Exercise Prevents Maternal High-Fat Diet–Induced Hypermethylation of the \( \text{Pgc-1}\alpha \) Gene and Age-Dependent Metabolic Dysfunction in the Offspring

Abnormal conditions during early development adversely affect later health. We investigated whether maternal exercise could protect offspring from adverse effects of a maternal high-fat diet (HFD) with a focus on the metabolic outcomes and epigenetic regulation of the metabolic master regulator, peroxisome proliferator-activated receptor \( \gamma \) coactivator-1\( \alpha \) (\( \text{Pgc-1}\alpha \)). Female C57BL/6 mice were exposed to normal chow, an HFD, or an HFD with voluntary wheel exercise for 6 weeks before and throughout pregnancy. Methylation of the \( \text{Pgc-1}\alpha \) promoter at CpG site \(-260\) and expression of \( \text{Pgc-1}\alpha \) mRNA were assessed in skeletal muscle from neonatal and 12-month-old offspring, and glucose and insulin tolerance tests were performed in the female offspring at 6, 9, and 12 months. Hypermethylation of the \( \text{Pgc-1}\alpha \) promoter caused by a maternal HFD was detected at birth and was maintained until 12 months of age with a trend of reduced expression of \( \text{Pgc-1}\alpha \) mRNA (\( P = 0.065 \)) and its target genes. Maternal exercise prevented maternal HFD-induced \( \text{Pgc-1}\alpha \) hypermethylation and enhanced \( \text{Pgc-1}\alpha \) and its target gene expression, concurrent with amelioration of age-associated metabolic dysfunction at 9 months of age in the offspring. Therefore, maternal exercise is a powerful lifestyle intervention for preventing maternal HFD-induced epigenetic and metabolic dysregulation in the offspring.

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The prevalence of maternal obesity is increasing at an alarming rate. Even more disturbing is that maternal obesity increases susceptibility of offspring to developing metabolic disease later in life and therefore contributes to a vicious cycle of transgenerational transmission of disease (1,2). Encouraging accumulating evidence has shown that maternal exercise has beneficial effects on offsprings’ metabolic outcomes (3–8). These benefits include improved glucose tolerance and increased glucose clearance in skeletal muscle and adipose tissue (3). However, it is unknown whether introduction of maternal exercise can protect offspring from maternal high-fat diet (HFD)–induced metabolic dysfunction and what
the underlying mechanism(s) of this developmental programming might be.

A promising candidate for parent-offspring transmission of metabolic dysfunction is the epigenetic modification of metabolically important genes through DNA methylation, histone modifications, or microRNA regulation (9–12). DNA methylation typically occurs in differentiated cells at the cytosine of CpG dinucleotide pairs. Methylation of the promoter region can block transcription and silence gene expression (13–15). Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), a transcriptional coactivator, is a master gene of mitochondrial biogenesis and oxidative metabolism (16,17). It has been shown that the PGC-1α promoter is hypermethylated, which negatively correlates with mRNA expression in skeletal muscle of patients with type 2 diabetes (18). Furthermore, hypermethylation of CpG site −260 is sufficient to reduce PGC-1α promoter activity (18). Thus, methylation of the PGC-1α promoter in skeletal muscle is an epigenetic modification with important consequences relevant to the development of metabolic disorders.

Here we used epigenetic analysis in well-established animal models of diet-induced obesity and voluntary wheel running to test in mice the hypothesis that maternal exercise would mitigate the metabolic and epigenetic abnormalities.

RESEARCH DESIGN AND METHODS

Animals

Female C57BL/6 mice (8 weeks old; n = 4 per group) were subjected to the following diet-activity interventions for 6 weeks before and throughout pregnancy: normal chow diet with sedentary activity (Sed-NC), 60% HFD (Research Diets, Inc., New Brunswick, NJ) with sedentary activity (Sed-HF), or HFD with exercise training (voluntary running; Ex-HF). Mice were housed individually in cages equipped with running wheels, which were locked 6 weeks before and throughout pregnancy: normal chow diet was placed in the cages overnight, for the sedentary groups. At the time of mating a sedentary male C57BL/6 mouse (14 weeks old; n = 6) eating a normal chow diet was placed in the cages overnight, and pregnancy was confirmed by vaginal plug. The female mice continued on the same diet-activity intervention until term. All dams and offspring were fed normal chow with sedentary activity during lactation and after weaning (21 days). Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were performed on the female offspring (8 Sed-NC offspring, 4 Sed-HF offspring, and 5 Ex-HF offspring) at 6, 9, and 12 months of age by measuring blood glucose in the tail vein following intraperitoneal injection of glucose (3.0 g/kg body weight) and insulin (1 unit/kg body weight), respectively (19).

At 12 months, dual-energy X-ray absorptiometry was performed to assess body composition (20). Muscles were harvested after the mice were humanely killed: all hind limb muscles from two pups per litter at birth and the quadriceps muscle from the remaining littermates at 12 months of age. All procedures were approved by the Animal Care and Use Committee of the University of Virginia.

DNA Methylation Analysis

Genomic DNA was isolated and bisulfite-converted using a MethylCode Bisulfite Conversion Kit (Invitrogen Life Technologies, Carlsbad, CA). PCR primers spanning the CpG site −260 of the Pgc-1α promoter were designed using PyroMark primer design software (Qiagen, Valencia, CA). PCR was performed using the PyroMark PCR kit (Qiagen) with forward TGGTTATTATGTGAGTAGG-GTTT and reverse CCAACCTCCCTTCTCCTATACA primers and the following conditions: 1 cycle at 95°C for 15 min, 50 cycles at 94°C for 30 s followed by 54°C for 30 s and then 72°C for 30 s, and final extension at 72°C for 10 min. The PCR product (3 μL) was resolved by electrophoresis on 2% agarose gel to confirm the identity of the product. Sequencing with the primer TGGTTAT-ATTATGTGAGTAGG-GTTT and reverse CCAACCTCCCTTCTCCTATACA primers and the following conditions: 1 cycle at 95°C for 15 min, 50 cycles at 94°C for 30 s followed by 54°C for 30 s and then 72°C for 30 s, and final extension at 72°C for 10 min. The PCR product (3 μL) was resolved by electrophoresis on 2% agarose gel to confirm the identity of the product. The data are reported as percentage methylation by determining the number of times the site exists as cytosine in the context of the total number of times the site is detected as thymine or cytosine. Data were analyzed using PyroMark Q24 software (Qiagen).

mRNA Analysis

PCR of total RNA was performed as previously described (19), using primers and a probe for Pgc-1α (Mm00470540_m1), glucose transporter 4 (Glut4; Mm00436615_m1), cytochrome c oxidase subunit 4 (Coxd4; Mm00446387_m1), cytochrome c (Cyt c; Mm00470540), myosin heavy chain 2a (Myh2a; Mm01332564_m1), superoxide dismutase 1 (Sod1; Mm01344233_g1), and hypoxanthine guanine phosphoribosyltransferase 1 (Hrpt1; Mm00446968_m1) (Applied Biosystems, Foster City, CA). mRNA expression was normalized by Hrpt1.

Statistical Analysis

Data are presented as mean ± SE. Comparisons were done using one-way ANOVA followed by the Student Newman-Kuels post hoc test; P < 0.05 was considered to be statistically significant. For GTT and ITT analyses, two-way ANOVA with repeated measures was conducted, and if an interaction was observed, one-way ANOVA was performed for each of the time points among different groups.
RESULTS
Maternal HFD Induces Muscle-Specific Hypermethylation of the Pgc-1α Promoter in the Offspring at Birth, Which Is Attenuated by Maternal Exercise

To investigate the epigenetic effect of maternal diet and exercise on the offspring, we assessed Pgc-1α promoter methylation at CpG site −260 (Fig. 1A) in muscle and liver from the offspring at birth. The Pgc-1α promoter was hypermethylated (P < 0.05) in skeletal muscle of Sed-HF offspring compared with Sed-NC offspring (Fig. 1B) and was attenuated in Ex-HF offspring (Fig. 1B). No differences in methylation levels were observed in the liver (Fig. 1C). Skeletal muscle Pgc-1α mRNA levels were similar among the groups (Fig. 1D), and there was no correlation between methylation and mRNA expression (Fig. 1E). These findings indicate that maternal diet and exercise impose muscle-specific epigenetic modification of Pgc-1α in the offspring.

Maternal Exercise Prevents Maternal HFD-Induced Pgc-1α Hypermethylation and Increases Pgc-1α mRNA in Skeletal Muscle of Adult Offspring

To determine whether maternal HFD-induced hypermethylation of the Pgc-1α promoter in offspring muscle was sustained to adulthood, we assessed Pgc-1α methylation in the 12-month-old littermates. Sed-HF offspring displayed hypermethylation of the Pgc-1α promoter (P < 0.05) compared with Sed-NC offspring (Fig. 2A); this was completely prevented in Ex-HF offspring (Fig. 2A). There was a trend (P = 0.056) for Pgc-1α methylation to negatively correlate (ρ = −0.48) with mRNA levels (Fig. 2C). Pgc-1α mRNA in the muscle of Ex-HF offspring was significantly higher (P < 0.05) than that in both Sed-NC and Sed-HF offspring (Fig. 2B), and there was a trend (P = 0.065) for reduced Pgc-1α mRNA (~50%) in Sed-HF offspring compared with Sed-NC offspring (Fig. 2B). Glut4, Cox4, and Cyt c mRNA, but not Myh2a and Sod1 mRNA, exhibited an expression pattern similar to that of Pgc-1α, such that expression was significantly higher in skeletal muscle of 12-month-old Ex-HF offspring (Fig. 2D). In addition, Cox4 and Cyt c mRNA expression was lower (P < 0.05) in Sed-HF offspring, with a similar trend (P = 0.072) observed for Glut4 mRNA (Fig. 2D). There were no significant differences in Myh2a and Sod1 mRNA expression between the groups (Fig. 2D). Postnatal growth, body weight, and fat and lean body mass were similar between groups (Fig. 2E–G).

Maternal Exercise Protects Offspring From Maternal HFD-Induced Age-Dependent Metabolic Dysfunction

To investigate whether the epigenetic mark on Pgc-1α was associated with metabolic outcomes, we assessed glucose and insulin tolerance in the aging offspring. There were no differences in GTT and ITT analyses between groups at 6 months (Fig. 3A–C). At 9 months, Sed-HF offspring displayed glucose intolerance (P < 0.01 at 30 min and P < 0.05 at 60 min; Fig. 3D) with a greater area under the curve (P < 0.01; Fig. 3E) compared with Sed-NC offspring. Maternal exercise prevented maternal HFD-induced metabolic dysfunction at this age (Fig. 3D–F). We did not find statistically significant differences at 12 months of age (Fig. 3G–I). In a separate cohort, maternal exercise without HFD as a negative control (Ex-NC) had no effect on Pgc-1α methylation in offspring’s skeletal muscle (Fig. 4A and B) or glucose tolerance at 18 weeks of age (Fig. 4C and D).

DISCUSSION
Our findings demonstrate a link between the maternal condition, epigenetic modifications to the gene of a master metabolic regulator in offspring, and later metabolic health outcomes. We observed that the Pgc-1α promoter was hypermethylated in the skeletal muscle,
but not in the liver, of newborns from dams exposed to an HFD. This epigenetic mark was maintained up to 12 months of age and exhibited a negative correlation with Pgc-1α and its target transcript levels. Importantly, these findings were accompanied by an age-dependent glucose intolerance at 9 months. Although a definitive cause and effect cannot be confirmed, our findings strongly support an epigenetic mechanism in the parent-offspring transmission of metabolic disease and suggest maternal exercise as an intervention with powerful positive epigenetic influences to halt the vicious cycle.

We have for the first time shown that maternal HFD induces hypermethylation of the Pgc-1α promoter in offspring skeletal muscle. Importantly, this occurred in a region of the Pgc-1α promoter known to be hyper-methylated in patients with type 2 diabetes (18). It is possible that systemic effects of maternal HFD, such as elevated circulating lipids and inflammatory cytokines that can enter the fetal circulation, impair the gestational environment and alter DNA (cytosine-5-)methyltransferase (DNMT) activity (9). Indeed, PGC-1α promoter methylation has been shown to be increased by tumor necrosis factor-α, palmitate, or oleate treatment in primary human myotubes (18). This epigenetic modification is likely a result of an altered DNMT3b isoform (18). Regulation of DNMT activity can be influenced by

Figure 2—Maternal HFD-induced Pgc-1α hypermethylation is maintained with reduced gene expression and abnormal metabolic function in aging mice. Pgc-1α promoter methylation and mRNA expression were assessed by pyrosequencing and real-time PCR, respectively, in offspring skeletal muscle at 12 months of age. Graphs show Pgc-1α promoter methylation at CpG site –260 (A), Pgc-1α mRNA expression (B), correlation between Pgc-1α methylation and gene expression (C), and mRNA expression of Glut4, Cox4, Cyt c, Myh2a, and Sod1 (D) in skeletal muscle at 12 months of age. Body weight and composition are presented as a growth profile from birth to 12 months (E); percentages of lean body mass (F) and fat mass (G) as measured by dual-energy X-ray absorptiometry at 12 months of age in female offspring are also shown. *P < 0.05; **P < 0.01; ***P < 0.001.
microRNA, phosphorylation, and translational activation and expression, and thus it will be important in future studies to dissect the precise influence of maternal HFD on skeletal muscle DNMT isoforms. The only current findings relevant to this issue are altered expression of DNMT isoforms in the liver of offspring of undernourished dams (20,21), providing a hint to their involvement in developmental programming.

Whether the epigenetic modification has functional consequences is of great significance for disease outcomes. In general, CpG methylation of a promoter region represses transcription. Although non-CpG methylation of PGC-1α has been associated with metabolic disease (18), the functional relevance is unclear and has yet to be elucidated. In this study we focused on methylation of CpG site −260 of the Pgc-1α promoter to ensure that the findings were functionally meaningful. Interestingly, the differences in Pgc-1α promoter methylation at birth in the skeletal muscle of offspring did not affect mRNA expression. We speculate that rapid proliferation and differentiation of myogenic cells during this critical period of growth requires active transcription of Pgc-1α. In contrast, in fully differentiated adult skeletal muscle, where myogenic cells are quiescent, DNA methylation may have more influence on gene transcription. Indeed, we observed that differences in Pgc-1α promoter methylation were associated with changes in gene expression by up to 50% in the adult offspring. Furthermore, mRNA expression of downstream target genes Glut4, Cox4, and Cyt c, but not Myh2a and Sod1, mirrored that of Pgc-1α.
epigenetic modification, such as demethylation. Since exercise training in mothers fed an HFD prevented the increase in body weight induced by HFD (data not shown), it is likely that the positive effect of exercise is mediated by suppression of dyslipidemia and associated systemic inflammation, which alter the gestational environment (9). Indeed, exercise training has positive effects on blood lipid profiles and inflammatory cytokines associated with obesity, as reported in adult male mice (22). We therefore speculate that a reduction in circulating factors that have been previously shown to increase PGC-1α promoter methylation (18) is responsible for the maternal exercise-mediated protection passed on to the offspring. Future studies will need to investigate the maternal HFD-induced factors that influence offsprings’ epigenetic regulators and the physiological changes induced by maternal exercise that are associated with the prevention of epigenetic modifications.

In summary, we have provided evidence that maternal HFD-induced metabolic dysfunction in aging offspring could be significantly ameliorated by maternal exercise. Methylation of the master metabolic regulator Pgc-1α at CpG site −260 in the offspring is sensitive to the maternal condition, and the epigenetic mark laid during embryonic development is maintained to adulthood. Hypermethylation of the Pgc-1α promoter has a negative effect on gene expression and metabolic outcomes as mice age. Our most novel finding is that exercise intervention protects the fetus from adverse epigenetic modifications induced by a maternal HFD, resulting in enhanced gene expression and preserved metabolic function in later life. The findings are critical to stem the cycle of developmental programming of disease and can be readily translatable to human practice, with significant implications for public health.

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**Author Contributions.** R.C.L performed the animal experiments, analyzed and interpreted data, and wrote the manuscript. T.S.L., M.O., M.Z., and K.L.H. provided technical support, contributed to the discussion, and reviewed the manuscript. J.J.C. and Z.Y. conceived the experiments, contributed to discussion and interpretation of data, and wrote the manuscript. R.C.L. and Z.Y. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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