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Exercise Prevents Maternal High-Fat Diet–Induced Hypermethylation of the *Pgc-1α* 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 γ coactivator-1α (*Pgc-1α*). 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 *Pgc-1α* promoter at CpG site −260 and expression of *Pgc-1α* 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 *Pgc-1α* 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 *Pgc-1α* mRNA (*P* = 0.065) and its target genes. Maternal exercise prevented maternal HFD-induced *Pgc-1α* hypermethylation and enhanced *Pgc-1α* 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

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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 HFD-induced, age-dependent metabolic dysfunction in offspring is linked to *Pgc-1α* promoter hypermethylation and reduced *Pgc-1α* mRNA expression and function. More important, we also tested whether 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 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 TGAGTTATTATGTGAGTAGGGTTT 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 TGAGTTATTATGTGAGTA was performed using a PyroMark Q24 pyrosequencing machine (Qiagen). Non-CpG cytosines acted as internal controls for bisulfite conversion efficiency since they are not methylated and are expected to have 100% conversion to uracil and be identified as thymine upon amplification. 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 (*Cox4*; Mm00446387_m1), cytochrome c (*Cyt c*; Mm00470540), myosin heavy chain 2a (*Myh2a*; Mm01332564_m1), superoxide dismutase 1 (*Sod1*; Mm01344233_g1), and hypoxanthine guanine phosphoribosyl transferase 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. 1*A*) 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. 1*B*) and was attenuated in Ex-HF offspring (Fig. 1*B*). No differences in methylation levels were observed in the liver (Fig. 1*C*). Skeletal muscle *Pgc-1α* mRNA levels were similar among the groups (Fig. 1*D*), and there was no correlation between methylation and mRNA expression (Fig. 1*E*). These findings indicate that maternal diet and exercise impose muscle-specific epigenetic modification of *Pgc-1α* in the offspring.

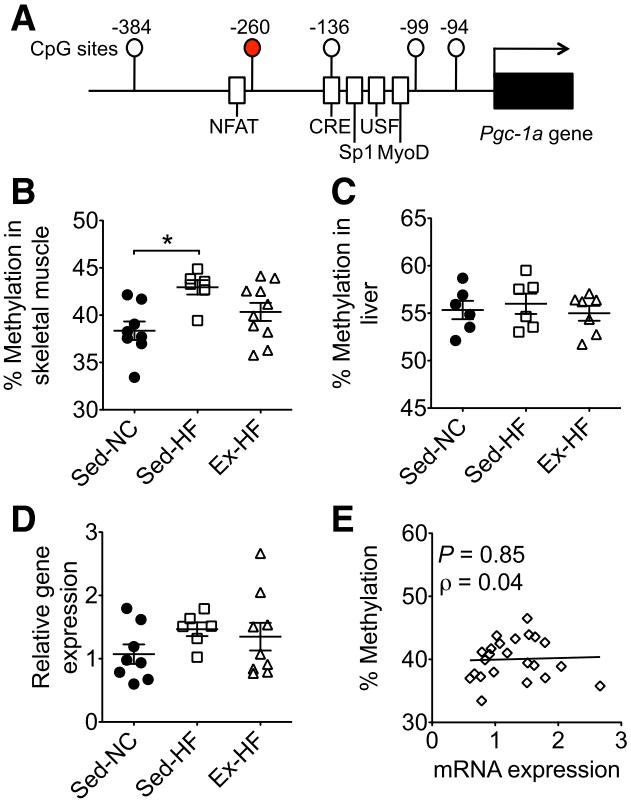

Figure 1—Maternal exercise prevents maternal HFD-induced hypermethylation of the *Pgc-1α* promoter in skeletal muscle in offspring. *Pgc-1α* promoter methylation and mRNA expression were assessed by pyrosequencing and real-time PCR, respectively, in offspring skeletal muscle and liver at birth. *A*: Schematic presentation of the structural feature of the *Pgc-1α* promoter. Circles represent CpG islands, labeled by the base pair number relative to the transcription start site, with site −260 highlighted in red. Open rectangles represent transcription factor binding sites. The arrow marks the transcription start site. Graphs show *Pgc-1α* promoter methylation at CpG site −260 in muscle (*B*) and liver (*C*). Graphs also show *Pgc-1α* mRNA in offspring skeletal muscle (*D*) and its correlation with *Pgc-1α* methylation status (*E*). *$P < 0.05$.

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. 2*A*); this was completely prevented in Ex-HF offspring (Fig. 2*A*). There was a trend ($P = 0.056$) for *Pgc-1α* methylation to negatively correlate ($\rho = -0.48$) with mRNA levels (Fig. 2*C*). *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. 2*B*), and there was a trend ($P = 0.065$) for reduced *Pgc-1α* mRNA (~50%) in Sed-HF offspring compared with Sed-NC offspring (Fig. 2*B*). *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. 2*D*). 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. 2*D*). There were no significant differences in *Myh2a* and *Sod1* mRNA expression between the groups (Fig. 2*D*). Postnatal growth, body weight, and fat and lean body mass were similar between groups (Fig. 2*E–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. 3*A–C*). At 9 months, Sed-HF offspring displayed glucose intolerance ($P < 0.01$ at 30 min and $P < 0.05$ at 60 min; Fig. 3*D*) with a greater area under the curve ($P < 0.01$; Fig. 3*E*) compared with Sed-NC offspring. Maternal exercise prevented maternal HFD-induced metabolic dysfunction at this age (Fig. 3*D–F*). We did not find statistically significant differences at 12 months of age (Fig. 3*G–I*). In a separate cohort, maternal exercise without HFD as a negative control (Ex-NC) had no effect on *Pgc-1α* methylation in offsprings' skeletal muscle (Fig. 4*A* and *B*) or glucose tolerance at 18 weeks of age (Fig. 4*C* 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,

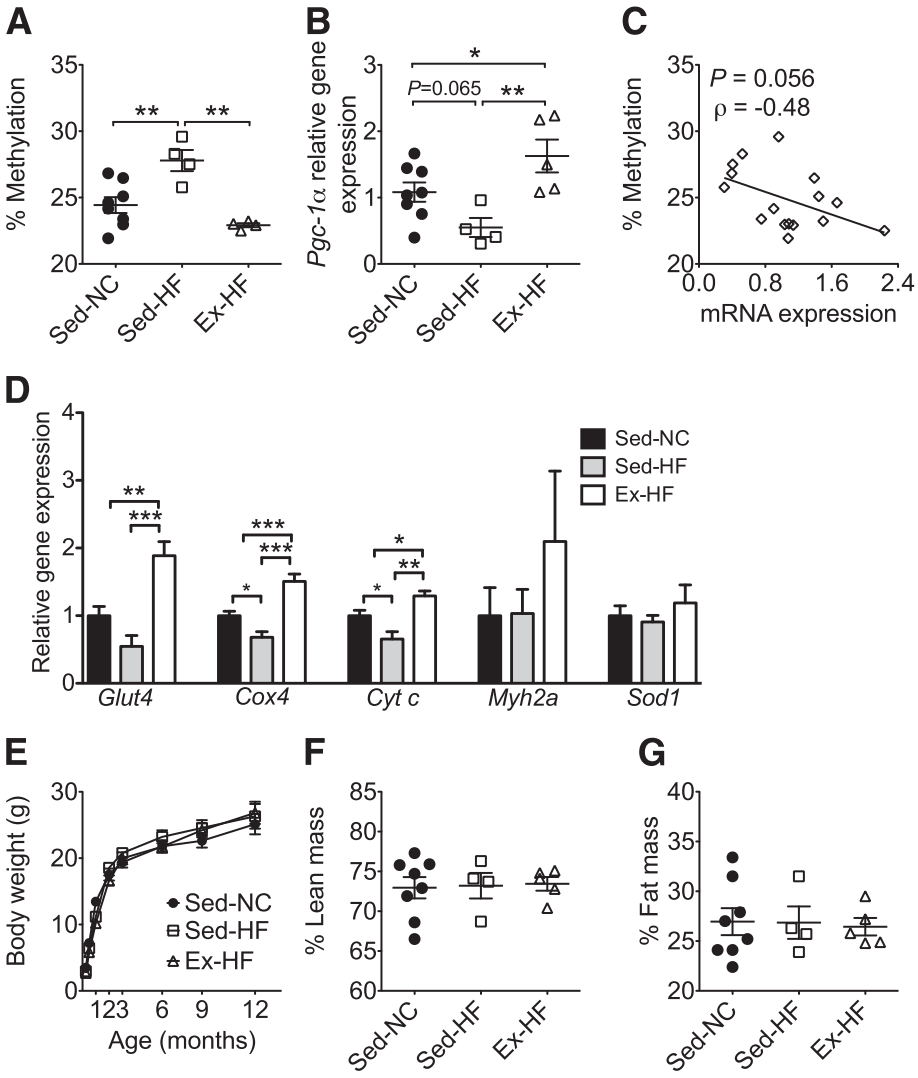

Figure 2—Maternal HFD-induced *Pgc-1α* hypermethylation is maintained with reduced gene expression and abnormal metabolic function in aging mice. *Pgc-1α* promoter methylation and mRNA expression were assessed by pyrosequencing and real-time PCR, respectively, in offspring skeletal muscle at 12 months 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. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

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 hypermethylated 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

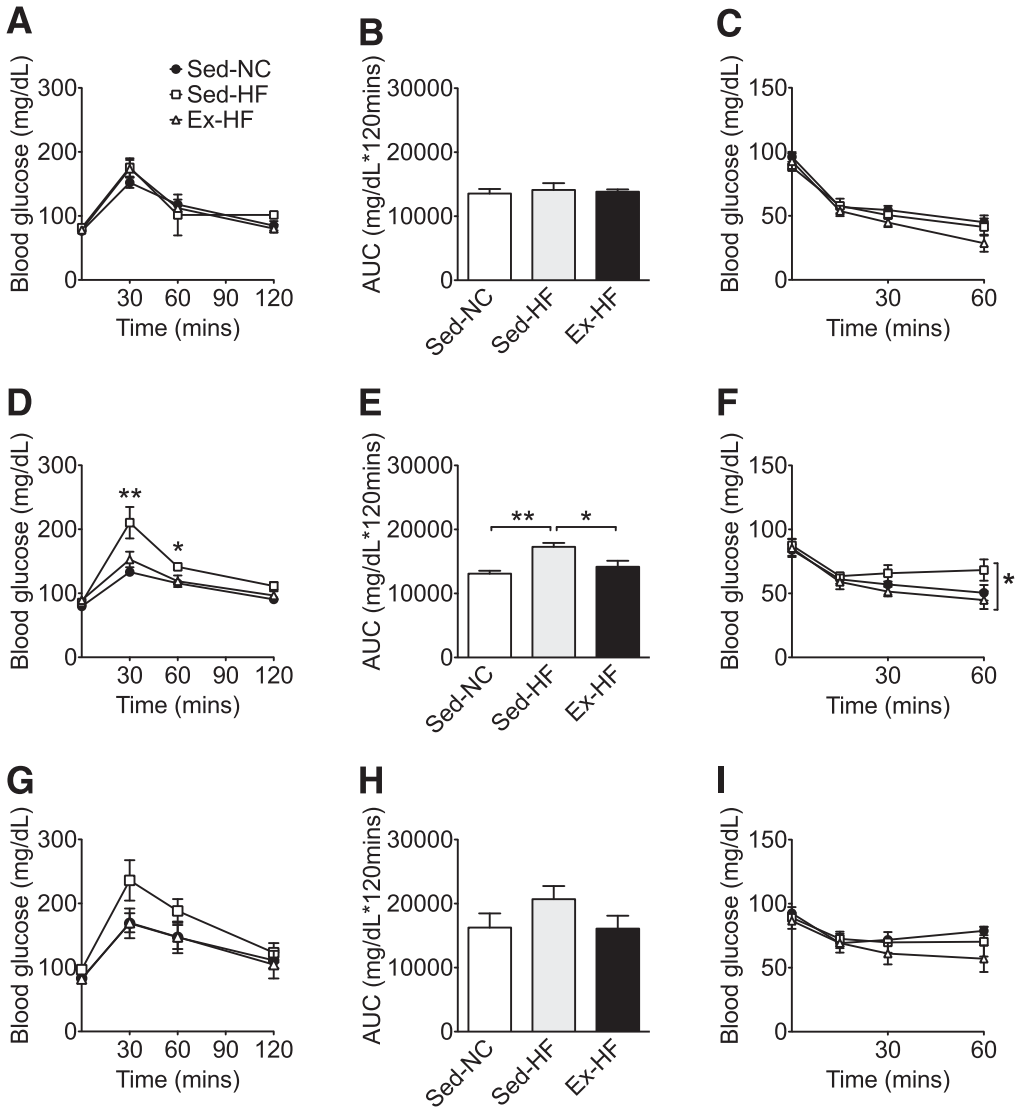

Figure 3—Maternal exercise protects offspring from maternal HFD-induced metabolic dysfunction. Whole-body glucose tolerance and insulin sensitivity were assessed in aging female offspring following a bolus intraperitoneal injection of glucose or insulin, respectively, by measuring blood glucose over time. Graphs show blood glucose levels during GTTs and areas under the curve (AUC) at 6 (*A* and *B*, respectively), 9 (*D* and *E*, respectively) and 12 (*G* and *H*, respectively) months of age. Blood glucose levels during ITTs at 6 (*C*), 9 (*F*), and 12 (*I*) months of age also are shown. $^{*}P < 0.05$; $^{**}P < 0.01$.

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*

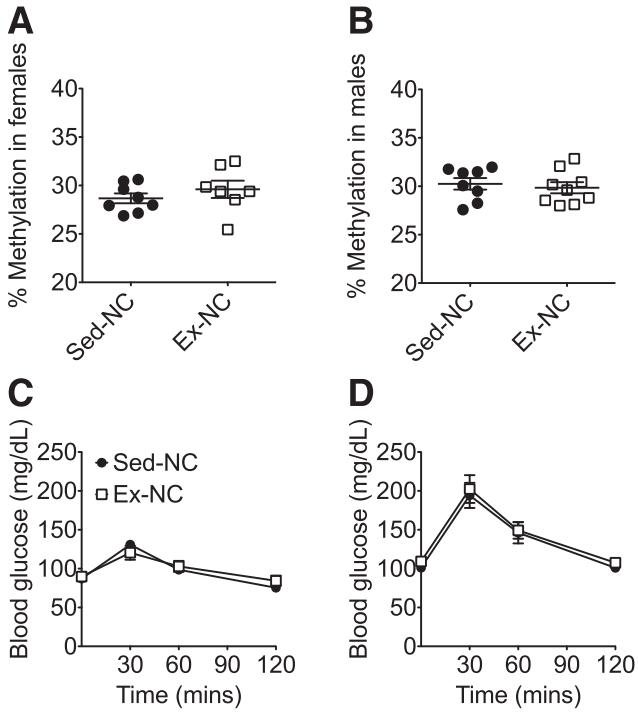

Figure 4— Maternal exercise alone does not affect *Pgc-1α* promoter methylation in the skeletal muscle or glucose tolerance in offspring. *Pgc-1α* promoter methylation was assessed by pyrosequencing in skeletal muscle and glucose tolerance was assessed following a bolus intraperitoneal injection of glucose by measuring blood glucose over time in 18-week-old female and male offspring. Graphs show *Pgc-1α* promoter methylation at CpG site −260 in females (*A*) and males (*B*) and blood glucose during GTTs in females (*C*) and males (*D*).

and provides further evidence of the functional importance of epigenetic regulation of *Pgc-1α*. Importantly, in skeletal muscle of humans with type 2 diabetes, a similar degree of *PGC-1α* hypermethylation corresponded to mRNA expression reduced by ~35% (18). Together, these data link hypermethylation of the *Pgc-1α* promoter to gene expression in adult offspring.

The most exciting finding of this study is that maternal exercise protects offspring from maternal HFD-induced metabolic dysfunction at 9 months of age. Because of the small sample size and increased variability within groups that naturally develops with aging, we did not achieve statistical differences in the metabolic phenotype between groups at 12 months of age. Regardless, our findings from offspring at 9 months of age paralleled the prevention of *Pgc-1α* promoter hypermethylation in skeletal muscle and preserved *Pgc-1α* mRNA later in life. These benefits seemed to be specific to the condition of maternal HFD because maternal exercise without HFD as a negative control had no effect on *Pgc-1α* methylation or glucose tolerance in adult offspring. These findings suggest that maternal exercise suppresses the maternal HFD-induced hypermethylation of *Pgc-1α* in the offspring rather than initiates an independent process of 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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References

1. Mingrone G, Manco M, Mora MEV, et al. Influence of maternal obesity on insulin sensitivity and secretion in offspring. Diabetes Care 2008;31:1872–1876
2. Dabelea D, Mayer-Davis EJ, Lamichhane AP, et al. Association of intrauterine exposure to maternal diabetes and obesity with type 2 diabetes in youth: the SEARCH Case-Control Study. Diabetes Care 2008;31:1422–1426
3. Carter LG, Lewis KN, Wilkerson DC, et al. Perinatal exercise improves glucose homeostasis in adult offspring. Am J Physiol Endocrinol Metab 2012;303:E1061–E1068
4. Carter LG, Qi NR, De Cabo R, Pearson KJ. Maternal exercise improves insulin sensitivity in mature rat offspring. Med Sci Sports Exerc 2013;45:832–840
5. Nathanielsz PW, Ford SP, Long NM, Vega CC, Reyes-Castro LA, Zambrano E. Interventions to prevent adverse fetal programming due to maternal obesity during pregnancy. Nutr Rev 2013;71(Suppl 1):S78–S87
6. Vik KL, Romundstad P, Nilsen TIL. Tracking of cardiovascular risk factors across generations: family linkage within the population-based HUNT study, Norway. J Epidemiol Community Health 2013;67:564–570
7. Vega CC, Reyes-Castro LA, Bautista CJ, Larrea F, Nathanielsz PW, Zambrano E. Exercise in obese female rats has beneficial effects on maternal and male and female offspring metabolism. *Int J Obes Lond* 2013 Aug 15. [Epub ahead of print]
8. Bahls M, Sheldon RD, Taheripour P, et al. Mothers' exercise during pregnancy programs vasomotor function in adult offspring. Exp Physiol 2013;99:205–219
9. Laker RC, Wlodek ME, Connelly JJ, Yan Z. Epigenetic origins of metabolic disease: the impact of the maternal condition to the offspring epigenome and later health consequences. Food Sci Hum Wellness 2013;2:1–11
10. Park JH, Stoffers DA, Nicholls RD, Simmons RA. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J Clin Invest 2008;118:2316–2324
11. Thompson RF, Einstein FH. Epigenetic basis for fetal origins of age-related disease. J Womens Health (Larchmt) 2010;19:581–587
12. Pinney SE, Simmons RA. Epigenetic mechanisms in the development of type 2 diabetes. Trends Endocrinol Metab 2010;21:223–229
13. Wolffe AP, Matzke MA. Epigenetics: regulation through repression. Science 1999;286:481–486
14. Vaissière T, Sawan C, Herceg Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. Mutat Res 2008;659:40–48
15. Schübeler D, Lorincz MC, Cimbora DM, et al. Genomic targeting of methylated DNA: influence of methylation on transcription, replication, chromatin structure, and histone acetylation. Mol Cell Biol 2000;20:9103–9112
16. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr Rev 2003;24:78–90
17. Lira VA, Benton CR, Yan Z, Bonen A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. Am J Physiol Endocrinol Metab 2010;299:E145–E161
18. Barrès R, Osler ME, Yan J, et al. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab 2009;10:189–198
19. Lira VA, Okutsu M, Zhang M, et al. Autophagy is required for exercise training-induced skeletal muscle adaptation and improvement of physical performance. FASEB J 2013;27:4184–4193
20. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. Br J Nutr 2007;97:1064–1073
21. Burdge GC, Hoile SP, Uller T, et al. Progressive, transgenerational changes in offspring phenotype and epigenotype following nutritional transition. PLoS One 2011;6:e28282
22. Huang P, Li S, Shao M, et al. Calorie restriction and endurance exercise share potent anti-inflammatory function in adipose tissues in ameliorating diet-induced obesity and insulin resistance in mice. Nutr Metab (Lond) 2010;7:59