Research Article

Quercetin and Quercetin-Rich Red Onion Extract Alter Pgc-1α Promoter Methylation and Splice Variant Expression

Prasad P. Devarshi, Aarin D. Jones, Erin M. Taylor, Barbara Stefanska, and Tara M. Henagan

Department of Nutrition Science, Purdue University, West Lafayette, IN, USA

Correspondence should be addressed to Tara M. Henagan; thenagan@purdue.edu

Received 27 July 2016; Revised 6 November 2016; Accepted 18 December 2016; Published 16 January 2017

Academic Editor: Rozalyn M. Anderson

Copyright © 2017 Prasad P. Devarshi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Pgc-1α and its various isoforms may play a role in determining skeletal muscle mitochondrial adaptations in response to diet. 8 wks of dietary supplementation with the flavonoid quercetin (Q) or red onion extract (ROE) in a high fat diet (HFD) ameliorates HFD-induced obesity and insulin resistance in C57BL/J mice while upregulating Pgc-1α and increasing skeletal muscle mitochondrial number and function. Here, mice were fed a low fat (LF), high fat (HF), high fat plus quercetin (HF + Q), or high fat plus red onion extract (HF + RO) diet for 9 wks and skeletal muscle Pgc-1α isoform expression and DNA methylation were determined. Quantification of various Pgc-1α isoforms, including isoforms Pgc-1α-a, Pgc-1α-b, Pgc-1α-c, Pgc-1α4, total NT-Pgc-1α, and FL-Pgc-1α, showed that only total NT-Pgc-1α expression was increased in LF, HF + Q, and HF + RO compared to HF. Furthermore, Q supplementation decreased Pgc-1α-a expression compared to LF and HF, and ROE decreased Pgc-1α-a expression compared to LF. FL-Pgc-1α was decreased in HF + Q and HF + RO compared to LF and HF. HF exhibited hypermethylation at the −260 nucleotide (nt) in the Pgc-1α promoter. Q and ROE prevented HFD-induced hypermethylation. −260 nt methylation levels were associated with NT-Pgc-1α expression only. Pgc-1α isoform expression may be epigenetically regulated by Q and ROE through DNA methylation.

1. Introduction

Peroxisome proliferator activated-receptor gamma coactivator 1 alpha (Pgc-1α) is a transcriptional coactivator that coordinates gene expression from the nuclear and mitochondrial genomes in order to determine mitochondrial biogenesis and function [1–4]. Environmentally induced upregulation of Pgc-1α plays a major role in determining skeletal muscle mitochondrial adaptations that are important in attenuating obesity and insulin resistance [1, 4–11]. Energy and nutritional status, such as the obese and diabetic state, and environmental cues, such as cold exposure, exercise training, and various dietary components, determine Pgc-1α expression [1, 2, 4–6, 12]. For example, high fat diet (HFD) feeding and palmitate treatment decrease Pgc-1α expression [6, 12, 13], whereas antiobesogenic and diabetic dietary supplements increase Pgc-1α expression [7, 13]. Traditionally, the molecular mechanisms regulating Pgc-1α have focused on posttranslational regulation of the protein affecting protein stability and its ability to bind target genes to induce transcriptional activation [1, 14]; however, more recent investigations have shown a major role of epigenetic modifications to the Pgc-1α promoter to play a role in its transcriptional regulation and mRNA expression in response to environmental and energy/nutritional inputs [5, 6, 13, 15, 16]. Exercise training, dietary fat content, long chain fatty acid exposure, and the presence of diabetes and overweight or obesity have all been shown to epigenetically regulate Pgc-1α mRNA expression and downstream mitochondrial adaptations in skeletal muscle [5, 6, 13, 15, 16].

Not only is Pgc-1α regulated posttranslationally and transcriptionally, but also several Pgc-1α isoforms with novel and specific activities have been identified [17–19]. The various expression patterns of these Pgc-1α isoforms are also regulated by environmental stimuli, including cold exposure and exercise training, and by the obese state [15, 17–22]. To date, more than ten isoforms of Pgc-1α are known to exist, arising from a combination of distinct promoter start sites.
The most extensively studied isoforms in skeletal muscle are Prx-1α-a, Prx-1α-b, Prx-1α-c, Prx-1α-d, the n-truncated spliced variant NT-Prx-1α, and the full length variant FL-Prx-1α [17–19, 21, 23, 24]. While many isoforms possess overlapping functions, several have been shown to have distinct functions in skeletal muscle [23]. For example, exercise training preferentially upregulates expression of the isoforms arising from the alternative, distal promoter region, including isoforms Prx-1α-b, Prx-1α-c, Prx-1α-d, total NT-Prx-1α, and FL-Prx-1α [23]. Additionally, Prx-1α-a, Prx-1α-b, Prx-1α-c, total NT-Prx-1α, and FL-Prx-1α are all known to play a role in exercise-induced mitochondrial adaptations in skeletal muscle [19]. Distinct from the other exercise-induced isoforms, Prx-1α-a plays a role in promoting muscle hypertrophy [21]. More recently, various isoforms of NT-Prx-1α (NT-Prx-1α-a, NT-Prx-1α-b, and NT-Prx-1α-c), derived from either the proximal or distal promoters, have been characterized. All NT-Prx-1α isoforms have been shown to be induced during either high intensity (NT-Prx-1α-a) or low intensity (NT-Prx-1α-b and NT-Prx-1α-c) exercise, contributing to upregulation of total NT-Prx-1α that occurs at all intensities of exercise [19]. Additionally, all NT-Prx-1α isoforms and total NT-Prx-1α are upregulated in skeletal muscle in response to AICAR treatment, a pharmacological activator of AMPK known to increase mitochondrial biogenesis and fatty acid oxidation in skeletal muscle [19]. Collectively, the isoforms most notably known to play roles in skeletal muscle mitochondrial adaptations to date include Prx-1α-a, Prx-1α-b, Prx-1α-c, Prx-1α-d, total NT-Prx-1α, and FL-Prx-1α. Expression patterns of these various splice variants may have the ability to prevent disease, as overexpression of total NT-Prx-1α is also known to attenuate HFD-induced obesity [25]. Interestingly, recent studies show that epigenetics may play a role in determining mRNA splicing [26] in addition to its role in determining transcription initiation and −1 nucleosome positioning may play a role in determining FL-Prx-1α and total NT-Prx-1α expression in skeletal muscle in relation to cardiovascular disease during overweight and obesity [15]. Thus, Prx-1α isoform expression may be epigenetically regulated during disease and in response to environmental stimuli, including dietary inputs.

Quercetin (Q) is a bioflavonoid that protects against mitochondrial dysfunction and attenuates HFD-induced obesity and insulin resistance when supplemented in a HFD at low concentrations [7, 27–32]. We have recently shown that a low dose (50 μg/day) of dietary Q supplementation or dietary supplementation with red onion extract (ROE), containing equivalent amounts of Q glycosides (50 μg/day), increases skeletal muscle mitochondrial number and function, leading to more complete fatty acid oxidation [7, 32], similar to the effects of exercise training on skeletal muscle. However, the effects of Q or ROE appear to occur through regulation of differential molecular mechanisms at the level of mitochondrial gene transcription [32], a process that may be controlled by the transcriptional coactivation abilities of Prx-1α.

To investigate the role of dietary fat, purified dietary Q, and ROE in determining Prx-1α DNA methylation in skeletal muscle in association with HFD-induced obesity and insulin resistance and elaborate on how these epigenetic effects associate with splice variant expression, we used diet-induced obese C57BL/6J mice as a model system. We hypothesized that the mitochondrial gene expression patterns previously observed and published [32] would be associated with differential diet-induced methylation patterns in the PGC-1 promoter and regulation of those specific Prx-1α isoforms known to play a role in skeletal muscle mitochondrial adaptations in response to environmental stimuli [17, 18, 21, 25].

2. Materials and Methods

2.1. Animals and Diets. The protocols for animal and diets have been previously published [32]. Briefly, ROE was prepared as previously described [32] and formulated into a purified high fat diet (Research Diets D12451, 45% kcal fat) to yield 17 mg/kg of quercetin equivalents [32]. 5-week-old C57BL/6J mice (Jackson Laboratories, Bar Harbor, MN, USA) were weaned onto low fat diet (LF; Research Diets 12450B, 10% kcal fat) for 1 week and then randomized into 4 dietary treatment groups (N = 10/group): LF (Research Diets 12450B, 10% kcal fat); high fat (HF; Research Diets D12451, 45% kcal fat); HF + Q (Research Diets D08072305, 45% kcal fat) with 17 mg/kg quercetin aglycone (Enzo Life Technologies ALX-385-001-G005; Farmingdale, NY, USA); or HF + RO (Research Diets D08072306). Mice were fed respective diets for 9 wks, during which time 48 h food consumption and body weight and composition via nuclear magnetic resonance (Bruker Minispec, Billerica, MA, USA) were assessed weekly. Mice were euthanized after 9 wks of feeding and quadriceps muscle was extracted and snap frozen in liquid nitrogen for further analyses. All experiments were reviewed and approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

2.2. DNA Isolation and Bisulfite Treatment. To provide a more homogenous sample, frozen quadriceps muscle was ground under liquid nitrogen using a mortar and pestle. Ground muscle was then used for the extraction of genomic DNA with a DNeasy Blood and Tissue Kit (Qiagen 69581) via the manufacturer’s protocol. The quantity and quality of the gDNA extracted were determined by spectrophotometry using a NanoDrop (Thermo Scientific, Wilmington, DE, USA). Approximately 200 ng of gDNA was subjected to bisulfite conversion using the EpiTect Bisulfite Kit (Qiagen 59104) via the manufacturer’s protocol.

2.3. Bisulfite Sequencing. Bisulfite sequencing was performed on the Qiagen PyroMark Q24 platform (Qiagen 900154). Bisulfite converted gDNA was PCR amplified using the EpiTect MSP kit (Qiagen) and biotinylated primers targeting the Prx-1α gene. Human primer sequences that amplify the region of the Prx-1α promoter surrounding the −260 nucleotide site have been previously published [6]. Here, primer sequences (IDT) that amplified and biotinylated the corresponding region within the mm10 reference genome that surrounds the methylation site were used with the PyroMark PCR kit (Qiagen 978703) per the manufacturer's
Table 1: Pgc-1α isoform primer pair sequences are shown for the forward (F) and reverse (R) primers used to measure isoform expression via qRT-PCR. Primer pairs have previously been reported by Miura et al. 2008, Ruas et al. 2012, and Zhang et al. 2009.

| Primer target | Primer sequence (5’ to 3’) |
|---------------|---------------------------|
| Pgc-1α-α F    | GCTTGACTGGGTCATTTCG       |
| Pgc-1α-α R    | ACAGAGTCTGGGTCAGATGT      |
| Pgc-1α-β F    | GACATGGATTTGGGATTGCA      |
| Pgc-1α-β R    | ACCAACCAGGACGACATTT       |
| Pgc-1α-γ F    | AGTGCATGGATGGGATGTTG      |
| Pgc-1α-γ R    | GAATTGCTGCCGGTACTCAGT     |
| Pgc-1α-δ F    | TCCACACAAACCACACAGAA      |
| Pgc-1α-δ R    | CTG GAA GAT ATG GCA CAT   |
| FL-Pgc-1α F   | TGCCATGTTAAGGCGA          |
| FL-Pgc-1α R   | CCAGATCAACCAATGACC        |
| total NT-Pgc-1α F | TGCCATGTTAAGGCGA         |
| total NT-Pgc-1α R  | CCATATCTTCCAGTAGGCC     |
| total Pgc-1α F | TGATGGAATGAGCTGGGATACAGCA |
| total Pgc-1α R | GCT CAT TGT TGT ACT GGT TGG ATA TG |

instructions. Biotinylated DNA then subjected to pyrosequencing on the PyroMark Q24 (Qiagen 9001514) with PyroMark Gold Reagents (Qiagen), following the manufacturer’s instructions. PyroMark analysis software was used to determine the DNA methylation percentage at the −260 nucleotide (nt).

2.4. RNA Isolation. Total RNA was extracted from quadriceps muscle tissue using Tri-Reagent (Molecular Research Center, Cincinnati, OH, USA) followed by further purification with a RNeasy mini kit (Qiagen, Valencia, CA, USA), as previously described [32]. The quantity and quality of the RNA were analyzed by spectrophotometry (NanoDrop, ND-1000, Thermo Scientific, Wilmington, DE, USA). RNA was reverse transcribed into a cDNA library using M-MLV reverse transcriptase (Promega, Madison, WI, USA).

2.5. qRT-PCR. qRT-PCR was performed using previously published primer pairs that targeted various Pgc-1α isoforms [17, 18, 21]. Primer sequences are shown in Table 1. All samples were run in duplicate on the ABI QuantStudio 6 Flex platform (Applied Biosystems, Foster City, CA, USA) using SYBR Green MasterMix (Applied Biosystems, Foster City, CA, USA). Gene expression was analyzed using a standard curve and normalization to cyclophilin B as the endogenous control. Data are expressed as arbitrary units (AU).

2.6. Statistical Analysis. The data were analyzed with GraphPad Prism 5.0 statistical analysis software. Results are expressed as means ± standard error. Food consumption and body weight composition parameters were analyzed by repeated measures ANOVA. All other measurements were analyzed by one-way ANOVA. A Tukey test was used post hoc as necessary. A P value < 0.05 was used to determine significance.

3. Results

3.1. Mouse Dietary Model. We have previously published the phenotypic data showing that 50 μg/g of dietary quercetin or ROE supplementation attenuates HFD-induced obesity and insulin resistance in C57BL/6J mice [32]. Interestingly, the antiobesogenic and antiadipogenic effects of quercetin and ROE occur in association with ~50% increase in skeletal muscle mitochondrial number and more complete beta oxidation in skeletal muscle [32]. In our previous studies, C57BL/6J mice received Q supplementation for 8 wks and this resulted in an increase in skeletal muscle Pgc-1α expression in skeletal muscle [32]. Thus, in the present study, we also aimed to determine if ROE acts similarly to Q to upregulate Pgc-1α in association with previously observed beneficial mitochondrial adaptations. Contrary to our previous findings, in the present study we found that, after 9 wks of supplementation, neither Q nor ROE altered total Pgc-1α expression despite the previously published improvements in skeletal muscle mitochondrial number in these animals (Figure 1).

3.2. Pgc-1α Isoform Expression. Several splice variants of Pgc-1α have recently been described, with some splice variants showing overlapping transcriptional coactivator activities and downstream physiological changes in metabolic and mitochondrial adaptations in response to environmental inputs [17–19, 21]. Due to the lack of change observed in total Pgc-1α (Figure 1) despite the significant increase in mitochondrial number, we aimed to determine if Q or ROE may act to upregulate specific splice variants of Pgc-1α using our model with known skeletal muscle mitochondrial adaptations [7, 32]. A schematic of the various Pgc-1α variants is shown in Figure 1.
measured in the present study is shown in Figure 2. Pgc-1α-a, Pgc-1α-b, Pgc-1α-c, and Pgc-1α4 differ in their N-termini due to transcription starting within either the distal, alternative (exon 1b), or proximal (exon 1a) promoters and also due to alternative splicing of exon 1b to produce exon 1b′, whereas NT-Pgc-1α and FL-Pgc-1α both contain the full exonic regions from 1b, 1a, and 2 (Figure 2(a)). In addition to splicing at the 5′ region, Pgc-1α may undergo further splicing at the 3′ end, resulting in differential C-termini. As depicted in Figure 2(a), Pgc-1α-a, Pgc-1α-b, Pgc-1α-c, and FL-Pgc-1α contain exons 6 and 7 within the respective, mature transcripts, whereas Pgc-1α4 and NT-Pgc-1α undergo alternative splicing resulting in an insertion between exons 6 and 7 and subsequent termination of translation before exon 7. Interestingly, both HF + Q and HF + RO exhibited decreases in FL-Pgc-1α compared to LF and HF groups (Figure 3(a)). HF + Q and HF + RO also showed a decrease in Pgc-1α-a compared to LF and HF, although the decrease in HF + RO compared to HF did not reach statistical significance (Figure 3(b)). HF + RO but not HF + Q showed decreased Pgc-1α-b expression compared to LF (Figure 3(c)). No change was observed in any groups for Pgc-1α-c or Pgc-1α4 (Figures 3(d) and 3(e)). Interestingly, the only differences observed between LF and HF occurred with a decrease in total NT-Pgc-1α expression (Figure 3(f)). HFD-induced decreases in total NT-Pgc-1α were prevented in HF + Q and HF + RO, who showed a significant increase in total NT-Pgc-1α expression compared to HF (Figure 3(f)).  

3.3. Pgc-1α DNA Methylation at the –260 Nucleotide. Pgc-1α has recently been shown to be hypermethylated in the skeletal muscle of type 2 diabetic individuals, in response to palmitate and oleate treatment of myocytes in vitro and by 10 wks of HFD feeding in C57BL/6J mice [6, 13]. Specific DNA methylation at the –260 nt is sufficient to decrease Pgc-1α expression and leads to detrimental skeletal muscle mitochondrial adaptations [6, 13]. To determine if Q and ROE may work to ameliorate HFD-induced skeletal muscle mitochondrial maladaptations through epigenetic regulation of Pgc-1α, bisulfite sequencing of the Pgc-1α promoter was performed, specifically measuring the percentage of DNA methylation at the regulatory –260 nt site. The bisulfite converted DNA sequence and location of the pyrosequencing primer and known DNA methylation site are shown in Figure 2(a). Pgc-1α-a, Pgc-1α-b, Pgc-1α-c, and Pgc-1α4 all contain exons 1b:1b′, 1a, and 2 as indicated. Total Pgc-1α contains both exons 6 and 7, as indicated, (b) Schematic representation of the start and stop of each Pgc-1α isoform is shown. Primer pairs have previously been designed and published, and the corresponding reference for each pair is indicated. ∗ is the alternative splice site between exons 6 and 7.

4. Discussion

DNA methylation of the Pgc-1α promoter decreases skeletal muscle Pgc-1α expression and mitochondrial number and function, both of which may be important in determining insulin sensitivity during obesity [5, 6, 8, 9, 13, 33, 34].
Environmental inputs, such as diet and exercise, alter Pgc-1α expression to determine skeletal muscle mitochondrial adaptations [1, 2, 4, 14]. Dietary supplementation with the flavonoid Q and/or the botanical extract from red onions prevents HFD-induced obesity and insulin resistance by increasing skeletal muscle Pgc-1α expression and mitochondrial function and number [7, 31, 32]. Due to their effects on Pgc-1α and mitochondrial adaptations and the ability of Pgc-1α to be epigenetically regulated, it is possible that the effects of Q and ROE occur through an epigenetic mechanism involving DNA methylation. Here, we show that 9 wks of HFD feeding, which causes increases in adiposity and insulin resistance in C57BL/6J mice [32], leads to hypermethylation at the −260 nt of the Pgc-1α promoter. Both Q and ROE attenuate HFD-induced obesity and insulin resistance while preventing HFD-induced hypermethylation of the −260 nt in Pgc-1α. Although we have previously found that 50 μg/day of Q supplementation upregulates skeletal muscle Pgc-1α in association with increased mitochondrial function in the form of more complete beta oxidation of fatty acids [7] and the mice in the present study showed an ∼50% increase in skeletal muscle mitochondrial number [32], in the present study we observed no difference in Pgc-1α expression (measured as the total Pgc-1α expression) between HF and HF + Q or HF + RO. Importantly, the effects of dietary Q supplementation on skeletal muscle Pgc-1α expression and mitochondrial adaptations occur in a dose- and time-dependent manner [7, 31]. Our previous study showed an increase in Pgc-1α at 8 wks of supplementation [7] and, in the present study, Pgc-1α was measured after 9 wks of supplementation. Despite the lack of increase in total Pgc-1α, we continued to observe Q- and ROE-induced increases in energy expenditure and mitochondrial number [32], which may partially be the result of prior Pgc-1α upregulation at 8 wks. However, mitochondrial turnover occurs rapidly, ranging from ∼1 to 17 days in a tissue-specific manner, and this turnover rate can be increased by dietary interventions, such as caloric restriction [35, 36]. Thus, one would expect that if the effects of Q and ROE are no longer beneficial after 9 wks due to the lack of change in total Pgc-1α expression, the continuous impetus of the HFD would lead to similar skeletal muscle physiologies in HF, HF + Q, and HF + RO groups; yet at
9 wks, we still observed increased mitochondrial number [32] and decreased Pgc-1α methylation in HF + Q and HF + RO. Thus, it appears that although total Pgc-1α remains unchanged, other mechanisms may compensate for this loss of Pgc-1α upregulation to perpetuate beneficial mitochondrial adaptations in HF + Q and HF + RO.

Interestingly, DNA methylation has been shown to drive mRNA splicing and Pgc-1α has several known isoforms that result from alternative splicing and alternate promoter start sites [17–19, 21, 26]. These isoforms have unique and complementary or overlapping functions, specific to each variant [17, 18, 21, 25]. Thus, it is possible that the effects of Q and ROE may be due to epigenetic regulation of mRNA splicing and subsequent alterations in Pgc-1α splice variant expression. Indeed, we show here that Q and ROE decreased expression of FL-Pgc-1α and isoform a, without changing isoforms b, c, or 4. Additionally, Q but not ROE decreased Pgc-1α isoform B in comparison to LF but HF. Here, none of the treatments had an effect on Pgc-1α isoforms 4 and c. The only isoform shown to decrease after 9 wks of HFD feeding was NT-Pgc-1α; and, both Q and ROE were able to prevent this HFD-induced decrease. These results are consistent with changes in DNA methylation status at the −260 nt, with HF increasing in methylation but HF + Q and HF + RO showing similar levels of methylation as LF. Thus at 9 wks of feeding, it is possible that the beneficial effects of Q and ROE on mitochondrial number and function are mediated via upregulation of NT-Pgc-1α and that isoform expression is dependent on epigenetic regulation of Pgc-1α. Indeed, NT-Pgc-1α has known complementary and overlapping functions to FL-Pgc-1α and may compensate for loss of FL-Pgc-1α or other isoforms [17, 25]; and it has recently been shown that Pgc-1α isoform expression is epigenetically regulated by histone methylation in response to exercise, with expression of Pgc-1α-b and of Pgc-1α-c but not of Pgc-1α-a being upregulated in conjunction with increased histone methylation in the promoter of exon 1b compared to exon 1a [16].

Here, we measured the percentage of −260 nt DNA methylation, a methylation site known to regulate Pgc-1α expression and skeletal muscle mitochondrial adaptations which is located within the proximal promoter upstream of exon 1a. Given that Q and ROE decreased Pgc-1α isoforms Pgc-1α-a and Pgc-1α-b without changing Pgc-1α-c, and Pgc-1α-a includes exon 1a, Pgc-1α-b includes exon 1b, and Pgc-1α-c includes exon 1b′, it appears that −260 nt methylation does not play a role in regulating 5′ splicing or transcription initiation within Pgc-1α proximal versus alternative, distal promoters. This is consistent with the recent publication by Lochmann et al. 2015, who similarly showed that Pgc-1α methylation was not related to Pgc-1α isoform a, b, or c expression [16]. Interestingly, total Pgc-1α expression was also not associated with DNA methylation at the −260 nt.
However, it is unclear in previous publications what isoforms of Pgc-1α were measured and correlated with −260 nt DNA methylation. It is possible that previous reports use primers that measure isoforms which are differentially spaced at the 3′ prime end; as in the present study, although we did not observe associations between −260 nt methylation and 5′ splicing or specific promoter initiation, we did see alterations in isoform expression based on spacing at the 3′ region in association with DNA methylation levels at the −260 nt. Notably, FL-Pgc-1α decreased in HF + Q and HF + RO and total NT-Pgc-1α increased. Although alternative splicing that produces these isoforms occurs at exons 6 and 7 and the intronic region between these exons [17] and DNA methylation occurs within the promoter region, promoter structure is known to be important for alternative splicing [26]. Promoter methylation regulates splicing through a complex process involving epigenetic modifications and promoter occupation of transcriptional activators [26]. DNA methylation not only alters transcription factor binding, but also directly regulates alternative splicing by recruiting RNA-binding proteins that can be transferred to the mRNA to alter the splicing pattern [26]. Although the present study is limited by measuring only the −260 nt methylation status, others have shown that methylation at the −260 nt occurs when the entire Pgc-1α promoter is hypermethylated [6]. Thus, hyper- and hypomethylation of the −260 nt may serve as a surrogate measure to indicate methylation status across the entire promoter or gene, whose methylation may play a role in determining alternative splicing and isoform expression.

In conclusion, Q and ROE show similar effects in preventing Pgc-1α−260 nt methylation induced by HFD feeding, upregulating total NT-Pgc-1α splice variant expression in skeletal muscle. Thus, the perpetuating beneficial effects of Q and ROE on body weight, body composition, energy expenditure, insulin sensitivity, and skeletal muscle mitochondrial number and function in mice after 9 wks of supplementation [32] may be mediated via upregulation of total NT-Pgc-1α.

There is a further need to study the functions and epigenetic regulation of total NT-Pgc-1α and other isoforms of Pgc-1α to determine their specific roles in regulating skeletal muscle adaptations and contribute to a lean, insulin sensitive state.

5. Conclusions

HFD causes Pgc-1α hypermethylation at the −260 nt in the Pgc-1α proximal promoter in conjunction with alterations in Pgc-1α splice variant expression in skeletal muscle. Q and ROE similarly prevent HFD-induced hypermethylation of Pgc-1α and increase NT-Pgc-1α expression in skeletal muscle. Q- and ROE-induced upregulation of NT-Pgc-1α may occur through an epigenetic mechanism and may be sufficient to increase skeletal muscle mitochondrial number and complete beta oxidation of fatty acids, contributing to attenuation of obesity and insulin resistance.

Competing Interests

The authors declare no competing interests.

Authors’ Contributions

Prasad P. Devarshi and Aarin D. Jones contributed equally to this work. Aarin D. Jones performed experiments, analyzed data, and edited the manuscript. Prasad P. Devarshi analyzed data and wrote and edited the manuscript. Erin M. Taylor and Barbara Stefanska performed experiments and edited the manuscript. Tara M. Henagan conceptualized, designed, and performed experiments, analyzed data, wrote and edited the manuscript, and provided funding.

Acknowledgments

This work was funded by Indiana CTSI Core Pilot Funding UL1TR001108 and NIH NCCAM 5P50-AT002776-09 grants.

References

[1] D. Knutti and A. Kralli, "PGC-1, a versatile coactivator," Trends in Endocrinology & Metabolism, vol. 12, no. 8, pp. 360–365, 2001.
[2] H. Liang and W. F. Ward, "PGC-1α: a key regulator of energy metabolism," Advances in Physiology Education, vol. 30, no. 4, pp. 145–151, 2006.
[3] J. Lin, H. Wu, P. T. Tarr et al., "Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres," Nature, vol. 418, pp. 797–801, 2002.
[4] Z. Wu, P. Puigserver, U. Andersson et al., "Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1α," Cell, vol. 98, no. 1, pp. 115–124, 1999.
[5] R. Barrès, J. Yan, B. Egan et al., "Acute exercise remodels promoter methylation in human skeletal muscle," Cell Metabolism, vol. 15, no. 3, pp. 405–411, 2012.
[6] R. Barrès, M. E. Osler, J. Yan et al., "Non-CpG methylation of the PGC-1α promoter through DNMT3B controls mitochondrial density," Cell Metabolism, vol. 10, no. 3, pp. 189–198, 2009.
[7] T. M. Henagan, N. R. Lenard, T. W. Gettys, and L. K. Stewart, "Dietary quercetin supplementation in mice increases skeletal muscle PGC1α expression, improves mitochondrial function and attenuates insulin resistance in a time-specific manner," PLoS ONE, vol. 9, no. 2, Article ID e89365, 2014.
[8] I. Pagel-Langenickel, J. Bao, L. Pang, and M. N. Sack, "The role of mitochondria in the pathophysiology of skeletal muscle insulin resistance," Endocrine Reviews, vol. 31, no. 1, pp. 25–51, 2010.
[9] M.-E. Patti and S. Corvera, "The role of mitochondria in the pathogenesis of type 2 diabetes," Endocrine Reviews, vol. 31, no. 3, pp. 364–395, 2010.
[10] J.-A. Kim, Y. Wei, and J. R. Sowers, "Role of mitochondrial dysfunction in insulin resistance," Circulation Research, vol. 102, no. 4, pp. 401–414, 2008.
[11] J. C. Bournat and C. W. Brown, "Mitochondrial dysfunction in obesity," Current Opinion in Endocrinology, Diabetes and Obesity, vol. 17, no. 5, pp. 446–452, 2010.
[12] L. M. Sparks, H. Xie, R. A. Koza et al., "A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle," Diabetes, vol. 54, no. 7, pp. 1926–1933, 2005.
[13] T. M. Henagan, B. Stefanska, Z. Fang et al., "Sodium butyrate epigenetically modulates high-fat diet-induced skeletal muscle mitochondrial adaptation, obesity and insulin resistance
through nucleosome positioning," *British Journal of Pharmacology*, vol. 172, no. 11, pp. 2782–2798, 2015.

[14] P. J. Fernandez-Marcos and J. Auwerx, "Regulation of PGC-δ, a nodal regulator of mitochondrial biogenesis," *American Journal of Clinical Nutrition*, vol. 93, no. 4, pp. 884S–890S, 2011.

[15] T. M. Henagan, L. K. Stewart, L. A. Forney, L. M. Sparks, N. Johanssen, and T. S. Church, "PGC1-1 nucleosome position and splice variant expression and cardiovascular disease risk in overweight and obese individuals," *PPAR Research*, vol. 2014, Article ID 895734, 7 pages, 2014.

[16] T. L. Lochmann, R. R. Thomas, J. P. Bennett Jr., and S. M. Taylor, "Epigenetic modifications of the PGC-δ promoter during exercise induced expression in mice," *PLoS ONE*, vol. 10, no. 6, Article ID e0129647, 2015.

[17] Y. Zhang, P. Huypens, A. W. Adamson et al., "Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1α," *Journal of Biological Chemistry*, vol. 284, no. 47, pp. 32813–32826, 2009.

[18] S. Miura, Y. Kai, Y. Kamei, and O. Ezaki, "Isoform-specific increases in murine skeletal muscle peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA in response to β2-adrenergic receptor activation and exercise," *Endocrinology*, vol. 149, no. 9, pp. 4527–4533, 2008.

[19] X. Wen, J. Wu, J. S. Chang et al., "Effect of exercise intensity on isoform-specific expressions of NT-PGC-1α mRNA in mouse skeletal muscle," *BioMed Research International*, vol. 2014, Article ID 402175, 11 pages, 2014.

[20] D. V. Popov, E. A. Lysenko, I. V. Kuzmin, O. L. Vinogradova, and A. I. Grigoriev, "Regulation of PGC-δ isoform expression in skeletal muscles," *Acta Naturae*, vol. 7, no. 1, pp. 48–59, 2015.

[21] J. L. Ruas, J. P. White, R. R. Rao et al., "A PGC-δ isoform induced by resistance training regulates skeletal muscle hypertrophy," *Cell*, vol. 151, no. 6, pp. 1319–1331, 2012.

[22] M. Ydfors, H. Fischer, H. Mascher, E. Blomstrand, J. Norrbom, and T. Gustafsson, "The truncated splice variants, NT−PGC−δ and PGC−δA4, increase with both endurance and resistance exercise in human skeletal muscle," *Physiological Reports*, vol. 1, Article ID e00140, 2013.

[23] V. Martinez-Redondo, A. T. Pettersson, and J. L. Ruas, "The hitchhiker’s guide to PGC-δ isoform structure and biological functions," *Diabetologia*, vol. 58, no. 9, pp. 1969–1977, 2015.

[24] M. Tadaishi, S. Miura, Y. Kai, Y. Kano, Y. Oishi, and O. Ezaki, "Skeletal muscle-specific expression of PGC-δb, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake," *PLoS ONE*, vol. 6, no. 12, Article ID e28290, 2011.

[25] H.-J. Jun, Y. Joshi, Y. Patil, R. C. Noland, and J. S. Chang, "NT−PGC−δ activation attenuates high-fat diet-induced obesity by enhancing brown fat thermogenesis and adipose tissue oxidative metabolism," *Diabetes*, vol. 63, no. 11, pp. 3615–3625, 2014.

[26] A. R. Kornblüth, M. De La Mata, J. P. Fededa, M. J. Muñoz, and G. Nogués, "Multiple links between transcription and splicing," *RNA*, vol. 10, no. 10, pp. 1489–1498, 2004.

[27] J. M. Davis, E. A. Murphy, M. D. Carmichael, and B. Davis, "Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance," *American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 296, no. 4, pp. R1071–R1077, 2009.

[28] C. Carrasco-Pozo, M. L. Mizgier, H. Speisky, and M. Gotteland, "Differential protective effects of quercetin, resveratrol, rutin and epigallocatechin gallate against mitochondrial dysfunction induced by indomethacin in Caco-2 cells," *Chemico-Biological Interactions*, vol. 195, no. 3, pp. 199–205, 2012.

[29] N. Rayamajhi, S.-K. Kim, H. Go et al., "Quercetin induces mitochondrial biogenesis through activation of HO-1 in HEPG2 cells," *Oxidative Medicine and Cellular Longevity*, vol. 2013, Article ID 154279, 10 pages, 2013.

[30] L. Rivera, R. Morón, M. Sánchez, A. Zarzuelo, and M. Galisteo, "Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats," *Obesity*, vol. 16, no. 9, pp. 2081–2087, 2008.

[31] L. K. Stewart, J. L. Soileau, D. Ribnicky et al., "Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet," *Metabolism: Clinical and Experimental*, vol. 57, supplement 1, pp. S39–S46, 2008.

[32] T. M. Henagan, W. T. Cefalu, D. M. Ribnicky et al., "In vivo effects of dietary quercetin and quercetin-rich red onion extract on skeletal muscle mitochondria, metabolism, and insulin sensitivity," *Genes & Nutrition*, vol. 10, article 2, 2015.

[33] K. Hojlund, M. Mogensen, K. Sahlin, and H. Beck-Nielsen, "Mitochondrial dysfunction in type 2 diabetes and obesity," *Endocrinology and Metabolism Clinics of North America*, vol. 37, no. 3, pp. 713–731, 2008.

[34] T. R. Koves, J. R. Ussher, R. C. Noland et al., "Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance," *Cell Metabolism*, vol. 7, no. 1, pp. 45–56, 2008.

[35] S. Miwa, C. Lawless, and T. Von Zglinicki, "Mitochondrial turnover in liver is fast in vivo and is accelerated by dietary restriction: application of a simple dynamic model," *Aging Cell*, vol. 7, no. 6, pp. 920–923, 2008.

[36] R. A. Menzies and P. H. Gold, "The turnover of mitochondria in a variety of tissues of young adult and aged rats," *Journal of Biological Chemistry*, vol. 246, no. 8, pp. 2425–2429, 1971.