Loss of Pgc-1α expression in aging mouse muscle potentiates glucose intolerance and systemic inflammation

Sarah Szcelecki,1,3 Aurèle Besse-Patin,1,4 Alexandra Abboud,1 Sandra Kleiner,5 Dina Laznik-Bogoslavski,5 Christiane D. Wrann,5 Jorge L. Ruas,6 Benjamin Haibe-Kains,2 and Jennifer L. Estall1,3,4

1Divisions of Cardiovascular and Metabolic Disease and 2Systems Biology and Medicinal Chemistry, Institut de Recherches Cliniques de Montréal, Montreal, Quebec, Canada; 3Department of Experimental Medicine, McGill University, Montreal, Quebec, Canada; 4Faculty of Medicine, University of Montréal, Montreal, Quebec, Canada; 5Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts; and 6Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

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Loss of Pgc-1α expression in aging mouse muscle potentiates glucose intolerance and systemic inflammation. Am J Physiol Endocrinol Metab 306: E157–E167, 2014. First published November 26, 2013; doi:10.1152/ajpendo.00578.2013.—Diabetes risk increases significantly with age and correlates with lower oxidative capacity in muscle. Decreased expression of peroxisome proliferator-activated receptor-γ coactivator-1α (Pgc-1α) and target gene pathways involved in mitochondrial oxidative phosphorylation are associated with muscle insulin resistance, but a causative role has not been established. We sought to determine whether a decline in Pgc-1α and oxidative gene expression occurs during aging and potentiates the development of age-associated insulin resistance. Muscle-specific Pgc-1α knockout (MKO) mice and wild-type littermate controls were aged for 2 yr. Genetic signatures of skeletal muscle (microarray and mRNA expression) and metabolic profiles (glucose homeostasis, mitochondrial metabolism, body composition, lipids, and indirect calorimetry) of mice were compared at 3, 12, and 24 mo of age. Microarray and gene set enrichment analysis highlighted decreased function of the electron transport chain as characteristic of both aging muscle and loss of Pgc-1α expression. Despite significant reductions in oxidative gene expression and succinate dehydrogenase activity, young mice lacking Pgc-1α in muscle had lower fasting glucose and insulin. Consistent with loss of oxidative capacity during aging, Pgc-1α and Pgc-1β expression were reduced in aged wild-type mouse muscle. Interestingly, the combination of age and loss of muscle Pgc-1α expression impaired glucose tolerance and led to increased fat mass, insulin resistance, and inflammatory markers in white adipose and liver tissues. Therefore, loss of Pgc-1α expression and decreased mitochondrial oxidative capacity contribute to worsening glucose tolerance and chronic systemic inflammation associated with aging.

Address for reprint requests and other correspondence: J. L. Estall, IRCM, 110 Ave. Des Pins Ouest, Montreal, QC, Canada H2W 1R7 (e-mail: jennifer.estall@ircm.qc.ca).

Generation of a new Pgc-1α floxed mouse line. Previously generated male Pgc-1α floxed mice are sterile (29), prohibiting generation of homozygous floxed mice (19). As reduced Pgc-1α in multiple
dicial health play a significant causative role or whether they are simply a consequence of disease progression.

Altered mitochondrial function can lower ATP synthesis, decrease lipid oxidation, and increase reactive oxygen species, causing energy imbalances and oxidative damage. Many genes encoding mitochondrial electron transport chain (ETC) components are decreased in aged muscle (56), consistent with observed decreases in oxidative capacity (47). Aged muscle has lower mitochondrial density (7), and mitochondria are often depolarized or nonfunctional (41). Similarly, patients with diabetes and/or insulin resistance have decreased expression of gene pathways involved in oxidative phosphorylation (34, 38). Thus, diabetic and aging muscle share similar deficiencies in mitochondrial function, linking ETC dysfunction to glucose intolerance.

Peroxisome proliferator-activated receptor-γ coactivator-1α (Pgc-1α) expression is decreased in aging human muscle (13, 44), and dysregulation of this transcriptional coactivator may underlie lower mitochondrial function and muscle pathologies associated with age (8). PGC-1α is considered a master regulator of mitochondrial biogenesis and function, acting as an upstream regulatory switch for gene pathways controlling oxidative phosphorylation, fatty acid oxidation, reactive oxygen species detoxification, and mitochondrial density. Overexpression of PGC-1α in muscle protects aging mice from age-related muscle wasting and glucose intolerance (50, 52), and yet tight regulation of PGC-1α expression is required to maintain efficient glucose and lipid handling in obese mice (6, 19, 51). Expression of Pgc-1α in muscle is low in type 2 diabetics and related family members (2, 21, 33, 38) but can be increased with exercise to possibly prevent or reverse metabolic abnormalities (3, 25, 44, 49). Thus, a decline in Pgc-1α expression in aging muscle (8, 13), brought on by factors such as diet, obesity, or decreased physical activity, may play a significant role in the age-associated development of glucose intolerance.

In the current study, we address whether decreasing muscle Pgc-1α and oxidative gene expression can serve as a foundation for age-related glucose intolerance. We hypothesized that long-term reductions in muscle Pgc-1α expression, causing persistent yet moderate loss of mitochondrial content and function, would exacerbate glucose intolerance later in life.

METHODS

Generation of a new Pgc-1α floxed mouse line. Previously generated male Pgc-1α floxed mice are sterile (29), prohibiting generation of homozygous floxed mice (19). As reduced Pgc-1α in multiple
tissues can have potentially confounding effects on whole body glucose metabolism. (27, 29), a new line of floxed Pgc-1α mice lacking the neomycin selection cassette was generated. Briefly, floxed Pgc-1α mice (29) were bred to mice expressing low-efficiency Cre recombinase (Ella-cre, no. 003724; Jackson Laboratories). Selected progeny with intact Pgc-1α floxed loci but lacking the neomycin cassette were bred to C57B/6N mice to remove the Ella-cre transgene. Exclusion of exons 3–5 in these floxed mice by Cre recombinase prevents protein expression of all currently identified PGC-1α isoforms (B6.129-PparcIa1m2Brsp/J, no. 009666; Jackson Laboratories).

Muscle-specific Pgc-1α knockout mice. The regenerated line of Pgc-1αfloxed mice was bred to a myogenin-Cre line (28) to generate skeletal muscle-specific Pgc-1α knockout (MKO) mice (Pgc-1αfloxed/myo-Cre). Test groups consisted of male mice homozygous for the floxed Pgc-1α alleles expressing myogenin-Cre (MKOs; 50%) or no transgene (50%, age-matched, littermate controls). Aging groups started with n = 13–17. Mice with the myogenin-Cre transgene alone responded similarly to wild types (WT; not shown). Mice were on a mixed C57B/6J/6N/129 background and maintained on a rodent chow (12:12-h light-dark cycle) over 3 days using the Comprehensive Laboratory Animal Monitoring System (Columbus Instruments). Air consumption, carbon dioxide production, and fractional excretion of water were measured using the Oxymax (Columbus Instruments). Oxygen consumption, carbon dioxide production, and fractional excretion of water were measured using the Oxymax (Columbus Instruments).

Serum glucose, lipids, body composition, and glucose/insulin tolerance tests. Blood glucose was measured in tail blood using a standard glucometer at the times indicated. Serum insulin, IL-6, and TNFα were determined by ELISA and triglycerides and nonesterified fatty acids by colorimetric assay (Specialized Assay Core; Joslin Diabetes Center). Mice were fasted for 16 or 6 h prior to intraperitoneal (ip) injection of 1 g/kg D-glucose or 0.8 U/kg insulin (Humulin), respectively. Fat and lean mass was determined by nuclear magnetic resonance in live mice (EchoMRI).

Islet histology and insulitis score. Formalin-fixed pancreata were mounted and sectioned to capture maximal surface area. Following hematoxylin and eosin staining, lymphocyte infiltration for all islets was scored and an insulitis score calculated as described previously (17). Briefly, scoring was assigned as follows: 0 = lymphocytes being present; 1 = peri-islet infiltration (lymphocytes on the periphery, not fully surrounding the islet); 2 = immune infiltration surrounding the entire islet, but with the islet appearing mostly intact; 3 = one-half of the islet being infiltrated (engulfed) by lymphocytes; and 4 = the entire islet being engulfed (few β-cells evident). Insulitis per islet was calculated as

\[ \text{inflammation score} \times \text{no. of islets} \]

representing the average inflammation state of each islet per mouse. Two independent reviewers scored blinded sections from each mouse.

Statistical analysis. Statistical significance (P < 0.05) for tolerance tests and mRNA expression was assessed by two-way ANOVA (with repeated measures for glucose tolerance tests), followed by Fisher’s least significant difference post hoc test. Area under the curve (AUC) was calculated with baseline set at fasting glucose. Comparisons between two independent groups were performed using either a two-tailed unpaired Student t-test or a Mann-Whitney U-test if the distribution was not Gaussian (by Shapiro-Wilk normality test). Two-way ANOVA was used when two factors’ (age and genotype) effect on gene expression was assessed, followed by a Holm-Sidak’s multiple comparison test for significance of each gene. To determine effect of one factor (genotype or age) on multiple genes, we performed an unpaired Student t-test for each gene and corrected our analysis for multiple comparisons (FDR = 10%). Analysis was completed using GraphPad Prism. All data points represent mean ± SE.
RESULTS

Aging abolishes compensatory increases in skeletal muscle Pgc-1α expression. As expected, young male MKO mice exhibited a >95% knockdown of Pgc-1α mRNA in skeletal muscle compared with WT littermates (Fig. 1A). Interestingly, there was a compensatory increase in Pgc-1β mRNA in all skeletal muscles that were tested (Fig. 1B). PGC-1α is a closely related family member that may share significant overlap in function to PGC-1α (45, 57). Expression of Pgc-1α and Pgc-1β was unchanged in other tissues examined, including cardiac muscle, confirming specificity of the knockdown to skeletal muscle only. We next compared muscle RNA from young (6 mo old) and old (24 mo old) WT and MKO mice to determine whether Pgc-1 gene expression was impacted by age. Interestingly, expression of both Pgc-1α and Pgc-1β mRNA decreased in aged WT skeletal muscle (Fig. 1C), and the compensatory increase in Pgc-1β seen in young MKO muscle was lost in all skeletal muscle groups with aging (quadriceps; Fig. 1C [other muscles not shown]).

Decreased mitochondrial oxidative capacity is a major effector pathway shared by aged muscle and muscle lacking PGC-1α. To determine whether biological processes impacted by loss of Pgc-1α intersect with those altered during aging, global changes in muscle mRNA expression in gastrocnemius muscle from young (10 wk old) and old (24 mo old) WT and MKO mice were compared using genome-wide Affymetrix GeneChip arrays.

Although >2,500 genes were differentially expressed in young vs. old muscle samples (FDR <25%; Fig. 2A and Supplemental Data Set S1), only 11 molecular pathways were enriched following GSEA (FDR <25%; Fig. 2B and Supplemental Data Set S3). In comparison, 475 individual genes were different between WT and MKO mice regardless of age (FDR <25%; Fig. 2A), with GSEA identifying 95 gene sets (FDR <25%; Fig. 2B). Strikingly, 43% of the genes affected by loss of Pgc-1α were also changed in aging muscle (8% of total age-affected genes). Within this pool of 206 genes shared by both Pgc-1α loss and aging (Fig. 2A), 80% changed in the same direction (Supplemental Data Set S4), suggesting that a significant proportion of genes regulated by Pgc-1α (~35%) may be involved in the molecular program of muscle aging.

Although PGC-1α has been implicated in multiple aspects of muscle biology, the top eight gene sets affected by Pgc-1α loss were pathways of mitochondrial metabolism (Fig. 2C). Of note, only two gene sets were shared by genotype and age, “electron transport chain” function and “regulation of TGFβ signaling” (Fig. 2C), illustrating that altered oxidative phosphorylation is indeed a hallmark of both reduced Pgc-1α and aging muscle in mice.

Aging exaggerates decreased oxidative capacity in muscle. As predicted by expression array data, mitochondrial genes were sensitive to both aging and loss of Pgc-1α (Fig. 3A). It is already established that PGC-1α is an important regulator of nuclear-encoded mitochondrial gene and protein expression (12, 55). To illustrate whether aging exaggerated decreases in MKO mice, we directly compared young vs. old mice by normalizing data to age-matched controls (Fig. 3B). This demonstrated that aging further lowered expression of many nuclear-encoded mitochondrial genes. Although Tfam expression was not significantly affected by Pgc-1α loss, mtDNA-encoded Nd1 and Atp6 were decreased in young MKO muscle, suggesting an early decrease in mitochondrial mass (Fig. 3A). Aging alone decreased mtDNA transcripts (Fig. 3A); however, unlike nuclear-encoded mitochondrial genes, aging did not further exacerbate mtDNA decreases caused by Pgc-1α ablation (Fig. 3B).

SDH activity (an indicator of mitochondrial oxidative capacity) in muscle was decreased by age alone and loss of Pgc-1α in both young and old gastrocnemius muscle (Fig. 3C).

The combination of reduced muscle Pgc-1α and aging worsened glucose intolerance. Because advanced age is a significant risk factor for diabetes, and low muscle ETC activity is linked to insulin resistance, we tested whether long-term loss of muscle Pgc-1α aggravated glucose intolerance. Chow-fed,
WT, and MKO mice were followed over a period of 2 yr. At 3 mo of age, MKO mice had significantly decreased fasting glucose and circulating insulin (Table 1), and blood glucose was significantly lower 20 min following an ip glucose challenge (Fig. 4A). At 12 mo of age, although glycemia remained lower in MKO mice at fasting and 20 min post-glucose challenge, overall glucose clearance tended to worsen (Fig. 4B). For both 3- and 12-mo-old mice, despite similar AUCs (Fig. 4, A and B), the shapes of the MKO glucose disposal curves were different compared with WT controls (significant interaction between time and genotype, 2-way ANOVA, P < 0.05), suggesting that loss of skeletal muscle Pgc-1α impacted glucose excursion kinetics. After 24 mo, MKO mice were significantly less glucose tolerant than controls (Fig. 4C), and total AUC was increased. Thus, despite reduced mitochondrial gene expression and oxidative capacity at all ages, significant glucose intolerance manifested only after prolonged aging.

MKO mice developed insulin resistance with advanced age.

We next assessed peripheral insulin sensitivity by insulin tolerance test. Similar to published reports (20, 57), younger MKOs (6 mo old) showed no differences in response to exogenous insulin (Table 1), and blood glucose was significantly lower 20 min following an ip glucose challenge (Fig. 4A). At 12 mo of age, although glycemia remained lower in MKO mice at fasting and 20 min post-glucose challenge, overall glucose clearance tended to worsen (Fig. 4B). For both 3- and 12-mo-old mice, despite similar AUCs (Fig. 4, A and B), the shapes of the MKO glucose disposal curves were different compared with WT controls (significant interaction between time and genotype, 2-way ANOVA, P < 0.05), suggesting that loss of skeletal muscle Pgc-1α impacted glucose excursion kinetics. After 24 mo, MKO mice were significantly less glucose tolerant than controls (Fig. 4C), and total AUC was increased. Thus, despite reduced mitochondrial gene expression and oxidative capacity at all ages, significant glucose intolerance manifested only after prolonged aging.

**Fig. 2. mRNA expression and gene set enrichment analysis in MKO vs. aging muscle. A and B:** unique and overlapping transcript no. (A) and enriched gene sets (B) identified by multivariant analysis of expression arrays (n = 3; P < 0.05, FDR = 25%) of gastrocnemius muscle RNA from WT vs. MKO (genotype) or 10-wk-old vs. 24-mo-old (age) mice. C: the top 10 significantly enriched gene sets [ranked by false discovery rate (FDR)] for genotype or age. Gray (genotype) or black (age) bars represent relative strengths of gene set enrichment (FDR, x-axis). Gray dashed line represents cutoff of significance (FDR = 25%), and light gray boxes denote pathways shared across genotype and age data.

Body composition was not different in young mice, old MKO mice had increased fat and decreased lean masses (Fig. 5C), with no significant difference in circulating triglycerides or free fatty acids in serum (Fig. 5, D and E). Despite decreased fasting insulin (Table 1) in MKOs, fed levels of serum insulin were similar (Fig. 5F), suggesting no defects in postprandial insulin secretion. Despite decreased oxidative capacity in MKO muscle, intramuscular triglyceride levels trended lower in old MKOs (Fig. 5G), arguing against insulin resistance due to high intramuscular lipids. The GLUT4 insulin-sensitive glucose transporter is a target of PGC-1α (32); however, Glut4 mRNA levels were unchanged in young mice and significantly lower only in aged MKO soleus, not gastrocnemius or other metabolically active tissues (Fig. 5, H and I). No significant differences were noted in total body weight between WT and MKO mice (Table 1), although the MKOs tended to be heavier.

There were also no significant differences between genotypes in VO2, VCO2, heat production, activity, or food intake by indirect calorimetry, regardless of whether data were corrected for total body weight (Fig. 6, A–E) or lean body mass (data not shown). However, aged MKO mice exhibited significantly lower RERs during the dark phase only (Fig. 6F), indicating a shift toward lipid metabolism. Because mice generally eat during the dark phase, increased catabolism of lipids when...
circulating insulin levels are highest can support insulin resistance (4, 15). And while defects in postprandial insulin secretion could also contribute to the shift in fuel utilization, fed insulin levels in 6-mo-old MKOs were similar to WT (Fig. 5F).

Loss of muscle Pgc-1α causes age-dependent inflammation in white adipose and liver tissue. Low-grade, chronic inflammation is associated with aging and insulin resistance. Because loss of muscle Pgc-1α may increase circulating IL-6 (19), we hypothesized that increased inflammatory signaling in muscle contributes to glucose intolerance in old MKO mice. However, there was no significant increase in mRNA levels of Il-6, Tnfα, Socs3, or Mcp-1 (Ccl2) in sedentary young or aged MKO muscle (Fig. 7A). Cd68 expression (a marker of macrophage infiltration) and circulating TNFα were also similar between genotypes (Fig. 7, A and B).

Since it was hypothesized that loss of muscle Pgc-1α leads to inflammatory signaling in islets (19), we assessed lymphocytic infiltration. The number of inflamed islets, the severity of lymphocyte infiltration, and the insulitis score of young MKO

Table 1. Metabolic parameters in WT vs. MKO mice at different ages

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<td>Weight, g</td>
<td>23.4</td>
<td>0.9</td>
<td>25.7</td>
<td>0.7</td>
<td>36.6</td>
<td>1.2</td>
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<td>Fasting glucose, mg/dl</td>
<td>96.4 ± 5.7</td>
<td>79.3 ± 6.8</td>
<td>97.6 ± 4.3</td>
<td>83.8 ± 2.8</td>
<td>91.0 ± 6.0</td>
<td>76.6 ± 9.5</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>0.49</td>
<td>± 0.05</td>
<td>0.37</td>
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<td>1.06</td>
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Data are expressed as means ± SE (n = 9–12 mice for 3- to 12-mo-old groups, n = 5 for 24-mo-old group). WT, wild type; MKO, muscle-specific Pgc-1α knockout. *P < 0.05, comparing WT and MKO of similar age.
islets trended higher in younger mice but was not significant (Fig. 7C). Unexpectedly, quantitative histological analysis demonstrated that old MKO islets were actually significantly less inflamed than old WT littermates. No overt differences in islet size were noted (not shown). Thus, to investigate other possible mechanisms contributing to glucose intolerance and insulin resistance in aged MKO mice, we investigated inflammatory gene expression in other tissues that play a role in glucose clearance. In contrast to muscle, there was a significant increase in Il-6 and Mcp-1 expression in the white adipose tissue of young MKOs that became more pronounced with age (Fig. 7D). Similarly, inflammatory markers increased with age in livers of mice lacking Pgc-1α/H9251 in skeletal muscle (Fig. 7E).

DISCUSSION

Reduced expression of Pgc-1 coactivators in muscle leads to significant defects in mitochondrial oxidative capacity (45, 55, 57). We show that this is not sufficient to worsen insulin sensitivity, but when combined with advanced age, it promotes glucose intolerance, insulin resistance, and inflammation in white adipose and liver. Thus, both genotype and chronological age played a role, highlighting an interaction between the two variables. Defects in mitochondrial function in muscle as a cause of insulin resistance are widely debated (14, 22). In fact, we show that young MKO mice had modestly improved metabolic parameters (fasting glucose, insulin, and glycemia immediately following glucose challenge) despite lower mitochondrial gene expression and function. This is consistent with other mouse models of disrupted mitochondrial function, including muscle-specific knockout of the Tfam and Aif genes (43, 53). The mechanism for improved glucose homeostasis in young MKOs and these other mouse models remains unclear but may involve increased glucose uptake or AMP-activated protein kinase activity.

It is important to note that most studies are performed in young mice (2–4 mo), whereas we investigated the long-term effects of modestly reduced muscle mitochondrial function. Our model provides mechanistic evidence that decreased Pgc-1α and deficiencies in electron transport chain function can indeed precede and lead to insulin resistance and impaired glucose clearance over time, which is particularly relevant, as age is a significant risk factor for diabetes. Consistent with our observations, overexpression of Pgc-1α in muscle improves glucose handling in sedentary aged mice (52) but causes insulin resistance in young high-fat-fed mice (6) that can be reversed by exercise (50). Although these studies support targeting muscle PGC-1s and mitochondria to improve insulin sensitivity, they highlight the importance of considering confounding variables such as physical activity, diet, and age. In fact, detrimental effects of age on metabolism can be improved by regular exercise (25, 31). In our current study, we limited the ability of the mice to exercise to better mimic the long-term sedentary lifestyle associated with metabolic disease. However, since muscle PGC-1α is intimately linked to exercise physiology, it would be interesting to evaluate how increased physical activity impacts the metabolic homeostasis of aging MKOs.

Our results in young MKOs contrast those of Handschin et al. (19), who reported decreased body weight and fat mass, increased energy expenditure, and resistance to diet-induced obesity despite glucose intolerance. One potential explanation for the discrepancy is their use of control mice lacking one allele of Pgc-1α in all tissues (due to breeding problems of their floxed line). Reduced Pgc-1α in metabolically active tissues such as liver, adipose, and brain has multiple confounding effects on baseline whole body glucose metabolism (11, 24, 26, 29, 30). In fact, aged whole body Pgc-1α knockout mice have increased circulating TNFα and IL-6 with no appreciable increase in muscle inflammatory signaling (36). We do note a trend toward increased islet lymphocyte infiltration in our young MKOs, consistent with evidence of pancreatic inflammation. However, we did not observe altered islet size or trends.
Fig. 5. Aging promoted insulin resistance, increased adipose tissue mass, and lowered respiratory exchange ratio (RER) in MKO mice. A and B: insulin tolerance tests (ITT) in 6- (A) and 24-mo-old (B) WT and MKO mice (n = 5). C: body composition of mice by NMR (n = 6–8). D–G: triglyceride (D), free fatty acid (FFA; E), insulin (F), and intramuscular triglyceride (TG) content (G) measured by colorimetric assay using serum from fed WT and MKO mice at 24 mo of age (n = 5–9). G and I: Glut4 mRNA expression in tissues from young (3 mo, n = 8–9; H) and aged (24 mo, n = 5; I) WT vs. MKO mice. Data points represent means ± SE. *P < 0.05 WT vs. MKO. Gene expression is normalized to mean expression of corresponding tissue in age-matched WT mice. WAT, white adipose tissue; BAT, brown adipose tissue.
morphology, and interestingly, old MKO islets had less age-induced lymphocyte infiltration. Although these data suggest that MKO islets are protected from increased systemic inflammatory signaling associated with aging, we did not assess β-cell function in aged MKOs, and it is possible that secretory capacity was also affected.

mRNA expression analysis in muscle uncovered a potentially cooperative role for PGC-1αβ in muscle glucose homeostasis. Pgc-1αβ mRNA levels were higher in all skeletal muscle groups of young MKO mice. Although the mechanism for this seemingly compensatory increase is not known, increased Pgc-1αβ expression in young mice may limit or delay the consequences of losing Pgc-1α. Since complete loss of both Pgc-1α and -1β decreases the oxidative capacity of muscle but does not in itself worsen glucose tolerance in young mice (45, 57), our data suggest that a combination of advanced age and reduction of both Pgc-1α and -1β is required for impairment of glucose handling. This is consistent with a growing body of evidence suggesting that reduced mitochondrial function or content in muscle does not directly impair glucose tolerance but may intersect with other genetic or environmental factors to worsen disease (25). It is also possible that loss of muscle Pgc-1αβ impacts unique gene targets not shared with Pgc-1α and not involved in mitochondrial oxidation. Thus, it will be important to evaluate the individual and/or synergistic contributions of each family member to the observed phenotype.

Our model suggests inflammation in white adipose and liver tissues as an underlying mechanism of insulin resistance related to decreased mitochondrial oxidative capacity in aging muscle. Increased inflammation in these tissues is believed to contribute to the pathogenesis of type 2 diabetes and the metabolic syndrome (9, 16, 39, 48) and provides a plausible explanation for the age-dependent development of glucose intolerance, insulin resistance, and decreased circulating insulin in aged MKO mice. Important unanswered questions are how loss of Pgc-1α in muscle impacts inflammatory signaling in other tissues and whether these effects are due to defects in mitochondrial metabolism or nonmitochondrial actions of PGC-1. Overexpressed Pgc-1α or -1β inhibits NF-κB signaling in muscle cells (1, 10), and forced exercise potentiates inflammation in muscle lacking muscle Pgc-1α (18, 54). However, lack of increased inflammatory markers in muscle of sedentary MKO mice suggests that muscle may not be the primary site of inflammation and implicates alternative mechanisms of interorgan cross-talk. Recently, PGC-1α was shown to regulate the expression of myokines such as FNDC5, myostatin, and IGF-I (5, 35, 46), and our GSEA analysis identifies altered muscle TGFβ signaling in both aged and MKO muscle, indicating that alterations in hormone expression, secretion, or
action may mediate the non-cell-autonomous effects of losing muscle Pgc-1α on whole body glucose homeostasis.

Our gene expression analysis also illustrated that a significant proportion of muscle Pgc-1α activity (~35%) may be involved in molecular mechanisms underlying aging. However, only 6% of total age-related gene changes were mimicked by loss of Pgc-1α. Thus, although decreased Pgc-1α may not be sufficient to drive muscle aging, it appears to play a significant role in age-associated loss of muscle oxidative capacity and glucose intolerance. Of note, GSEA identified only altered ETC function within aging muscle, whereas other pathways of mitochondrial biology (lipid oxidation, ATP metabolism, or tricarboxylic acid cycling) were not significantly enriched. Although this may be a function of the large aging data set and limits of our analysis, it is consistent with studies specifically identifying oxidative phosphorylation as a major player in deregulated muscle metabolism during aging (40, 56).

Diabetes is a progressive and chronic disorder in humans, with diagnosis commonly made in midlife to late life. Evidence suggests that decreased physical activity, increased adiposity,
and gradually declining mitochondrial function act synergistically to regulate insulin sensitivity during aging. Importantly, reduced mitochondrial function in MKO mice preceded increased adiposity, glucose intolerance, and insulin resistance. Our study highlights that preexisting, modest disruption of mitochondrial gene expression and function early in life, when combined with a sedentary lifestyle, can disrupt glucose homeostasis in later years. Identification of mitochondrial deficiencies in young adults may help predict diabetes risk, and augmentation of mitochondrial function could be a valid approach to delay or prevent disease progression.

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DISCLOSURES

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS


REFERENCES


