucose transporter 4; PDK4, pyruvate dehydrogenase kinase-4; CHO, carbohydrate; COX I, III, and IV, cytochrome oxidase I, III, and IV; ALAS, α-aminolevulinate synthase; ERRα, estrogen-related receptor-α; NRF-1 and -2, nuclear respiratory factor-1 and -2; TFAM, mitochondrial transcription factor A; CPT I, carnitine palmitoyl transferase I; MHCIIa, myosin heavy chain IIa; EDL, extensor digitorum longus; i-NAMe, N^5^-nitro-L-arginine methyl ester; FOXO1, forkhead box-containing protein O1; NOS, nitric oxide synthase; ENOS and nNOS, endothelial and neuronal isoforms of NOS, respectively; KO, knockout; AICAR, 5-aminimidazole-4-carboxamide-1-β-D-ribofuranoside; β-HAD, β-hydroxyacyl-CoA dehydrogenase; AMPK, AMP-activated protein kinase; MKK6, mitogen-activated protein kinase kinase-6. * Reduction; ‡no change; ‡increase; *1 Refer to text and original references for details. *2 Results for basal levels of PGC-1α and skeletal muscle phenotype under basal conditions are in comparison with untreated wild-type mice or wild-type littermates. *3 Results for exercise-induced PGC-1α upregulation are in comparison with exercised wild-type mice (pharmacological treatments) or exercised wild-type littermates (genetic manipulations) submitted to the specified exercise protocol in square brackets. *4 Only the main findings are listed. *5 Results for skeletal muscle phenotype in response to exercise are in comparison with the same genotype when sedentary and/or not nerve stimulated (unless when specified in brackets). *6 Findings suggest that COX I and COX IV mRNA upregulation was normal, but some sort of toxicity imposed by cyclosporine A prevented these proteins from being upregulated. *7 Only results for the EDL muscle are presented. *8 Cantó et al. (29) demonstrated that PGC-1α is activated by sirtuin 1-mediated deacetylation following PGC-1α phosphorylation by AMPK (via AICAR stimulation). *9 Jäger et al. (93) demonstrated that AMPK (via AICAR stimulation) directly phosphorylates and activates PGC-1α. *10 Trangenic mice expressing the mutant AMPKγ3 R225Q that increases the activity of the AMPK complex, preferentially expressed in glycolytic muscle. *11 At basal levels, PGC-1α expression was not affected, but PGC-1α upregulation in response to β-guanidinopropionic acid (a creatine analog) was prevented. *12 Only results for the quadriceps muscle are presented.
constitutively active form of CaMKIV andmediate skeletal muscle plasticity.

CaMKII, the main CaMK isoform in skeletal muscle (36, 170, 171), is activated (phosphorylated) by endurance exercise (160, 170, 171). Caffeine treatment of adult skeletal muscle ex vivo at levels that increase cytosolic calcium but are insufficient to cause contraction activated CaMKII (224) along with p38 MAPK activation, PGC-1α upregulation, and mitochondrial biogenesis (222). These adaptations appeared to be dependent on both CaMKII and p38 MAPK activities because the CaMKII inhibitor KN93 blocked p38 MAPK activation, and the p38 MAPK inhibitor SB-202190 prevented mitochondrial biogenesis (222). These ex vivo studies provide insights into Ca²⁺-dependent signaling in skeletal muscle by circumventing other cellular events that occur during contraction. However, CaMKII requirement for exercise-induced upregulation of PGC-1α and adaptation remains to be determined. More recently, endurance exercise in humans has been shown to lead to nuclear export of class IIa histone deacetylase (HDAC)4 and HDAC5 along with activation of CaMKII and AMP-activated protein kinase (AMPK) in skeletal muscle (130). HDAC5 normally inhibits MEF2 activity and represses PGC-1α transcription in skeletal muscle (46, 72). These observations suggest a potential pathway for CaMKII regulation of PGC-1α during exercise. Finally, CaMKK has been shown to be a kinase of AMPK (96, 221) responsible for AMPK activation during contractions in skeletal muscle (96). Therefore, CaMKII and CaMKK may function as upstream kinases in exercise-induced regulation of PGC-1α via regulation of p38 MAPK, HDAC5, and AMPK activation. However, these hypotheses would benefit from experiments with loss-of-function approaches in animal models of exercise in vivo.

Reactive nitrogen and oxygen species-dependent signaling. Nitric oxide (NO) production is increased during contractile activity (13, 149, 185). Both endothelial and neuronal isoforms of NO synthase (eNOS and nNOS, respectively) have been shown to be activated by Ca²⁺/calmodulin and AMPK (34, 35, 47, 161, 197). Several studies suggest a role of endogenous NO in AMPK- and Ca²⁺-dependent regulation of PGC-1α, GLUT4, and mitochondrial genes (119, 128). NO is also required for functional overload-induced upregulation of slow myosin heavy chain (MHC; type I/β) (182) through modulation of Akt and glycogen synthase kinase-3β (GSK-3β) activities and amplification of CnA/NFAT-dependent signaling (51). In support of this, eNOS⁻/⁻ muscle cells have reduced type 1 MHC expression (52), and low levels of NO donors promote mitochondrial biogenesis, PGC-1α, and GLUT4 expression in cultured muscle cells (119, 145) through a mechanism involving AMPKα1 activation (Lira VA and Criswell DS, unpublished data). However, neither pharmacological inhibition (by N²-nitro-L-arginine methyl ester) in rats nor genetic deletion of eNOS or nNOS genes in mice prevents endurance exercise-induced PGC-1α mRNA expression in skeletal muscle (210, 211). Altogether, these studies support a role for NO in PGC-1α regulation, mitochondrial biogenesis, and fast-to-slow fiber type transformation but not in the exercise-induced increase in PGC-1α.

There is also ample evidence for increased reactive oxygen species (ROS) in the form of superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH⁻) in skeletal muscle during contraction (41, 101, 126, 127, 149, 162, 186). Peroxynitrite (ONOO⁻), an oxidant formed by a reaction between NO and O₂⁻, is also found in the extracellular fluid of muscle during contraction (149). Most studies point toward H₂O₂ as an important signaling molecule for metabolic adaptations in skeletal muscle. Silveira et al. (186) showed that H₂O₂ produced by contracting skeletal muscle cells was required for PGC-1α upregulation. Irrcher et al. (91) observed that treatment of skeletal muscle cells with H₂O₂ reduced cellular ATP levels, activated AMPK, and increased PGC-1α mRNA, suggesting that H₂O₂ can promote PGC-1α expression through AMPK. Interestingly, increased H₂O₂ production has been proposed as an indirect mechanism by which lactate, a by-product of glycolysis, upregulates PGC-1α (75). Most importantly, ROS has been shown to be functionally important for endurance exercise-induced PGC-1α expression and metabolic adaptation in skeletal muscle. High-dose antioxidant supplementation blocks endurance exercise-induced upregulation of transcriptional regulators, including PGC-1α and genes involved in mitochondrial biogenesis (63, 165), and precludes the beneficial effect of endurance exercise on insulin sensitivity in humans (165). More recently, it has been reported that pharmacological inhibition of xanthine oxidase with allopurinol suppresses the exercise-induced upregulation in PGC-1α in parallel to blunted activation (i.e., phosphorylation) of p38 MAPK in rats. These findings suggest that ROS is involved in p38 MAPK activation and subsequent regulation of PGC-1α expression in response to contraction in vivo (101). Future studies should focus on the precise molecular mechanism by which ROS influences exercise-induced PGC-1α regulation and muscle adaptation.

AMPK signaling. AMPK, a sensor of metabolic stress and energy deprivation, is also activated by contractile activity in skeletal muscle (55, 69, 214, 217, 218, 220). Multiple mechanisms for AMPK-mediated PGC-1α regulation have been postulated. First, exercise-induced AMPK activation is associated with HDAC5 phosphorylation, nuclear export, and derepression of PGC-1α transcription in human skeletal muscle (130, 209). Second, AMPK has been shown to stimulate the cAMP response element-binding protein (202), the upstream stimulatory factor, and possibly GATA4 in transcriptional activation of the PGC-1α gene in cultured cells (72, 92). Third, both phosphorylation and deacetylation of PGC-1α are required for upregulation of the PGC-1α gene and mitochondrial genes (29, 93). Mechanistically, AMPK directly activates PGC-1α by phosphorylating Thr172 and Ser388 (93), which is required for PGC-1α deacetylation by HDAC sirtuin 1 (SIRT1) (29). Interestingly, AMPK activates SIRT1 indirectly by stimulating β-oxidation and subsequently increasing the NAD⁺/NADH ratio (29) and by promoting the expression of nicotinamide phosphoribosyltransferase in NAD⁺ biogenesis (45, 56). Endurance exercise in humans can also stimulate SIRT1 through this latter mechanism (45, 56), and a single bout of exercise induces PGC-1α deacetylation especially in the glycolytic EDL and gastrocnemius muscles, correlating with upregulation of PGC-1α target genes such as PDK4, GLUT4, and carnitine palmitoyl transferase 1b (29).

Pharmacological activation of AMPK by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) increases PGC-1α gene expression and mitochondrial biogenesis in skeletal muscle (195, 218). In addition, AICAR enhances the running ability of mice even in the absence of exercise training (141). Mice expressing a gain-of-function mutation in the regulatory γ3-subunit of
AMPK, expressed predominantly in glycolytic fibers, present increased expression of PGC-1α and other transcription factors associated with mitochondrial biogenesis (58). More recently, Cantó et al. (30) have demonstrated that exercise-induced PGC-1α deacetylation was blunted in AMPKδ3-knockout (KO) mice. On the other hand, animals expressing a dominant-negative form of AMPK (dnAMPKα2) in skeletal muscle present normal PGC-1α mRNA levels but are unable to upregulate PGC-1α, mitochondrial DNA, and enzyme activity in response to β-guanidinopropionic acid, a creatine analog that causes metabolic stress (230). These findings are consistent with the notion that AMPK serves as a metabolic sensor that regulates PGC-1α expression and promotes mitochondrial biogenesis in skeletal muscle. However, a muscle-specific expression of a dominant-negative form of AMPK blunt IIb-to-IIa/IIX fiber type transformation in response to voluntary running without affecting the induction of PGC-1α expression or mitochondrial enzyme activity (168). Furthermore, genetic deletion of functional AMPK isoforms failed to block exercise-induced PGC-1α gene expression in skeletal muscle (98). Therefore, it remains to be fully determined whether endurance exercise-induced skeletal muscle adaptation depends on functional AMPK.

p38 MAPK signaling. Various types of exercise lead to activation of the stress-activated protein kinases, including c-Jun-NH2-terminal kinases, p38 MAPKs, and ERKs in skeletal muscle of humans and rodents (9, 10, 66, 216). Of those, early evidence pointed toward p38 MAPK as the one most likely involved in PGC-1α regulation. p38 MAPK phosphorylates and activates PGC-1α (157) as well as MEF2 (228) and activating transcription factor 2 (ATF2) (31), which are transcription factors that bind to the PGC-1α promoter. In this context, Akimoto et al. (3) demonstrated that p38 MAPK acts through ATF2 phosphorylation to induce PGC-1α expression in skeletal muscle and that muscle-specific overexpression of the upstream kinase (mitogen-activated protein kinase kinase kinase-6) of p38 MAPK resulted in upregulation of PGC-1α and mitochondrial proteins in glycolytic muscles. In a subsequent study, Akimoto et al. (2) observed that PGC-1α transcriptional control in response to motor nerve stimulation required functional interaction of ATF2 with the PGC-1α promoter. These studies provided initial genetic evidence for the role of p38 MAPK in exercise-induced skeletal muscle metabolic phenotype.

Wright et al. (223) observed that p38 MAPK and ATF2 phosphorylation occur in parallel with PGC-1α translocation to the nucleus shortly after an exhaustive bout of swimming exercise. These events coincided with increases in citrate synthase and cytochrome c mRNAs and preceded the upregulation in PGC-1α protein. These observations allude to another potential mechanism of p38 MAPK-mediated activation of PGC-1α, which seems to occur prior to increased PGC-1α transcription. The signals responsible for PGC-1α activation and translocation to the nucleus remain to be determined, but it is likely that phosphorylation and/or deacetylation of PGC-1α is required for this process.

More recently, Pogozelski et al. (154) demonstrated that p38α MAPK, but not p38β MAPK or p38δ MAPK, is required for PGC-1α upregulation in mitochondrial biogenesis and angiogenesis in response to voluntary wheel running and nerve stimulation in mice. None of the p38 MAPK isoforms was required for endurance exercise-induced IIb-to-IIa fiber type transformation in these mice. Of note, a parallel study employing muscle-specific genetic disruption of the PGC-1α gene (61) showed that these mice had the same phenotype as the p38γ MAPK muscle-specific KO mice with impaired metabolic adaptations but normal contractile adaptation to exercise (see below for details). These findings provide functional confirmation for the p38γ MAPK-PGC-1α regulatory axis in exercise-induced skeletal muscle adaptation (Fig. 1) and genetically separate the metabolic adaptation from contractile adaptation in a physiological model of exercise in mice.

**PGC-1α and Exercise-Induced Adaptations**

Animal and cellular genetic models provide much of the evidence for a functional role of PGC-1α in fiber type specification (72, 116), GLUT4 (132), PDK4 (215), and mitochondrial protein expression (72, 109, 111) as well as angiogenesis (5) in skeletal muscle. However, few studies have examined whether PGC-1α is indeed required for exercise-induced adaptations and what aspects of the adaptive responses, if not all, are affected by the absence of functional PGC-1α in skeletal muscle. Leick et al. (110) employed a mouse model of global gene disruption of the PGC-1α gene (PGC-1α-KO) and showed that PGC-1α-KO mice had increased in hexokinase II, aminolevulinyl synthase 1, and cytochrome oxidase (COX) I protein expression in response to endurance exercise despite the fact that these mice had reduced basal level expression of these metabolic enzymes. They concluded that PGC-1α is not mandatory for exercise- and training-induced adaptive gene responses. Adhihetty et al. (1) extended these findings, confirming that PGC-1α-KO mice have normal voluntary running activity and reduced basal level mitochondrial respiratory function, which can be corrected by endurance exercise training. However, other reports document that PGC-1α-KO mice suffer from hyperactivity due to lesions in the central nervous system, and that these animals have circadian rhythm abnormalities, and present AMPK activation in resting skeletal muscle (70, 117, 121). Therefore, an approach with skeletal muscle-spec-
specific gene disruption was then desirable to minimize potential confounding factors of the global PGC-1α-KO models.

Recently, muscle-specific PGC-1α-KO (PGC-1α-MKO) mice were generated (70, 71), and these animals presented reduced locomotive activity, maximal exercise tolerance, and impaired muscle function, consistent with reduced oxidative capacity in skeletal muscles. Despite the fact that PGC-1α-MKO mice exhibited normal voluntary running activity and normal IIb-to-IIa fiber type transformation in skeletal muscles that are recruited during exercise, endurance exercise-induced expression of mitochondrial enzymes (cytochrome c and COX IV) and increases in platelet endothelial cell adhesion molecule-1-positive endothelial cells were significantly attenuated (61). These findings from PGC-1α-MKO underscore the importance of PGC-1α in mitochondrial biogenesis and angiogenesis, but not fiber type transformation, in response to endurance exercise training.

Exercise, Insulin Sensitivity, and PGC-1

For more than two decades, a number of studies have demonstrated the benefits of exercise and muscle contraction on improving insulin sensitivity in rodents and in humans (24, 50, 77, 78, 143, 163, 181, 205, 212, 229). More recently, a key prospective study has shown conclusively that a modest program of moderate exercise (“brisk walking”, 150 min/wk) along with a modest weight loss (−7%, or −5.6 kg) over ~3 yr markedly reduced the incidence of type 2 diabetes (104). With the discovery of PGC-1α (227) and its exercise-mimetic effects on muscle metabolism (cf. Ref. 73) and the subsequent discovery of PGC-1β (114), a PGC-1α homolog, considerable interest arose as to whether these coactivators had a role in skeletal muscle insulin sensitivity (73, 113).

PGC-1α and PGC-1β share a similar tissue distribution and are expressed in tissues characterized with a high mitochondrial content, including skeletal muscle (108, 114, 131). There seems to be considerable redundancy of function between PGC-1α and PGC-1β. For example, PGC-1β, like PGC-1α, activates genes involved with fatty acid oxidation and mitochondrial biogenesis (7, 100, 114, 115, 131, 176, 190), and in muscle cells PGC-1α and -β increased the mRNAs of GLUT4 (132, 139). However, in lean and insulin-resistant (obese) human muscle, PGC-1α and -β protein expression were not correlated, and only PGC-1α protein correlated with rates of palmitate oxidation and citrate synthase activity, an index of mitochondrial content (85). With exercise training only PGC-1α mRNA was increased (105, 131, 140), whereas PGC-1β mRNA was not altered (105, 131) or was reduced (140). With respect to changes in skeletal muscle with aging (118) and type 2 diabetes (148), some have observed reductions in both PGC-1α and -β mRNA (118, 148), whereas others have observed only a reduction in skeletal muscle PGC-1β mRNA in senescence-prone animals (169) and reductions in PGC-1α mRNA, but not PGC-1β mRNA, in type 2 diabetes (204). Collectively, these observations begin to suggest that although PGC-1β and PGC-1α may share similar functions, they also appear to have some different roles. However, only skeletal muscle PGC-1α expression is consistently down-regulated in type 2 diabetes. Indeed, work in cell lines has shown that PGC-1α induces exercise-mimetic effects, increasing mitochondrial biogenesis, fatty acid oxidation, GLUT4 expression, and insulin signaling (132), leading to speculations (73, 105, 113) that the molecular basis for the exercise-mediated improvement in glucose transport is attributable, in part, to PGC-1α.

Lipids, Glucose Transport, and PGC-1α

There is a strong association between skeletal muscle insulin resistance and circulating plasma fatty acid concentrations (18–20) and intramuscular triacylglycerol accumulation (94, 107, 147, 192). However, in athletes intramuscular triacylglycerol depots are also increased, yet they are insulin sensitive (208), possibly as a result of an enhanced capacity for fatty acid oxidation (22, 25, 64, 87). This anomaly between increased intramuscular triacylglycerol depots and improved insulin sensitivity was termed the “athlete paradox.” However, recent work indicates that this is much less of a paradox than was once thought. It is the more soluble, bioactive lipid metabolites such as ceramide, diacylglycerol, and long-chain fatty acyl-CoAs, not intramuscular triacylglycerol storage depots (22, 81, 120), that impair postreceptor insulin signaling, thereby interfering with GLUT4 translocation and glucose transport (32, 33, 80, 193, 194). The increase in intramuscular triacylglycerol depots, whether in athletes or in insulin-resistant muscles, appears to be an attempt to limit intramuscular muscle lipotoxicity, since intracellular triacylglycerol provides a cytoprotective role in lipid-overload states (120, 122).

In insulin resistance and type 2 diabetes, intramuscular lipid accumulation does not result from a reduced intrinsic capacity for fatty acid oxidation by mitochondria (83, 84, 86, 135, 207), as had been widely believed (103, 152). Instead, intramuscular lipids accumulation stems, in part, from a FAT/CD36-mediated increase in fatty acid uptake (23, 62, 83, 124). The excess fatty acids taken up cannot be oxidized away (23, 83, 106), because there is an insufficient compensatory increase in mitochondrial mass (68, 83) or an insufficient capacity for fatty acid oxidation (106), or alternatively, the mitochondrial mass may be reduced (86, 138, 152). These observations may be the reason that an improvement in fatty acid oxidation (e.g., with exercise), which allows excess fatty acids to be oxidized away, has so often been associated with improved insulin sensitivity (26, 87, 150). Indeed, exercise training improves fatty acid oxidation not only during exercise (151, 191, 196) but also at rest (65, 188), since under basal conditions there are training-induced increases in mitochondrial carnitine palmitoyl transferase I activity, resulting in an increased rate of fatty acid oxidation by isolated mitochondria under basal conditions (27). A comparable effect could well be provoked by PGC-1α since, similarly to exercise training, PGC-1α induces mitochondrial biogenesis and fatty acid oxidation (cf. Ref. 73 and 113), thereby integrating the upregulation in fatty acid oxidation with improvements in insulin sensitivity.

Whether fatty acids regulate PGC-1α expression in muscle has been controversial. Palmitate (0.75 mM), but not oleate, reduced PGC-1α mRNA by 66% in C2C12 muscle cells and appeared to involve MAPK-ERK 1/2 and nuclear factor-κB activation (44). Similarly, in humans, prolonged (48 h) lipid infusion increased circulating fatty acids 3.6-fold (0.48–1.73 mM) and reduced muscle PGC-1α mRNA by 30% (164). In contrast, prolonged fasting for 24 or 48 h, which increased circulating fatty acids 1.6- and 2.1-fold, respectively, did not alter muscle PGC-1α mRNA or protein (206). Contradictory results have also been observed when circulating fatty acids...
were reduced by acipimox (12) or nicotinic acid (214), since these treatments reduced (12) or increased PGC-1α mRNA (214), respectively. Taken altogether, it is difficult to conclude that fatty acids influence the expression of PGC-1α.

Type 2 Diabetes and PGC-1α

Given 1) the linkage between the dysregulation in fatty acid metabolism and impairment in insulin sensitivity and 2) that PGC-1α induces mitochondrial biogenesis (cf. Ref. 73) and the expression of key enzymes involved in skeletal muscle fatty acid utilization (for review, see Refs. 16, 73, and 113), it was of considerable interest to examine whether PGC-1α expression was altered in type 2 diabetes.

Oxidative phosphorylation genes and PGC-1α are reduced in type 2 diabetes. Based on multiple statistical comparisons of high-density oligonucleotide arrays and quantitative real-time PCR, it has been concluded that differences in muscle gene expression between type 2 diabetes patients and healthy humans are quite modest (i.e., ≤34% reduction in type 2 diabetes), involving only a small number of selected genes in fatty acid transport and metabolism and oxidative phosphorylation (OXPHOS) (137, 148). Concurrently, PGC-1α mRNA expression was also reduced modestly (20–36%) in muscles of type 2 diabetes patients (137, 148) as well as in asymptomatic individuals with a family history of type 2 diabetes (−34%) (148). Reductions in PGC-1α have also been observed when fasting insulin concentrations are increased and with an increased body mass index in diabetes-prone humans (8, 155).

Thus, there appears to be a potential role for PGC-1α in the etiology of insulin resistance in human skeletal muscle. This linkage is strengthened by the fact that 1) PGC-1α is also downregulated in selected animal models of insulin resistance and type 2 diabetes (99, 189), 2) PGC-1α is known to regulate genes involved in lipid metabolism and OXPHOS (cf. Ref. 16, 73, and 113), and 3) PGC-1α transfection in a mouse skeletal muscle cell line upregulated almost precisely a subset of OXPHOS genes that were downregulated in muscles of type 2 diabetes patients (137). Importantly, in type 2 diabetes, the reductions in the expression of skeletal muscle PGC-1α and its associated OXPHOS genes were quite modest (≤34%) (137, 148). This suggests that a modest increase in PGC-1α may be sufficient to improve insulin sensitivity.

Does the reduction in PGC-1α in type 2 diabetes reflect reduced muscle activity? To date, little recognition has been given to the fact that reductions in PGC-1α and its associated OXPHOS genes could be spuriously associated with type 2 diabetes. Individuals with type 2 diabetes have low levels of physical activity (76, 180), and PGC-1α and OXPHOS genes are downregulated, when physical activity is reduced (59, 172, 204). Indeed, there is a highly significant positive relationship between maximal aerobic capacity (VO2max, an index of low physical activity) and the expression of OXPHOS genes among individuals with type 2 diabetes and those with normal and impaired glucose tolerance (137). Close inspection of these data revealed that individuals with type 2 diabetes had a 25% lower VO2max and were clustered in the lowest third of the correlational analysis (OXPHOS vs. VO2max). Others have shown that PGC-1α mRNA and its associated genes are positively correlated with VO2max (59) and that “diabetes-like” reductions in PGC-1α mRNA are induced by muscle inactivity (204). Thus, it is unclear to what extent the observed reductions in skeletal muscle PGC-1α mRNA and its associated OXPHOS genes in type 2 diabetes (137, 148) are 1) the result of type 2 diabetes per se or 2) due to reduced physical activity patterns in individuals with type 2 diabetes.

Effects of PGC-1α on Glucose Utilization and Lipid Metabolism

Experimental studies to examine the role of PGC-1α in insulin sensitivity have been conducted in cell lines and in PGC-1α-KO and -overexpressing mice. In general, these studies, except for a report in muscle cells (132), have yielded unexpected results (Table 2).

Cell lines. In L6 and C2C12 myotubes, but not in the COS-1 kidney epithelial cell line, PGC-1α overexpression increased insulin-stimulated glucose transport largely via an increase in GLUT4 expression through a PGC-1α and MEF2C interaction at the promoter of GLUT4 (132). This study forged the notion that PGC-1α can have insulin-sensitizing effects.

PGC-1α-null mice. Two models of PGC-1α-null mice have been created (111, 117). In one model the expression of selected mitochondrial genes was reduced by 20–50% (117), but surprisingly, the animals were hyperactive, possibly accounting for their unexpected lean phenotype (6, 117). Other PGC-1α-null mice were not hyperactive (111) and exhibited most of the expected metabolic changes, including 1) abnormally increased body fat (only in female mice), 2) diminished mitochondrial number and respiratory capacity of soleus muscle, 3) reduced muscle performance and exercise capacity, and 4) short-term, starvation-induced hepatic steatosis (111). Yet, contrary to the expectation that the PGC-1α-null mice were susceptible to insulin resistance, both models were resistant to diet-induced obesity (111, 117).

Muscle-specific PGC-1α-null mice. Muscle-specific PGC-1α-null mice exhibited the expected reductions in selected mitochondrial genes (73). Moreover, the animals were hypoadipic and exercise intolerant (70, 73). PGC-1α ablation provoked a reduction in GLUT4 mRNA, yet surprisingly, peripheral (i.e., muscle) insulin sensitivity and Akt activity were increased (71).

PGC-1α-overexpressing mice. In PGC-1α-transgenic mice there was the expected increase in selected mitochondrial genes, a robust increase in mitochondrial proliferation in muscle (116) and heart (109, 173), and a resistance to muscle fatigue (116). However, anomalies were also reported, including the loss of cardiac sarcomeric structure due to excess mitochondria (109, 173). Moreover, whereas a lean phenotype was expected, these animals were severely obese (109). In addition, neither the expected increase in GLUT4 expression nor the expected increase in insulin-stimulated glucose transport was observed. Instead, a >10-fold increase in PGC-1α mRNA unexpectedly reduced GLUT4 mRNA and whole body insulin sensitivity (133).

Muscle-specific PGC-1α overexpression. Limiting PGC-1α overexpression to skeletal muscle also induced deleterious effects. These muscle-specific PGC-1α-overexpressing mice were not protected from diet-induced insulin resistance as had been expected. Instead, these mice developed insulin resistance when provided with a high-fat diet (39). This was attributed to reduced peripheral (muscle) insulin sensitivity (−25%) and...
Table 2. *Comparison of glucose and lipid metabolism responses in different models of PGC-1α ablation and overexpression*

<table>
<thead>
<tr>
<th>Metabolic Parameter</th>
<th>PGC-1α-Null</th>
<th>Large (600–2,000%) PGC-1α Overexpression</th>
<th>Modest (25%) Muscle-Specific PGC-1α Overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole body, Refs. 111, 117**</td>
<td>Muscle specific, Ref. 71**</td>
<td>Healthy muscle (25%), Ref. 15</td>
</tr>
<tr>
<td></td>
<td>Muscle specific, Ref. 109, 133</td>
<td>Muscle specific (600%), Ref. 39</td>
<td>Zucker obese muscle (25%), Ref. 14</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>†Improved*</td>
<td>No difference</td>
<td>†Reduced</td>
</tr>
<tr>
<td></td>
<td>†Improved*</td>
<td>†Intolerant*</td>
<td>†Reduced</td>
</tr>
<tr>
<td>Glucose tolerance</td>
<td>Improved</td>
<td>Improved</td>
<td>†Reduced</td>
</tr>
<tr>
<td>Muscle glucose transport</td>
<td>†Reduced</td>
<td>†Reduced</td>
<td></td>
</tr>
<tr>
<td>Protein expression</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Muscle GLUT4</td>
<td>√/Reduced</td>
<td>†Reduced</td>
<td>√Increased</td>
</tr>
<tr>
<td>Muscle IRS-1</td>
<td></td>
<td></td>
<td>√Increased</td>
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<tr>
<td>Muscle PI 3-kinase</td>
<td></td>
<td></td>
<td>No difference</td>
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<tr>
<td>Muscle Akt2 protein</td>
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<td></td>
<td>No difference</td>
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<tr>
<td>Muscle AS160 protein</td>
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<td>No difference</td>
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<tr>
<td>Insulin signaling</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Muscle p-IRS-1</td>
<td>No difference</td>
<td>Reduced</td>
<td>Increased</td>
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<tr>
<td>Muscle p-Akt or activity</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
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<tr>
<td>Muscle p-AS160</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
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<tr>
<td>Lipid metabolism</td>
<td></td>
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<tr>
<td>Fatty acid oxidation</td>
<td>†No difference</td>
<td>Increased</td>
<td>√/Increased</td>
</tr>
<tr>
<td>Intramuscular lipids</td>
<td>†No difference</td>
<td>Increased</td>
<td>√/Decreased</td>
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<tr>
<td>Lipid metabolism gene</td>
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<tr>
<td>AMPKα2 protein</td>
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<td>Increased</td>
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<td>p-AMPKα2 or activity</td>
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<td>No difference</td>
<td>√/Large increase†</td>
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<tr>
<td>CD36 mRNA/protein</td>
<td></td>
<td></td>
<td>√/Increased</td>
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<tr>
<td>CPT I mRNA, protein or activity</td>
<td></td>
<td></td>
<td>√/Increased†</td>
</tr>
<tr>
<td>ACC2 protein</td>
<td></td>
<td></td>
<td>√/Increased†</td>
</tr>
<tr>
<td>mtGPAT mRNA</td>
<td></td>
<td></td>
<td>√/Increased†</td>
</tr>
<tr>
<td>DGAT1 mRNA</td>
<td></td>
<td></td>
<td>√/Increased†</td>
</tr>
<tr>
<td>Diet-induced obesity</td>
<td>†No (Ref. 117)</td>
<td>†No</td>
<td>†Severely (Ref. 109)</td>
</tr>
<tr>
<td></td>
<td>√/Yes (Ref. 111)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass</td>
<td>†Reduced</td>
<td>No difference</td>
<td>No difference</td>
</tr>
</tbody>
</table>

IRS-1, insulin receptor substrate-1; PI, phosphatidylinositol; ACC2, acetyl-CoA carboxylase-2; mtGPAT, mitochondrial glycerol-3-phosphate acyltransferase-1; DGAT1, diacylglycerol acyltransferase 1. Comparisons are relative to wild-type mice or muscles not transfected with PGC-1α. †Response observed is the expected response in the PGC-1α model. ‡Response observed is contrary to the expected response in the PGC-1α model. *PGC-1α-null mice in Ref. 117 are hyperactive and exhibit neurological disorders characteristic of Huntington’s disease. **PGC-1α muscle-specific null mice in Ref. 71 are hypoactive. ***Results are observed primarily in female mice. *Observations after a period of high-fat feeding; †fatty acid oxidation was improved in subsarcolemma mitochondria only, not in intramyofibrillar mitochondria; †mRNA; †protein; †activity.
impaired muscle insulin-stimulated glucose transport, insulin receptor substrate-1 (IRS-1) phosphorylation, and Akt2 activity (39). Concurrently, there were increases in selected genes regulating lipid metabolism as well as in functional measurements of muscle fatty acid metabolism, including fatty acid oxidation (+30%), and 200–300% increases in intramuscular lipids (triacylglycerol, diacylglycerol, lysophosphatidic acid, long-chain fatty acyl-CoA) (39), which have been implicated in interfering with insulin signaling.

Are PGC-1α-Null and -Transgenic Animals Suitable for Revealing PGC-1α Insulin-Sensitizing Effects In Vivo?

Based on the foregoing studies with PGC-1α ablation or overexpression, it appears that 1) PGC-1α does not contribute to regulating insulin sensitivity. However, this contrasts with 2) experiments in muscle cells in which GLUT4 and insulin signaling were increased by PGC-1α (132) and 3) gene array data in human type 2 diabetes (137, 148) in which reductions in muscle PGC-1α expression have been interpreted as evidence that PGC-1α has a role in regulating insulin sensitivity. Taken altogether these observations may suggest that 1) the associative evidence between reductions in PGC-1α and type 2 diabetes in humans cannot be interpreted causally or, 2) alternatively, the use of genetic approaches, rather than physiological/metabolic considerations, for examining the insulin-sensitizing role of PGC-1α in vivo may have inadvertently obscured the positive metabolic effects of this coactivator.

In recent years, genetically altered animal models have proved to be extremely useful to examine the function of specific genes, particularly those with a specific restrictive function dedicated to a single process. However, genes involved in broader metabolic functions and actions in multiple tissues have, not unexpectedly, led to unanticipated results (e.g., various murine PPARγ models) (for review, see Ref. 42). Hence, the appropriateness of PGC-1α-null or -overexpressing animal models to examine insulin sensitivity (39, 71, 109, 111, 117, 133) may be questioned, given the multiple metabolic effects induced by this coactivator among and within tissues.

Viewed from a physiological perspective, it is not entirely surprising that completely ablating or massively overexpressing PGC-1α has consistently provided unexpected results with respect to establishing a role for PGC-1α in improving insulin sensitivity in vivo (Table 2). PGC-1α overexpression by 600–2,000% (39, 133) is physiologically unrealistic, exceeding by five- to 10-fold the changes that can be expected to be induced in muscle by physiological stimuli such as cold exposure (146), acute exercise (200, 223), or exercise training (3, 189, 198). Just as studies with PPARγ animal models have shown that too much of a good thing causes harm (42), this may also apply to PGC-1α. Indeed, there is now ample evidence that a massive PGC-1α overexpression (600–2,000%) (39, 109, 116, 133, 173) can have unexpected pathophysiological consequences.

An Alternative Approach: PGC-1α Overexpression Within Physiological Limits

Since traditional genetic approaches, including insulin sensitivity, may not be suitable for examining the role of PGC-1α in muscle metabolism, another strategy has evolved. This approach aimed to 1) upregulate PGC-1α modestly and 2) mimic physiologically realistic changes in PGC-1α protein expression (i.e., 20–150%) (3, 125, 146, 172, 189, 198, 200, 223).

Overexpressing PGC-1α Within a Physiological Range Improves Lipid Metabolism and Glucose Transport in Healthy and Insulin-Resistant Muscle

When PGC-1α was modestly overexpressed (~25%) in rat muscle (15), using an electrotransfection approach (17, 40, 144), PGC-1α mRNA (28%) and protein (24%) were modestly upregulated (15). Typically, only 30–40% of the muscle fibers are electrotransfected (15, 17, 26, 40, 144, 178). Hence, PGC-1α upregulation in the transfected muscle fibers was 60–75%, which is within a physiological range. This level of PGC-1α overexpression is accompanied by a visible increase in the capacity for oxidative metabolism, namely, the redder coloration of PGC-1α-transfected muscle [see Fig. 5 in Benton et al. (15)].

The modest or physiological overexpression of PGC-1α induced a number of positive metabolic changes in healthy animals, including improvements in insulin-stimulated glucose transport, mitochondrial fatty acid oxidation (subsarcolemmal mitochondria only, not intermyofibrillar mitochondria), and mitochondrial density (15). These changes were accompanied by PGC-1α-induced upregulation of some proteins (FAT/CD36, GLUT4, AMPKα2), but not others (plasma membrane-associated fatty acid binding protein, IRS-1, phosphatidylinositol 3-kinase, Akt2, hormone-sensitive lipase), improvements in insulin-stimulated phosphorylation of Akt2, and increased expression of COX IV and FAT/CD36 in isolated mitochondria (15). Similarly,
PGC-1α upregulation (20–27%) in insulin-resistant muscles (obese Zucker rats) (14) improved insulin-stimulated glucose transport, reduced intramuscular lipids (triaclylglycerol, diacylglycerol, ceramide), increased mitochondrial fatty acid oxidation (subsarcolemmal mitochondria only, not intermyofibrillar mitochondria), and increased expression of FAT/CD36 and GLUT4, but not Akt2 or AS160, although their insulin-stimulated phosphorylation was increased (14). Modest PGC-1α overexpression also protected animals from diet-induced insulin resistance (Bonen A, unpublished data).

For all parameters examined, whether in healthy or insulin-resistant animals, the PGC-1α-induced alterations in selected parameters ranged from 15 to 85%, with most being in the range of 20 to 50%. Although these responses are not large when compared against those that can be induced in transgenic animals, these increases are metabolically meaningful, because in skeletal muscle of type 2 diabetic patients there are only l) small reductions in PGC-1α (≤36%) (137, 148) and 2) a small number of selected genes involved in fatty acid transport and metabolism and in OXPHOS (≤34%) (137, 148). Moreover, unlike with massive PGC-1α overexpression (Table 2), the changes with modest PGC-1α overexpression in all of the parameters collectively provide a metabolically coordinated response in lipid and carbohydrate metabolism such that insulin sensitivity was improved (Table 2) (14, 15). Thus, experiments based on physiological and metabolic rationales for controlling the overexpression of PGC-1α have demonstrated the insulin-sensitizing effects of this coactivator.

Comparing PGC-1α Overexpression With Improvements in Glucose Utilization

The effects of PGC-1α overexpression on insulin sensitivity in vivo can be strikingly different. When PGC-1α overexpression is within a physiological range (<100%), improvements in insulin sensitivity are observed (Table 2 and Fig. 2). However, when muscle-specific PGC-1α overexpression is far beyond normal physiological limits, insulin-stimulated glucose disposal is impaired (Fig. 2). Although this relationship between PGC-1α overexpression and insulin-stimulated glucose utilization (Fig. 2) does not reflect the complexity of PGC-1α’s effects or its mechanisms of action, this relationship (Fig. 2) does provide insight as to when PGC-1α upregulation is likely to be most effective.

Speculation Concerning Disparate Effects of PGC-1α-Mediated Glucose Utilization

Disparate changes in PGC-1α-mediated glucose utilization may be linked, in part, to the differential PGC-1α-induced upregulation of proteins involved with lipid metabolism. Key among these is the fatty acid transporter FAT/CD36. This PGC-1α-inducible gene (15, 39) has been linked to insulin resistance (21, 23, 62, 83), since it promotes excess uptake of fatty acids beyond the capacity to oxidize them (21, 23, 62, 83). Hence, intramuscular lipids accumulate. Because of this work, we (15, 16) had already suggested several years ago that it may be critically important to limit the PGC-1α-induced overexpression of FAT/CD36, even in the face of PGC-1α-induced improvements in fatty acid oxidation. Indeed, an excessively large PGC-1α overexpression (+600%) induced a large up-regulation of FAT/CD36 (+300%) (39), which not unexpectedly increased intramuscular lipids by 200–300% and therefore likely reduced insulin-stimulated glucose transport and activation of insulin-signaling proteins (39). In contrast, modest PGC-1α overexpression only moderately increased FAT/CD36 (20–35%) in muscle (Table 2) (14, 15), an increase that may be expected to improve fatty acid oxidation while not increasing intramuscular lipids (144). Thus, it seems that if PGC-1α is to be a therapeutic target, its upregulation in muscle must be modest, since this limits PGC-1α-induced FAT/CD36 upregulation, thereby preventing excess fatty acid uptake and intramuscular lipid accumulation.

Summary

Multiple signaling transduction pathways, including Ca²⁺-dependent signaling, ROS, NO, AMPK, and p38 MAPK, have been linked to the regulation of PGC-1α expression and function in skeletal muscle plasticity. Gene deletion studies have demonstrated that the CaN/NFAT signaling controls fiber type transformation, whereas p38 MAPK/PGC-1α signaling controls mitochondrial biogenesis and angiogenesis in response to endurance exercise in skeletal muscle. The insulin-sensitizing effects of exercise may be mediated, in part, via the exercise-induced upregulation of PGC-1α, since its modest overexpression within an exercise-like range is sufficient to improve both lipid metabolism and insulin sensitivity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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