Epinephrine and AICAR-induced PGC-1α mRNA expression is intact in skeletal muscle from rats fed a high-fat diet

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Frier BC, Wan Z, Williams DB, Stefanson AL, Wright DC. Epinephrine and AICAR-induced PGC-1α mRNA expression is intact in skeletal muscle from rats fed a high-fat diet. Am J Physiol Cell Physiol 302: C1772–C1779, 2012. First published April 11, 2012; doi:10.1152/ajpcell.00410.2011.—Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a master regulator of mitochondrial biogenesis and is controlled, at least in part, through AMP-activated protein kinase and p38-dependent pathways. There is evidence demonstrating that activation of these kinases and induction of PGC-1α in skeletal muscle are regulated by catecholamines. The purpose of the present study was to determine if consumption of a high-fat diet (HFD) impairs epinephrine and 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (AICAR) signaling and induction of PGC-1α in rat skeletal muscle. Male Wistar rats were fed chow or a HFD for 6 wk and then given a weight-adjusted bolus injection of epinephrine (20, 10, or 5 μg/100 g body wt sc) or saline, and triceps muscles were harvested 30 min (signaling) or 2 and 4 h (gene expression) postinjection. Despite blunted increases in p38 phosphorylation, the ability of epinephrine to induce PGC-1α was intact in skeletal muscle from HFD-fed rats and was associated with normal increases in activation of PKA and phosphorylation of cAMP response element-binding protein, reputed mediators of PGC-1α expression. The attenuated epinephrine-mediated increase in p38 phosphorylation was independent of increases in MAPK phosphatase 1. At 2 h following AICAR treatment (0.5 g/kg body wt sc), AMP-activated protein kinase and acetyl-CoA carboxylase phosphorylation were similar in skeletal muscle from chow- and HFD-fed rats. Surprisingly, AICAR-induced increases in PGC-1α mRNA levels were greater in skeletal muscle from HFD-fed rats. Our results demonstrate that the ability of epinephrine and AICAR to induce PGC-1α remains intact in skeletal muscle from HFD-fed rats. These results question the existence of reduced β-adrenergic responsiveness in diet-induced obesity and demonstrate that increases in p38 phosphorylation are not required for induction of PGC-1α in muscle from obese rats.

5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside; p38; peroxisome proliferator-activated receptor-γ coactivator 1α

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ COACTIVATOR 1α (PGC-1α) is a transcriptional coactivator that binds to and coactivates transcription factors that are involved in the regulation of mitochondrial and nuclear encoded mitochondrial enzymes (24, 25, 40). Gain- and loss-of-function studies have eloquently demonstrated the central role of PGC-1α in the control of skeletal muscle mitochondrial biogenesis. For instance, the overexpression of PGC-1α in cultured muscle cells (40) or rodent skeletal muscle (3) induces mitochondrial biogenesis, whereas the muscle-specific deletion of this gene attenuates exercise-induced increases in mitochondrial enzymes (14).

Given the key role of PGC-1α in regulating skeletal muscle mitochondrial content, much work has centered on elucidating the mechanisms controlling the expression of this molecule. There is good evidence that 5′-AMP-activated protein kinase (AMPK) (20, 32, 33) is involved in this process. For example, AMPK agonists such as 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (AICAR) (33) and metformin (32) increase PGC-1α expression. Similarly, AMPK phosphorylates and activates PGC-1α, leading to increases in the PGC-1α-dependent induction of the PGC-1α promoter (20). In addition to AMPK, it would appear that p38 mitogen-activated protein kinase (p38) is also involved in the regulation of PGC-1α. For example, the overexpression of MAPK kinase 3, an upstream p38 kinase, in cultured muscle cells increases PGC-1α promoter activity (1), while the muscle-specific overexpression of MAPK kinase 6 increases PGC-1α protein content in mouse fast-twitch skeletal muscle (1).

Although localized biochemical perturbations within contracting skeletal muscle are thought to be intimately involved in the activation of AMPK and p38 and the subsequent induction of PGC-1α, there is a growing appreciation for the involvement of systemic factors in this process. For instance, adrenergic agonists activate AMPK (22) and p38 (15) in skeletal muscle. Furthermore, β-adrenergic agonists increase the phosphorylation of cAMP response element-binding protein (CREB) (35), a transcription factor regulating the expression of PGC-1α (19). On the basis of these findings, it is not surprising that we (9) and others (23) reported β-adrenergic agonist-mediated increases in the expression of PGC-1α in rodent skeletal muscle.

Work from several groups has shown that mitochondrial content is increased in skeletal muscle from rodents fed a high-fat diet (HFD) (18, 36). These findings have demonstrated that “mitochondrial deficiency,” i.e., reductions in mitochondrial content, is not a causal event in the development of skeletal muscle insulin resistance. However, whether skeletal muscle from insulin-resistant animals remains responsive to perturbations that induce PGC-1α is less clear. Interestingly, the exercise-induced activation of AMPK (6, 31) and induction of PGC-1α (6) are attenuated in skeletal muscle from insulin-resistant individuals. These findings are consistent with recent studies reporting reductions in AICAR-stimulated glucose uptake in skeletal muscle from type 2 diabetic patients (2) and obese rats (4) and earlier work from Reznick et al. (27) showing an ablated AICAR-mediated activation of AMPK in skeletal muscle from aged mice (27). Although these investigations clearly demonstrate reductions in AMPK signaling, it...
is difficult to discern how large drops in ATP levels [exercise (6) and β-guanadinedoproprionic acid feeding (27)] or increases in AICAR (2, 27) fail to appreciably activate AMPK, especially since the content of AMPK was not reduced (2, 6). In contrast to the aforementioned results, Fillmore and colleagues (8) found that AICAR-induced AMPK/acetyl-CoA carboxylase (ACC) phosphorylation was intact in skeletal muscle from HFD-fed rats.

In addition to a blunted activation of AMPK, some have reported reduced responsiveness to β-adrenergic stimulation in various tissues from insulin-resistant individuals or rodents. For example, the ability of epinephrine to stimulate lipolysis is reduced in adipocytes from HFD-fed mice (13), and catecholamine-induced hormone-sensitive lipase activation is decreased in skeletal muscle from insulin-resistant subjects (21). Similarly, we recently showed that fasting-induced increases in p38 phosphorylation and the induction of PGC-1-related coactivator were decreased in skeletal muscle from HFD-fed rats (10); however, this occurred independent of reductions in PKA signaling.

Given these discrepant results and the increasingly recognized role of catecholamines in the regulation of PGC-1α expression, the purpose of the present investigation was to examine the effects of diet-induced obesity on epinephrine- and AICAR-mediated signaling and the induction of PGC-1α. We hypothesized that a HFD would attenuate epinephrine-stimulated increases in the phosphorylation of p38 and the induction of PGC-1α, whereas the ability of AICAR to stimulate AMPK and induce PGC-1α would remain intact.

**METHODS**

**Materials**

Reagents, molecular weight marker, and nitrocellulose membranes for SDS-PAGE were purchased from Bio-Rad (Mississauga, ON, Canada). ECL Plus (catalog no. RPN2132) was a product of Amersham Pharmacia Biotech (Baie d’Urfe, PQ, Canada). Antibodies against phosphorylated p38 MAPK (catalog no. 9211), total p38 MAPK (catalog no. 9212), phosphorylated AMPK (catalog no. 2531), total AMPK (catalog no. 2793), phosphorylated ERK (catalog no. 9101), total ERK (catalog no. 4695), phosphorylated CREB (catalog no. 9191), and phosphorylated PKA substrate (catalog no. 9624) were purchased from Cell Signaling (Danvers, MA). Tubulin antibodies (ab7291) were purchased from Abcam (Cambridge, MA). Antibodies against mitogen-activated protein kinase phosphatase 1 (MKP1; catalog no. 07-535) were obtained from Millipore (Temecula, CA). Cytochrome oxidase subunit IV (COX IV; catalog no. MS407) and Core 1 (catalog no. MS303) antibodies were products of Mitosciences (Eugene, OR). Horseradish peroxidase-conjugated donkey anti-rabbit (catalog no. 711-005-152) and goat anti-mouse (catalog no. 115-005-003) IgG secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Fibrous tissue RNeasy extraction kits (catalog no. 74704) were purchased from Qiagen (Mississauga, ON, Canada). SuperScript II reverse transcriptase (catalog no. 18064-014) was a product of Invitrogen (Burlington, ON, Canada). Taqman gene expression assays for β-actin (Rn00667869_m1), PGC-1α (Rn00582041_m1), and MKP1 (Rn00587176) were obtained from Applied Biosystems (Foster City, CA). AICAR (catalog no. A611700) was purchased from Toronto Research Chemicals (Toronto, ON, Canada).

**Treatment of Rats**

All protocols followed Canadian Council on Animal Care guidelines and were approved by the Animal Use and Welfare Committee at the University of Alberta. Male Wistar rats (~200 g body wt; Charles River, St-Constant, PQ, Canada) were housed two per cage in a 12:12-h light-dark cycle with water and standard rat chow provided ad libitum. Rats were acclimated to the animal housing facility for 1 wk prior to the start of the diet manipulation. Rats continued to receive standard rat chow or were fed a HFD ad libitum for 6 wk. The standard chow diet (LabDiet, catalog no. 5001) contained 28.5% protein, 13.5% fat, and 58.0% carbohydrates (expressed as percentage of total energy) and was purchased from PMI Nutrition International. The HFD (catalog no. TD06414) contained 18.4% protein (casein), 60.3% fat, and 21.3% carbohydrate and was purchased from Harlan Laboratories (Madison, WI).

**Epinephrine experiments.** After an overnight (~12-h) fast, chow- or HFD-fed rats were injected subcutaneously with a weight-adjusted bolus of epinephrine (20, 10, or 5 μg/100 g body wt) or an equivalent amount of sterile saline. Animals were anesthetized with pentobarbital sodium (5 mg/100 g body wt), and triceps brachii muscles were harvested 30 min (signaling) or 2 or 4 h (RT-PCR) following epinephrine injections. The triceps muscle is predominantly (~96%) composed of fast-twitch muscle fibers (12). We chose to use this muscle, since p38 phosphorylation is increased to a much greater extent in response to β-adrenergic stimulation in fast- than slow-twitch muscle (16). Triceps muscle was washed in sterile saline to remove any blood and then snap-frozen in aluminum tongs cooled to the temperature of liquid nitrogen. Samples were stored at −80°C until analysis.

**AICAR experiments.** After an overnight (~12-h) fast, chow- or HFD-fed rats were injected subcutaneously with a weight-adjusted bolus of AICAR (0.5 gram/kg body wt) or an equivalent amount of sterile saline. Animals were anesthetized with pentobarbital sodium (5 mg/100 g body wt), and triceps muscles were harvested 2 h postinjection. Samples were snap-frozen in liquid nitrogen and stored at −80°C until analysis.

**Western Blot Analysis**

Muscle samples were homogenized in 10 volumes of ice-cold cell lysis buffer (catalog no. FN0021, Invitrogen) supplemented with protease inhibitor cocktail and PMSF. Homogenized samples were centrifuged for 15 min at 2,500 g at 4°C. The supernatant was collected, and protein concentration was determined by the bicinchoninic acid method (35). Equal amounts of protein were separated on a 7.5% (ACC), 10% (AMPK, p38, CREB, and ERK), or 12.5% (PKA substrate) gel. Proteins were wet-transferred to nitrocellulose membranes at 200 mA/tank and subsequently blocked in Tris-buffered saline + 0.1% Tween 20 (TBST) supplemented with 5% nonfat dry milk for 1 h at room temperature with gentle agitation. Membranes were incubated in TBST + 5% nonfat dry milk supplemented with appropriate primary antibodies overnight at 4°C with gentle agitation. On the following morning, membranes were briefly washed in TBST and then incubated in TBST + 0.1% nonfat dry milk supplemented with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Bands were visualized using ECL Plus and captured using a Typhoon imaging system (GE Health Care). Imagequant software (GE Healthcare) was used to quantify relative band intensities.

**Citrate Synthase Activity**

Citrate synthase activity was determined as described in detail by our laboratory previously (11, 38).

**Real-Time PCR**

RNA was isolated from skeletal muscle using a Fibrous RNeasy kit according to the manufacturer’s instructions. cDNA was synthesized from 1 μg of RNA using SuperScript II reverse transcriptase, oligo(dT), and dNTP. Real-time PCR was performed using a 7900HT
Fast Real-Time PCR system (Applied Biosystems). Taqman gene expression assays were used to determine the expression of PGC-1α and β-actin. Samples were run in duplicate in a 96-well plate format. Each assay (20 μl total volume) contained 1 μl of gene expression assay, 1 μl of cDNA template, 10 μl of Taqman Fast Universal PCR Master Mix, and 8 μl of RNase-free water. We used β-actin as our endogenous control, as the expression of this gene did not change following 6 wk on the HFD or acute epinephrine treatment. Differences in gene expression are expressed relative to the chow-fed saline-injected group. The 2−ΔΔCt method (23) was used for these analyses.

Statistical Analysis

Values are means ± SE. Comparisons between the group means of diet (chow and HFD) and treatment (saline and epinephrine/AICAR) were made using a two-way ANOVA followed by a least significant difference post hoc test. Differences between two groups were analyzed using an unpaired two-way Student’s t-test. Statistical significance was set at P < 0.05.

RESULTS

Physical Characteristics

In the same rats used in this study, we previously reported that HFD consumption for 6 wk increased body weight, epicardial fat pad mass, and fasting blood glucose levels (37).

HFD Consumption Increases Skeletal Muscle Mitochondrial Enzymes

To confirm the findings from Hancock et al. (18) and others (36), we measured the content of mitochondrial marker proteins in triceps muscles from HFD-fed rats. HFD consumption for 6 wk led to increases in the protein content of COX IV and Core 1 (Fig. 1). Similarly, there was a trend (P = 0.057) for increases in citrate synthase activity (73.8 ± 5.7 and 93.6 ± 8.1 μmol-min−1·g protein−1 in control and HFD, respectively).

HFD Consumption Alters Epinephrine Signaling

The phosphorylation of AMPK was not altered by diet or epinephrine treatment (Fig. 2A). Epinephrine significantly increased p38 phosphorylation in triceps muscles from Chow-fed, but not HFD-fed, rats (Fig. 2A). There was a trend (P = 0.11) for increases in p38 phosphorylation in triceps muscles from saline-treated HFD-fed compared with Chow-fed rats.

To determine if the attenuated epinephrine signaling was specific to p38 or a more general reduction in β-adrenergic signaling, we measured changes in the phosphorylation of ERK, CREB, and PKA substrates. Epinephrine increased ERK phosphorylation nearly twofold in triceps muscles from Chow-fed animals (Fig. 2A). ERK phosphorylation was increased in the HFD-fed rats and was not further increased by epinephrine. CREB phosphorylation was increased in muscles from HFD-fed rats and further increased by epinephrine in muscles from both diet groups. Using an antibody that recognizes proteins phosphorylated by PKA on serine/threonine residues with arginine at the −3 position, we found that epinephrine treatment increased the phosphorylation of −37-, 35-, and 20-kDa proteins. The phosphorylation of these proteins was similar in skeletal muscle from Chow- and HFD-fed rats (Fig. 2B). A summary of the changes in epinephrine-mediated signaling is presented in Table 1.

Altered p38 Signaling Is Not Associated With Changes in MKP1

MKP1 dephosphorylates p38 and has been reported to be increased in obesity and insulin resistance (28). In our model, MKP1 mRNA expression and protein content were not altered in triceps muscles from HFD-fed rats (Fig. 3).

Epinephrine-Induced PGC-1α Expression Is Similar in Skeletal Muscle from Chow- and HFD-Fed Rats

At 2 h following a bolus injection of epinephrine, PGC-1α mRNA expression was increased nearly threefold in Chow-fed rats. PGC-1α mRNA levels were similar in saline- and epinephrine-treated Chow- and HFD-fed rats (Fig. 4). PGC-1α mRNA remained elevated in Chow-fed (3.00 ± 0.74 fold increase vs. saline-injected Chow-fed rats) and HFD-fed (3.81 ± 1.55 vs. saline-injected Chow-fed rats) rats 4 h following epinephrine injection.

To ascertain if β-adrenergic resistance was being overridden by the high dose of epinephrine (20 μg/100 g body wt), we repeated the HFD intervention and treated rats with lower doses of the hormone. At 2 h following the injection of 10 μg/100 kg body wt epinephrine, the induction of PGC-1α was similar in triceps muscles from both diet groups (2.52 ± 0.38 and 2.39 ± 0.37 fold increase in Chow- and HFD-fed rats, respectively, compared with Chow-fed saline controls, n = 6, P > 0.05). Similarly, regardless of diet, injection of 5 μg/100 kg body wt epinephrine led to similar increases in PGC-1α (1.89 ± 0.35 and 3.36 ± 0.77 fold increase in Chow- and HFD-fed rats, respectively)
HFD-fed rats, respectively, compared with chow-fed saline controls, n = 6, P > 0.05).

**AICAR-Stimulated AMPK/ACC Phosphorylation Is Normal in HFD-Fed Rats**

AICAR increased the phosphorylation of AMPK on Thr172 in triceps muscles from both diet groups (Fig. 5A). Similarly, ACC phosphorylation was increased to a similar extent in chow- and HFD-fed rats (Fig. 5B).

**AICAR-Induced PGC-1α Expression Is Greater in HFD-Fed Rats**

AICAR increased the expression of PGC-1α nearly twofold in triceps muscles from chow-fed rats. Surprisingly, AICAR-induced increases in PGC-1α were greater in triceps muscles from HFD-fed rats (Fig. 5C).

**DISCUSSION**

PGC-1α is a critical regulator of skeletal muscle mitochondrial biogenesis. While elevations in cytosolic calcium concentration and reductions in high-energy phosphates are likely key events in the induction of PGC-1α, there is increasing evidence to suggest that adrenergic agonists also play a part in this process. Interestingly, it has been suggested that skeletal muscle from obese, insulin-resistant individuals is resistant to -adrenergic stimulation (21), and thus it is not clear if the ability of catecholamines to induce PGC-1α is intact. In the present study, we found that epinephrine-mediated increases in PGC-1α mRNA expression were similar in skeletal muscle from chow- or HFD-fed rats. These results are particularly intriguing, given the fact that epinephrine-stimulated increases in the phosphorylation of p38, a kinase previously shown to regulate PGC-1α in healthy skeletal muscle (1), were attenuated in skeletal muscle from HFD-fed rats.
Although not a universal finding (5), reductions in p38 signaling have been reported and linked to decreases in the expression of PGC-1α in skeletal muscle from obese mice (28). Reductions in p38 activation would appear to be related to increases in the expression of MKP1. In support of this contention, MKP1/f−/− mice are protected against HFD-induced reductions in p38 phosphorylation (28). In contrast to these results, neither the protein content nor mRNA expression of MKP1 was increased in HFD-fed rats in the present study, suggesting that the attenuation of epinephrine-induced p38 phosphorylation was independent of MKP1. Consistent with these findings, we found that p38 phosphorylation tended, although not significantly (P = 0.11), to be higher in HFD-fed saline-treated rats. If MKP1 accounted for the blunted epinephrine-induced phosphorylation of p38, then it would be expected that p38 phosphorylation would also be reduced in muscles from HFD-fed saline-treated rats. As this was not the case, the blunted increase in p38 phosphorylation following epinephrine injections could likely be explained, at least in part, by elevations in basal p38 phosphorylation.

The disconnect between increases in p38 phosphorylation and the induction of PGC-1α in skeletal muscle from HFD-fed rats suggests that additional signaling pathways are involved in the regulation of PGC-1α in skeletal muscle from obese rats. Alternatively, when the absolute levels of p38 phosphorylation following epinephrine treatment were examined, there were no differences between diet groups. Thus it could be argued that the absolute levels of p38 phosphorylation are a more important indicator than the fold increase in phosphorylation with epinephrine in regard to the induction of PGC-1α.

Given the apparent impairment of epinephrine-induced p38 phosphorylation in HFD-fed rats, we examined the effects of epinephrine stimulation on additional signaling pathways that could explain the increase in PGC-1α expression in muscle from HFD-fed rats. AMPK is an enzyme that induces PGC-1α (20, 32, 33) and is activated by α-adrenergic agonists (22). Surprisingly, epinephrine treatment did not increase AMPK phosphorylation in muscles from rats from either diet group, demonstrating that AMPK is not involved in the pathways through which epinephrine induces PGC-1α in skeletal muscle.

Catecholamine stimulation leads to the generation of cAMP and the activation of PKA. In the present study, we found that epinephrine-stimulated increases in the phosphorylation of PKA substrates were similar in skeletal muscle from Chow- or HFD-fed rats. The activation of PKA signaling, in the absence of increases in p38 and AMPK phosphorylation, suggests that PKA could mediate the effects of epinephrine on the induction of PGC-1α in skeletal muscle from HFD-fed rats. Furthermore, our results would question the premise that β-adrenergic responsiveness is reduced in skeletal muscle from obese, insulin-resistant rats.

The epinephrine injection protocol used in this study leads to increases in plasma epinephrine levels several times higher than those observed under most physiological conditions, i.e., fasting and exercise (7). Thus the existence of β-adrenergic “resistance” could potentially be overcome by these supra-physiological increases in epinephrine. Arguing against this, we demonstrated that the effect of a much lower dose of epinephrine (5 µg/100 g body wt, or 4 times less than the dose...
used in the initial experiments) on the induction of PGC-1α was similar between diet groups. Moreover, we recently showed that fasting-mediated increases in PKA substrate phosphorylation are normal in triceps muscles from HFD-fed rats (10). Fasting leads to an nearly twofold increase in plasma epinephrine levels (10). Thus, if the β-adrenergic activation of PKA was impaired, the marginal increase in epinephrine levels with fasting would not be expected to override it.

When activated, PKA phosphorylates a wide range of substrates, including CREB (34), a transcription factor regulating the expression of PGC-1α in skeletal muscle (39). Although CREB phosphorylation was markedly elevated in triceps muscles from saline-treated HFD-fed compared with chow-fed rats, the ability of epinephrine to increase CREB phosphorylation was similar between groups. The elevation in CREB phosphorylation in muscles from saline-treated HFD-fed rats is similar to that reported in adipose tissue from HFD-fed and db/db mice (26) and could be related to increases in the phosphorylation of ERK, an enzyme previously shown to be involved in CREB phosphorylation (17).

Despite the already elevated phosphorylation of CREB in triceps muscle from saline-treated HFD-fed rats, epinephrine led to a further increase in phosphorylation. While the relative increase in CREB phosphorylation with epinephrine in HFD-fed rats was approximately half that in chow-fed animals, it was still associated with an upregulation of PGC-1α, suggesting that it is sufficient to induce PGC-1α. However, PGC-1α was not increased in triceps muscles from HFD-fed rats, despite a robust increase in CREB phosphorylation. These findings suggest that epinephrine activates additional, yet to be identified, signaling pathways that allow for the induction of

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**Fig. 4.** Epinephrine-induced increases in peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA expression are not different in triceps muscles from chow- or HFD-fed rats. Values (means ± SE) are expressed as fold differences in gene expression relative to triceps muscles from chow-fed saline-treated rats. *Significant effect of epinephrine within a diet group (P < 0.05).

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**Fig. 5.** 5-Aminoimidazole-4-carboxamide-1β-D-ribofuranoside (AICAR)-mediated increases in phosphorylation of AMPK (A) and acetyl-CoA carboxylase (ACC) (B) are similar in triceps muscle from chow- and HFD-fed rats, yet induction of PGC-1α is greater in triceps muscle from HFD-fed rats (C). Values are means ± SE for 6–8 samples per group. *Significant effect of AICAR within a given diet group. #Significant difference between the AICAR effect in chow- and HFD-fed rats. Representative Western blots are shown above quantified data in C. p-ACC, phosphorylated ACC.
PGC-1α in skeletal muscle from HFD-fed rats. Collectively, these results clearly highlight the complexity of the signaling pathways and transcriptional regulators of PGC-1α, especially in the setting of obesity and insulin resistance.

Because epinephrine did not activate AMPK and we wanted to determine if AMPK signaling was perturbed in HFD-fed rats, we treated rats with AICAR, an AMPK agonist. In contrast to findings previously reported in human skeletal muscle from type 2 diabetic patients (2), we found that AMPK signaling was not attenuated in muscle from HFD-fed rats. Interestingly, not only was the ability of AICAR to induce PGC-1α mRNA expression maintained in triceps muscles from HFD-fed rats, but it was actually greater than the increase in muscles from chow-fed animals. As the degree of AMPK signaling appeared to be similar between groups, yet PGC-1α was increased to a greater extent, we speculate that HFD consumption leads to reductions in transcriptional repressors of PGC-1α, which could then sensitize the muscle to the effects of AICAR, resulting in a greater induction of PGC-1α. However, in preliminary experiments, we found that the content of receptor-interacting protein 140, a transcriptional regulator of mitochondrial enzymes (29), is not altered in skeletal muscle from HFD-fed rats (data not shown). Clearly, further work is needed to examine this hypothesis in further detail.

Regardless of the specific mechanisms involved in the potentiated effects of AICAR on the induction of PGC-1α, our results clearly demonstrate that AMPK signaling is intact in a model of diet-induced obesity. These results are in contrast to previous reports showing reductions in the effects of AICAR in skeletal muscle from type 2 diabetic patients or aged mice, a condition in which insulin resistance is present (30). As Babraj et al. (2) suggested that age, rather than diabetic status, explains the blunted effect of AICAR in human skeletal muscle, the use of younger animals in our study may account for the discrepant results.

In summary, this study has been the first to examine epinephrine and AICAR signaling and the induction of PGC-1α in skeletal muscle from HFD-fed rats. Our data demonstrate that not only is mitochondrial content increased in skeletal muscle from HFD-fed rats, but the ability of β-adrenergic and AMPK agonists to induce PGC-1α remains intact. In the setting of lipid oversupply, the maintained ability to induce PGC-1α would be advantageous, as it would allow for the induction of mitochondrial biogenesis, a process that would enhance skeletal muscle fatty acid oxidation and limit/reduce excessive lipid deposition in skeletal muscle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.C.F., D.B.W., A.L.S., and D.C.W. are responsible for conception and design of the research; B.C.F., Z.W., D.B.W., and A.L.S. performed the experiments; B.C.F., Z.W., and D.C.W. analyzed the data; B.C.F., Z.W., D.B.W., and D.C.W. interpreted the results of the experiments; B.C.F., Z.W., and D.C.W. prepared the figures; B.C.F., D.B.W., A.L.S., and D.C.W. edited and revised the manuscript; B.C.F., Z.W., A.L.S., and D.C.W. approved the final version of the manuscript; D.C.W. drafted the manuscript.

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