IL-15 Mediates Mitochondrial Activity through a PPARδ-Dependent-PPARα-Independent Mechanism in Skeletal Muscle Cells

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Molecular mediators of metabolic processes, to increase energy expenditure, have become a focus for therapies of obesity. The discovery of cytokines secreted from the skeletal muscle (SKM), termed “myokines,” has garnered attention due to their positive effects on metabolic processes. Interleukin-15 (IL-15) is a myokine that has numerous positive metabolic effects and is linked to the PPAR family of mitochondrial regulators. Here, we aimed to determine the importance of PPARα and/or PPARδ as targets of IL-15 signaling. C2C12 SKM cells were differentiated for 6 days and treated every other day with IL-15 (100 ng/mL), a PPARα inhibitor (GW-6471), a PPARδ inhibitor (GSK-3787), or both IL-15 and the inhibitors. IL-15 increased mitochondrial activity and induced PPARα, PPARδ, PGC1α, PGC1β, UCP2, and Nrf1 expression. There was no effect of inhibiting PPARα, in combination with IL-15, on the aforementioned mRNA levels except for PGC1β and Nrf1. However, with PPARδ inhibition, IL-15 failed to induce the expression levels of PGC1α, PGC1β, UCP2, and Nrf1. Further, inhibition of PPARδ abolished IL-15 induced increases in citrate synthase activity, ATP production, and overall mitochondrial activity. IL-15 had no effects on mitochondrial biogenesis. Our data indicates that PPARδ activity is required for the beneficial metabolic effects of IL-15 signaling in SKM.

1. Introduction

Obesity has become a modern epidemic and is growing in both prevalence and severity throughout the world. Obesity is one of the leading causes of preventable death, with more than one-third of all Americans considered overweight or obese [1, 2]. Current treatments for obesity include a calorie restricted diet and physical exercise, but sustaining long-term weight loss has proven to be a challenge for many [3]. A mainstay for treating obesity has been to increase metabolic rate, particularly through induction of mitochondrial activity in skeletal muscle (SKM). SKM is considered the largest organ in the body and acts to carry out bodily movement through generation of ATP, primarily via mitochondrial respiration [4]. Recently, SKM has attracted attention due to its newly identified ability to release cytokines, termed “myokines,” into circulation that act to increase overall energy expenditure [5–9]. Many myokines (FGF-21, Irisin, BDNF, interleukin-6, and interleukin-15, among others) increase in circulation following physical exercise, owing to their potential to reduce adiposity [9–11]. However, the downstream effectors of myokine signaling that positively influence metabolism remain elusive. In this regard, studies aimed at elucidating myokine signaling pathways, for the potential treatment and/or prevention of obesity, have come to the forefront of metabolic research.

Interleukin-15 (IL-15) is considered a myokine and is confirmed to increase mitochondrial activity, resulting in a decrease in overall adiposity [12–15]. Historically, IL-15 had
been extensively studied as an activator of natural killer (NK) cells with antitumorigenic potential and anti-inflammatory properties [11, 16]. Interestingly, in human and rodent studies, there is evidence that circulating levels of IL-15 increase following exercise, although this notion is somewhat controversial [17–21]. It is postulated that IL-15 acts to increase glucose uptake, fatty acid oxidation, and mitochondrial activity and lipolysis to reduce adiposity [14, 22–25]. Although the evidence is clear that IL-15 possesses positive metabolic effects, its direct downstream signaling pathways remain largely unknown. Many transcriptional regulators, responsible for inducing mitochondrial dynamics, have been linked to IL-15, such as peroxisome proliferator-activated receptors (PPARs), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), and silent mating type information regulation 2 homolog (SIRT1) [26–29].

PPARs are important transcriptional regulators linked to numerous beneficial metabolic effects, such as induction of mitochondrial biogenesis and fatty acid oxidation [30, 31]. Among the three isoforms, PPARα is highly expressed in oxidative tissues, such as liver, heart, and type I SKM fibers, while PPARδ appears to be ubiquitously expressed and acts to induce mitochondrial activity and lipid metabolism [30, 32, 33]. Both PPARα and δ have been suggested to promote induction of mitochondrial activity in many cell types and have garnered attention as potential antiobesogenic factors [30, 34–37]. PPARγ is most highly expressed in adipose tissue, both brown and white, and it plays an integral part in lipogenesis and adipogenesis [38]. The antidiabetic drug class, thiazolidinediones, acts to bind to and activate PPARγ to clear circulating lipids for the restoration of insulin sensitivity [39]. Much is known regarding the positive metabolic effects of PPARs but it is not known if myokines, such as IL-15, act to upregulate their transcriptional activity [30, 36]. Interestingly, PPARδ expression levels have been strongly linked to IL-15 signaling in SKM [26, 28]. However, the direct relationship between IL-15 and PPARδ transcriptional activity, to modulate mitochondrial processes, has yet to be firmly established. On the other hand, there are reports that IL-15 acts to increase PPARα expression levels in adipose tissue [29], but little is known regarding an IL-15-PPARα relationship in SKM. Taken together, it is clear that a relationship between IL-15 and PPARα and/or δ exits, but the depth of these relationships to induce metabolism, thereby, reducing adiposity is unknown.

Here we aimed to determine the necessity of PPARα and/or δ as downstream mediators of the metabolically beneficial effects of IL-15 action on mitochondrial activity in SKM. Here, we show that PPARδ is required for IL-15 mediated induction of mitochondrial activity independent of PPARα in SKM cells.

2. Methods

2.1. Reagents. C2C12 cells were obtained from Sigma (#91031101) along with Dulbecco’s Modified Eagle Medium (DMEM; #D6429), fetal bovine serum (FBS; #F0926), horse serum (#H1270), and insulin (#I9278). Recombinant IL-15 was from GenScript (#Z03309-50) and GW-6471 (#11697) and GSK-3787 (#15219) were obtained from Cayman Chemicals. Trizol (#15956), SYBR green (#A25742) and a SuperScript VILO reverse transcription kit (#11754050) were purchased from ThermoFisher. The mitochondrial dye, Mito Red, was from Santa Cruz Biotechnology (SC-#301164). Male C57BL6 mice were kept on a 12:12 light dark cycle and fed a standard diet and water ad libitum. Gastrocnemius muscle was obtained following euthanasia. All animal procedures were approved by the Institutional Animal Care and Use Committee at Chapman University.

2.2. C2C12 Cell Culture. The mouse immortalized SKM fibroblast cell line, C2C12, was cultured in DMEM and supplemented with 10% FBS, 1% penicillin-streptomycin (10,000 U/mL), and 0.1% amphotericin B. When the cells reached 80% confluence, they were induced to differentiate into mature myotubes by supplementing the DMEM with 2% horse serum and 1μM insulin for 6 days. Myotube formation was confirmed by visualization using an inverted microscope. Upon induction of differentiation, cells were treated every other day, for 6 days, with either vehicle control (DMSO), IL-15 (100 ng/mL), 10 μM of a PPARα inhibitor (GW-6471), 1μM of a PPARδ inhibitor (GSK-3787), IL-15 + GW-6471, IL-15 + GSK-3787, or IL-15 + GW-6471 + GSK-3787.

2.3. Western Blotting. Western blotting was performed as previously described [40]. Briefly, C2C12 cells were lysed in a modified RIPA buffer supplemented with protease inhibitors (Pierce). Approximately 20 μg of protein from the cell homogenate preparations was separated on a 4–12% gradient gel (GenScript) via SDS-PAGE. Proteins were transferred onto Immobilon-P polyvinylidene difluoride (PVDF) membranes and blocked with 5% BSA in TWEEN-TBS for 1 hour. The membranes were then incubated (4°C) in 5% BSA in Tween-TBS with antibodies (1:1000) against PPARα, PPARδ, or GAPDH (Sigma). Following overnight incubation, the membranes were then probed with a secondary antibody (GenScript, 1:2000; or Thermo, 1:10,000). Blots were then washed and subjected to enhanced chemiluminescence (Pierce). Membranes were stripped in 0.5 M NaOH and probed for total proteins and subsequently GAPDH (Sigma) was used as a loading control.

2.4. RNA Extraction and Reverse Transcription. Standard RNA isolation procedures were performed on the cells following the 6-day treatment protocol, as previously described [41, 42]. Mouse gastrocnemius muscle was used to verify IL-2 receptor expression. Briefly, cells or muscle tissue was lysed with Trizol reagent and chloroform was added to separate the RNA from the DNA and protein fractions. RNA was precipitated from the clear phase of the Trizol-chloroform mixture, followed by centrifugation at 12,000 × g at 4°C, with isopropanol. The RNA pellet was washed with 75% ethanol and centrifuged at 7,500 × g for 5 minutes, at 4°C, and the pellets were air-dried. Using RNase-free water, the pellets were resuspended and the RNA purity and concentration were quantified using a NanoDrop spectrophotometer. Reverse transcription of RNA to cDNA was performed on 2 μg of RNA using SuperScript reverse transcriptase VILO kit.
2.5. Mitochondrial DNA Assessments. Following the IL-15 treatment protocol, genomic DNA was isolated using a miniprep DNA isolation kit (Sigma). Briefly, 5 × 10^6 of cells in suspension, in lysis buffer, were mixed with RNase and proteinase K and incubated at 70°C for 10 minutes. Ethanol was added and then homogenates were transferred to the binding columns and subjected to a series of washes. The columns were air-dried and the DNA was eluted. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo). Real time qPCR was carried out and a mitochondrial DNA marker was compared relative to a nuclear encoded marker (18S) and the corresponding sequences are displayed in Table 1.

2.6. Real Time Quantitative PCR. Real time qPCR was performed on the cDNA, using SYBR green in a 96-well plate. The primers are displayed in Table 1. GAPDH was used as an internal control and the ddCT method was used to calculate gene expression levels.

2.7. Live Cell Mitochondrial Activation Assay. Following the IL-15 treatment protocol, as described above, live cells were stained using a dye that becomes sequestered in active mitochondria [42]. The cells were then fixed with phosphate buffered formalin and DAPI was used as a nuclear stain. Fluorescence levels were assessed using an inverted Zeiss microscope and images were captured using an Axiovision camera. Relative and absolute fluorescence levels were calculated using Image J software. Measurements were corrected for total cell fluorescence to account for the variation in myotube size.

2.8. Citrate Synthase and ATP Assays. Citrate synthase (CS) activity was measured using a previously described protocol with some modifications [42–44]. To assess CS activity, C2C12 cell lysates were added to a 96-well plate containing 100 μM 5, 5'-dithio-bis (2-nitrobenzoic acid) and 250 μM acetyl-CoA. To initiate the reaction, 500 μM oxaloacetate was added. The reaction was monitored in a microplate reader for 5 min at an ABS of 405. The specific activity was calculated as the absorbance rate per minute divided by the mercaptopeptide coefficient and expressed per μg of protein. ATP was measured on cell lysates using a fluorometric kit in a 96-well plate (Sigma) and normalized to total protein.

2.9. Statistical Analysis. Data are presented as mean ± SEM and all calculations were carried out using GraphPad Prism 6. A one-way ANOVA was calculated to determine multiple comparisons with a Fisher’s post hoc analysis. For comparisons between two groups Student’s t-test was performed. A P level of 0.05 was used to determine statistical significance.

### 3. Results

3.1. IL-15 Induces Mitochondrial Activity in Skeletal Muscle Cells. To verify total mitochondrial activation via IL-15 signaling, a mitochondrial activity assay was performed to analyze relative mitochondrial activity in live SKM cells, as indicated by red staining (Figure 1(a)). According to quantification of the fluorescence signal, IL-15 stimulated mitochondrial activity by 44% in the SKM cells (P < 0.05; Figure 1(b)). IL-15 treatment stimulated increases in both PPARα and PPARβ protein expression levels (Figure 1(c)). To verify that the IL-15 receptor, IL-2R [45], was present in the C2C12 cells mRNA levels in mouse gastrocnemius and C2C12 cells were measured (Figure 1(d)). IL-15 induced mRNA expression levels of PPARα (4-fold) and PPARβ (2-fold), along with their cofactors PGC1α (100%) and PGC1β (40%), when compared to control cells (P < 0.05; Figure 2(a)). Owing to the capability of IL-15 to induce mitochondrial activity, the mitochondrial uncoupling protein-2 (UCP2) mRNA expression levels were increased by 50% (P < 0.05. Figure 2(b)). An additional factor associated with transcriptional activity of PPARs, nuclear respiratory factor 1 (Nrf1), and mRNA level was increased (30%) with IL-15 treatment (P < 0.05; Figure 2(b)). However, IL-15 failed to alter the expression levels of SIRT1 in the SKM cells (P > 0.05; Figure 2(b)). In order to determine whether IL-15 stimulated increases in mitochondrial associated factors or activity was due to biogenesis, we assessed mtDNA and Tfam mRNA

| Gene | Sequence |
|------|----------|
| PPARα, F | ATGGGAGTATGGAGCTAATAG |
| PPARα, R | GGGTGACAGAAGGAGACAG |
| PPARδ, F | ACAAGGGCTCTAGGTTAAC |
| PPARδ, R | GCCGAAAGCGCCCTCACAG |
| PGC1α, F | ACTGACTACCTTGGATTG |
| PGC1α, R | TAAAGATTCCTGGTGACATA |
| PGC1β, γ | TGTCAAAACGCGAGGTAGT |
| PGC1β, α | GCTCTGTAGGGGAGATGA |
| UCP2, F | CCAATGCAAGAGAGGAAGGAT |
| UCP2, R | GTCTATGTTTGCTTTCAGAG |
| Nrf1, F | TTGGAACACGAGTGGCACA |
| Nrf1, R | CTCACCTTGCTATGTTATTCCTCCAT |
| IL-2Rγ, F | TACACGACATTTTGTTGAC |
| IL-2Rγ, R | GCCGCTGGATACAAAGTT |
| Tiam, F | CAAGTCGCTATGGGTATG |
| Tiam, R | TTTCCTGAGCGCAATCCATC |
| mtDNA, F | GGCTTTGGGAAACTGACTGT |
| mtDNA, R | TTGCCTGATAATTTATGCTG |
| Cox5b, F | GCAGCGACAGCCTGAA |
| Cox5b, R | GGTGACATCTGTGAAGAGGACAC |
| Cox7a1, F | CAGCTTGTAATGGGTCTCCAGAT |
| Cox7a1, R | CAGAGTCATGTCATGCTG |
| Cox8b, F | AAGAAGCCGGTTGGGACAGA |
| Cox8b, R | GAACATGAGCCCAACAGC |
| Gapdh, F | AGGTCGGTTGAGCATGGTTT |
| Gapdh, R | TGATAGACATGTAGTTTGAGTCA |

*Table 1: Primer sequences used for qPCR analysis.*
expression levels. IL-15 had no effect on mtDNA content or on Tfam expression levels ($P > 0.05$; Figures 3(a) and 3(b)).

3.2. The Involvement of PPARs in IL-15 Signaling. To verify the efficiency of the PPAR$\alpha$ inhibitor, GW, we confirmed a reduction in PPAR$\alpha$ mRNA expression levels by 57% when compared to vehicle control cells ($P < 0.05$; Figure 4(a)). PPAR$\delta$ mRNA levels were assessed to determine the specificity of GW and there were no reductions with PPAR$\alpha$ inhibition ($P > 0.05$; Figure 4(b)). Although PPAR$\alpha$ was inhibited, the stimulatory effects of IL-15 on PGC1$\alpha$ and UCP2 mRNA expression levels were maintained ($P < 0.05$; Figure 4(c)). Conversely, the IL-15 induced increases in PGC1$\beta$ and Nrf1 mRNA levels were abolished with PPAR$\alpha$ inhibition when compared to vehicle control cells ($P > 0.05$; Figure 4(c)). Next, PPAR$\delta$ mRNA levels were confirmed
Figure 2: Effects of IL-15 signaling on mitochondrial associated factors. (a) mRNA expression of PPARα, PPARδ, PGC1β, and PGC1α; (b) mRNA expression of UCP2, SIRT1, and Nrf1. Assessments were carried out on differentiated C2C12 myotubes following treatment with 100 ng/mL of IL-15 every other day for 6 days during the differentiation protocol. GAPDH was used as an internal control for qPCR analysis. All values are displayed as means ± SEM, n = 6–9 per group, *P < 0.05.

Figure 3: Effects of IL-15 signaling on mitochondrial biogenesis. (a) mitochondrial DNA (mtDNA) assessments; (b) mRNA expression of mitochondrial factor Tfam. Assessments were carried out on differentiated C2C12 myotubes following treatment with 100 ng/mL of IL-15 every other day for 6 days during the differentiation protocol. mtDNA was normalized to the total nuclearDNA (nucDNA) content. GAPDH was used as an internal control for qPCR analysis. All values are displayed as means ± SEM, n = 6.

to be reduced by 60%, when compared to vehicle control cells, with its inhibitor (GSK) (P < 0.05; Figure 5(a)). Conversely the PPARδ inhibitor had no effects on PPARα mRNA levels, confirming the specificity of GSK (P > 0.05; Figure 5(b)). Unlike the PPARα experiments, inhibition of PPARδ signaling resulted in a loss of IL-15 induced increases in PGC1α, PGC1β, UCP2, and Nrf1 when compared to vehicle control cells (P > 0.05, Figure 5(c)).

3.3. PPARδ Is Required for IL-15 Mediated Increases in Mitochondrial Activity. In the SKM cells, IL-15 stimulated increases in CS activity by 43%, when compared to vehicle
cells, \( P < 0.05 \) and these stimulatory effects were eliminated with PPARδ inhibition \( (P > 0.05; \text{Figure } 6(a)) \). Likewise, ATP content was elevated (30%) with IL-15 treatment and PPARδ inhibition abolished these effects \( (P < 0.05; \text{Figure } 6(b)) \). IL-15 induced increases in mRNA levels of cytochrome C oxidase (Cox) isoforms 5b, 7a1, and 8b were dependent on PPARδ activity \( (P < 0.05; \text{Figure } 6(c)) \). Mitochondrial activity was assessed directly in live SKM cells and the IL-15 induced increases in mitochondrial activity remained elevated (53%), when compared to vehicle control cells, with PPARα inhibition \( (P < 0.05; \text{Figures } 7(a) \text{ and } 7(b)) \). However, inhibition of PPARδ prevented the effects of IL-15 induced increases in mitochondrial activity \( (P > 0.05; \text{Figures } 7(a) \text{ and } 7(b)) \). To further solidify the PPARδ-dependent-PPARα-independent IL-15 mediated signaling, mitochondrial activity was abolished with IL-15 stimulation in the presence of both PPARα and PPARδ inhibitors \( (P > 0.05; \text{Figures } 7(a) \text{ and } 7(b)) \).

4. Discussion

Data from this study solidify the notion that IL-15 is directly involved in mediating mitochondrial activity in SKM cells. Importantly, our data indicate that PPARδ activation is required for IL-15 signaling to carry out its stimulatory effects on mitochondrial activity. Further, based on our findings, we have ruled out PPARα as a potential modulator...
of IL-15 induced mitochondrial activity. However, we have evidence for a role of an IL-15-PPARα signaling relationship in mediating PGC1β and Nrf1 expression levels. Altogether it is clear that PPARδ is required for IL-15 induced expression of mitochondrial regulators and activity in SKM cells.

In line with other reports in adipose tissue [25], treatment with IL-15 increased mitochondrial associated processes in SKM cells, as indicated by increased activity of CS and ATP production. Our data confirm other reports showing that IL-15 has the ability to induce activity of the key Krebs Cycle factor, CS, in SKM from mice [25]. It has been postulated that one route that IL-15 acts to reduce adiposity is through its ability to increase lipolysis and mitochondrial activity in adipocytes [25]. Additionally, IL-15 has been shown to increase the activity of mitochondrial processes, such as fatty acid oxidation [24, 46]. Here we show, for the first time, that IL-15 acts to directly increase overall mitochondrial activity in live SKM cells. On the other hand, it does not appear that IL-15 induces increases in mitochondrial biogenesis as indicated by the mtDNA and Tfam assessments in the C2C12 cells. Likewise, in the current study, activity of CS, ATP production, and Cox isoform expression were fully dependent on PPARδ activity with IL-15 stimulation in the SKM cells. Further, it has previously been established that IL-15 increases expression levels of key factors that function to increase mitochondrial activity and biogenesis in both white and brown adipose tissue as well as in SKM [22, 26, 27, 29, 47]. Our results are in line with those findings, as PPARα, PPARδ, PGC1α,
Figure 6: Involvement of PPARδ in IL-15 mediated mitochondrial activity. (a) Citrate synthase (CS) activity; (b) total intracellular ATP content; (c) mRNA expression of cytochrome C oxidase isoforms Cox5b, Cox7a1, and Cox8b. Assessments were carried out on total cell lysates from C2C12 SKM cells following treatment every other day, for 6 days, with either vehicle control (DMSO), IL-15 (100 ng/mL), 1μM of the PPARδ inhibitor (GSK-3787), or IL-15 + GSK-3787 (I + G). All values are displayed as means ± SEM, n = 6 per group, * different from vehicle control group; P < 0.05; ** different from all groups, P < 0.01; *** different from IL-15 group, P < 0.001; **** GSK different from IL-15 group, P < 0.001.

PGC1β, UCP2, and Nrf1 expression levels were all elevated with IL-15 stimulation. Conversely, our data do not support the notion that IL-15 stimulates mitochondrial biogenesis, which is in agreement with previously reported data in mouse SKM [48]. Unlike other studies, our treatment with IL-15 failed to increase SIRT1 expression levels [27, 28]. Here we employed an in vitro model to study the effects of IL-15 on SKM cells and the other reports linking IL-15 to SIRT1 were carried out in a transgenic mouse model overexpressing IL-15 [27, 28]. Therefore, it is a possibility that IL-15 induced SIRT1 expression levels are secondary to direct activation of the IL-15 signaling pathway in SKM. On the other hand, it cannot be ruled out that SIRT1 activity is regulated by IL-15, as we have only assessed mRNA expression levels. Taken together, it is clear that PPARδ is required for the IL-15 induced effects on mitochondrial associated factors and activity.

Here, our data point to a strong link between IL-15 and PPARδ in SKM cells, but PPARα activity involvement had not been fully assessed in SKM [24, 26–28]. PPARα has been associated with IL-15 signaling in adipose tissue and, with this in mind, we attempted to elucidate a potential IL-15-PPARα relationship in C2C12 cells [29]. Interestingly, IL-15 induced PPARα expression levels nearly 4-fold, while PPARδ expression was induced only 2-fold, suggesting that PPARα may be a more direct target of IL-15 signaling. However, with inhibition of PPARα, PGC1α and UCP2 mRNA expression...
Figure 7: Involvement of PPARα and PPARδ in IL-15 mediated mitochondrial activity in live cells. (a) Quantifiable fluorescence corrected for myotube size. Assessments were carried out on differentiated C2C12 myotubes following treatment with vehicle control (DMSO), IL-15 (100 ng/mL), IL-15 + GW-6471 (I + GW), IL-15 + GSK-3787 (I + GSK), and IL-15 + GW + GSK (I + G + G) every other day for 6 days during the differentiation protocol. (b) Representative images of mitochondrial activity assessment in live cells using a fluorescent probe sequestered into active mitochondria; ImageJ was used to quantify cell fluorescence. All values are displayed as means ± SEM, n = 6 per group, * different from all groups; P < 0.05.

levels were maintained with IL-15 treatment. Conversely, Nrf1 mRNA expression levels were equivocal to the vehicle control cells with IL-15 treatment when PPARα was inhibited. Nrf1 has been shown to be directly regulated by PPARα, with a greater affinity than PPARδ, which may explain the reduction in its expression levels with IL-15 treatment and PPARα inhibition [49]. Furthermore, PGC1β expression levels were not statistically different from vehicle control cells with both IL-15 and PPARα inhibitor. On the other hand, with PPARα inhibition, the stimulatory effect of IL-15 on mitochondrial activity was maintained. Although we show a potential connection between IL-15-PPARα mediated increases in some mitochondrial associated factors, these relationships do not appear to translate to functional assays, such as mitochondrial activity. It should be noted that addition of the vehicle control (DMSO) in the inhibitor studies yielded alterations in baseline mRNA expression levels when compared to mRNA levels in the absence of DMSO. Further, our data is dependent on pharmacological inhibitors of PPARs. Genetic knockdown studies would provide additional support for our data. However, the functionality of IL-15 induced increases in mitochondrial activity is relevant in mature fully differentiated SKM cells. Therefore, methodological constraints do not allow for genetic knockdown studies on fully differentiated cells with repeated treatments.

Even though our data indicate that PPARα activity is not required for IL-15 mediated mitochondrial activity in SKM cells, we definitively show that PPARδ activity is a requirement. Indeed, the master mitochondrial regulators, PGC1α and PGC1β, mRNA expression levels were both
reduced with PPARδ inhibition in combination with IL-15 stimulation. Both PGC1α and PGC1β are responsible for the numerous beneficial effects of exercise on mitochondrial processes and biogenesis and signal in concert with PPARs [50, 51]. Additionally, PGC1α is responsible for regulating mitochondrial uncoupling, via UCP2, and our data are in support of this pathway, as indicated by the reductions of UCP2 expression with inhibition of PPARδ with IL-15 treatment [52]. The importance of IL-15 induced PPARδ activation for the regulation of mitochondrial activity is further supported by the reduction of Nrf1 mRNA expression levels with PPARδ inhibition and IL-15 treatment. In this regard, not only does IL-15 signal directly through PPARδ but also its effects initiate a master metabolic regulation pathway, including UCP2 and Nrf1 as downstream targets.

5. Conclusions

It is widely accepted that PPARs play an important role in mediating mitochondrial processes to prevent and/or treat metabolic disorders [30, 32, 34, 53, 54]. We provide evidence for the requirement of PPARδ as a direct target of IL-15 signaling to carry out mitochondrial processes in SKM. Additionally, our data indicate that PPARα is not necessary for the beneficial effects of IL-15 signaling on mitochondrial activation in SKM. Although we have shown the importance of PPARδ in IL-15 signaling, the signals directly downstream the IL-2 receptor remain unknown in SKM. Therefore, examining the effects of IL-15 on IL-2R targets such as Akt and the Jak/STAT pathway is required in SKM. In order to define the complex relationship of IL-15 signaling and PPARs further in vivo studies are warranted. Overall, understanding the players involved in IL-15 signaling will give rise to potential therapies for obesity and its associated disorders.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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