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Original Article

Paternal lifestyle influence susceptibility to high-fat diet-induced metabolic disorders among male offspring of C57BL/6 mice

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Abstract: Previous evidences have indicated that parental lifestyle is critical for offspring's susceptibility to obesity and has a risk of developing an altered metabolic profile. In the present work, we examined whether offspring of fathers with high-fat diet (HFD) has a greater susceptibility to HFD-induced metabolic derangements or not and whether paternal exercise has protective effects against HFD damages or not. C57BL/6 male mice (3-weeks-old, F0, $n = 34$) were divided into 3 groups: group fed with standard-chow (C 12% kcal from fat), group fed with standard-chow accompanied with treadmill running (E), and group fed with HFD (HF 60% kcal from fat). After intervention, each male mouse was mated with a female sibling. Furthermore, after weaning, all pups (F1) were given HFD for 4 weeks. Our results showed that paternal HFD resulted in a significantly increase in body weight ($P < 0.05$), epididymal (+70%, $P < 0.001$) and perirenal (+100%, $P < 0.05$) fat pad mass, serum lipid concentrations and impaired glucose tolerance. Meanwhile, paternal HFD can significantly down-regulated the expressions of PGC-1α ($P < 0.05$), Glut4 ($P < 0.05$) and Nrf1 ($P < 0.05$) in epididymal adipose tissues, but no significant difference was observed in skeletal muscle of male pups. In addition, there was no difference in oral glucose tolerance tests (OGTT) and body weight of female offspring. Additionally, paternal exercise also ameliorated the detrimental effects of HFD in male offspring. In conclusion, our results indicated that paternal lifestyle influence male offspring's susceptibility to HFD-induced metabolic disorders via regulating Pgc-1α in skeletal muscle and adipose tissues.

Keywords: Paternal treadmill exercise, paternal high-fat diet, male pup, metabolic health, Pgc-1α

Introduction

Currently, it's reported that the global prevalence of obesity is increasing, especially in children. Obesity contributes to early emergence of the Type 2 Diabetes Mellitus (T2DM). It's reported that physical exercise could reduce body fat and attenuate the risk of T2DM [1, 2], and previous reports also revealed that the obesity, T2DM and other metabolic diseases in offspring is closely related to the parental lifestyle [3, 4].

Clinical and laboratory studies have revealed that maternal exercise exerts beneficial effects for the mother and fetus [5]. PGC-1α, an inducible transcriptional coactivator, regulates cellular energy metabolism and metabolic adaptation to environmental and nutritional stimuli [6, 7], and is reported to be closely related to obesity and T2DM [8, 9]. Currently, it's demonstrated that mitochondrial dys-regulation contributes to metabolic pathologies including obesity and T2DM. Mitochondria undergo constant biogenesis that is controlled primarily by the gene expressions and post-translational modifications of Pgc-1α [10]. In addition, it's also revealed that mother is the primary influencer of child's metabolic health; besides, dietary and environmental factors also influence offspring's health via paternal line. Furthermore, it's reported that food availability during the prepubertal phase of grandfathers is associated with an increased risk of cardiovascular diseases, diabetes, and mortality in grandsons [11,

12]. Additionally, exercise training is an environmental stimulus affecting many systems throughout the body, and it might be capable of inducing trans-generational modifications similar to nutritional interventions. Previously, we had found that paternal exercise had significant effects on the spatial learning and memory of male pups via up-regulating hippocampus BDNF and reeling [13]. It's suggested that obese fathers can improve reproductive health of their offspring via exercise interventions [14]. However, it is unknown that whether offspring of fathers with high-fat diet (HFD) has a greater susceptibility to HFD-induced metabolic derangements or not, and whether paternal exercise has protective effects against the damages of HFD challenging or not. Thus, our present study is aimed to explore whether paternal exercise or HFD can influence HFD-induced metabolic dysfunction of offspring by using a mice model of paternal exercise; and our present results demonstrated that HFD male offspring from sedentary fathers are more susceptible to developing metabolic dysfunction than offspring from exercised fathers.

Materials and methods

Animals

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Chinese Academy of Sciences. Protocols were approved by the Tianjin Medical University Animal Care and Use Committee. Eight-week-old C57BL/6 male (n = 10) and female (n = 10) mice were obtained from Beijing HFK Bioscience Limited Liability Company. Animals were housed under controlled conditions (20-23°C; 35-55% humidity; 12-h light/dark cycle), with food and tap water provided *ad libitum*. Mice were fed for 7 days to adapt to the environment before being mated. During mating, one male and one female were housed together. Females were checked daily for vaginal seminal plugs; the presence of a plug was used to indicate date of pregnancy. Litter size was adjusted to 8 pups with 4 males and 4 females to perform the test by either sacrifice for litters (n > 8), whereas litters with less than eight pups were excluded from analysis. All offspring were weaned at 3 weeks of age. After weaning, founder male mice (F0, n = 34) were divided into three groups: Group I mice served as controls (C, n = 10) group and were fed standard-chow (12% kcal from fat, 28% from protein and 60% from carbohydrate) for 12 weeks. Group II mice (HF, n = 12) were HFD-fed (60% kcal from fat, 20% from protein and 20% from carbohydrate) for 12 weeks. Group III mice saved as exercise (E, n = 12) group were fed standard-chow as above, as well as exercised on a motorized treadmill at the speed of 12 m/min (75% VO2 max), 60 min/day, and 5 days/week from 9-15 weeks of age (6 weeks) (**Figure 1**). After intervention, one male founder was paired with one normal-weight female sibling for a maximum period of 8 nights. Female mice were housed with founder males during the dark cycle only and separated. The females were kept sedentary and maintained on standard-chow during pregnancy and after birth. All offspring (F1) were weaned at 3 weeks of age. Offspring from the control diet group (CO, n = 7 male; n = 8 female), the exercise group (EO, n = 9 males; n = 8 female), and the high-fat diet group (HFO, n = 8 male; n = 6 female) were given normal chow for 1 week following HFD for 4 weeks.

Oral glucose tolerance test (OGTT)

OGTT was performed at 4 and 8 weeks of age for all offspring. Mice were fasted overnight (12 h), and blood was collected at 15, 30, 60, 90, and 120 min from the tail after glucose administration. OGTT was evaluated by calculating the total area under the curve (AUC) using the trapezoidal method.

Tissue collection

At 8 weeks of age, male mice were sacrificed under anaesthetization (100 mg/kg ketaminexzs and 10 mg/kg xylazine) after fasting for 6 h. Blood samples were rapidly obtained by extirpating eyeball. Gastrocnemius muscle and fat pads (inguinal, retroperitoneal and epididymal fat pads) were carefully dissected, weighed, immediately stored at -80°C (CO, n = 6; EO, n = 6; HFO, n = 6) for later analysis.

Gene expression assay

Total RNA was isolated from mouse tissue using TRIzol reagent (Invitrogen, UK) according to the manufacturer's instructions. Synthesize cDNA with SuperScript II reverse transcriptase

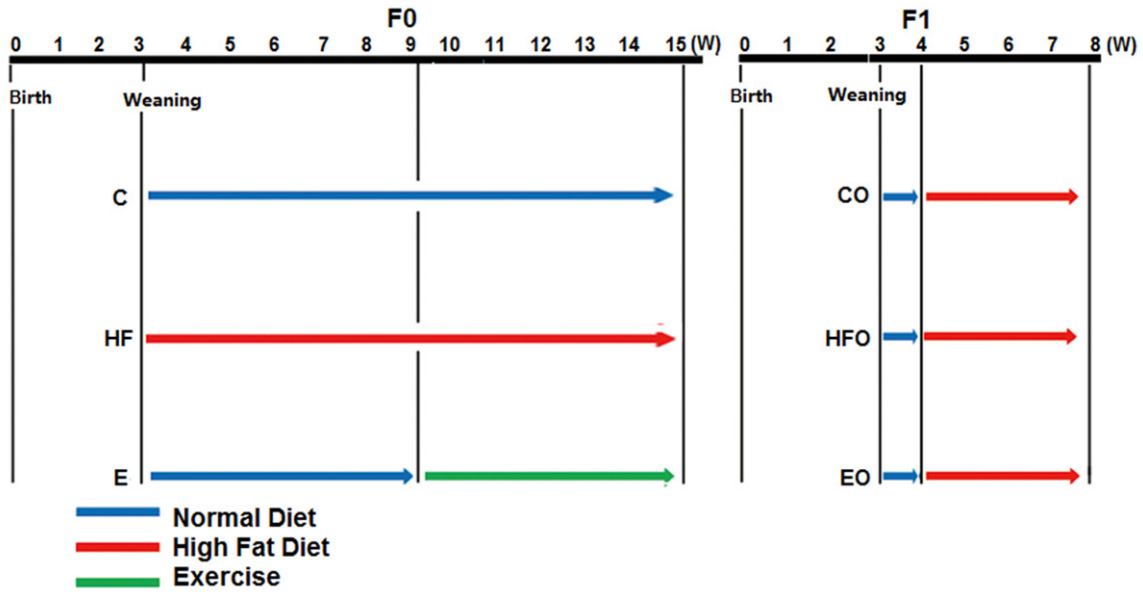

Figure 1. Experimental animal groups. Blue arrows: periods in which standard-chow was administered for F0 or F1. Red arrow: period in which F0 and F1 were fed a high-fat diet. Green arrow: period in which F0 were exercised.

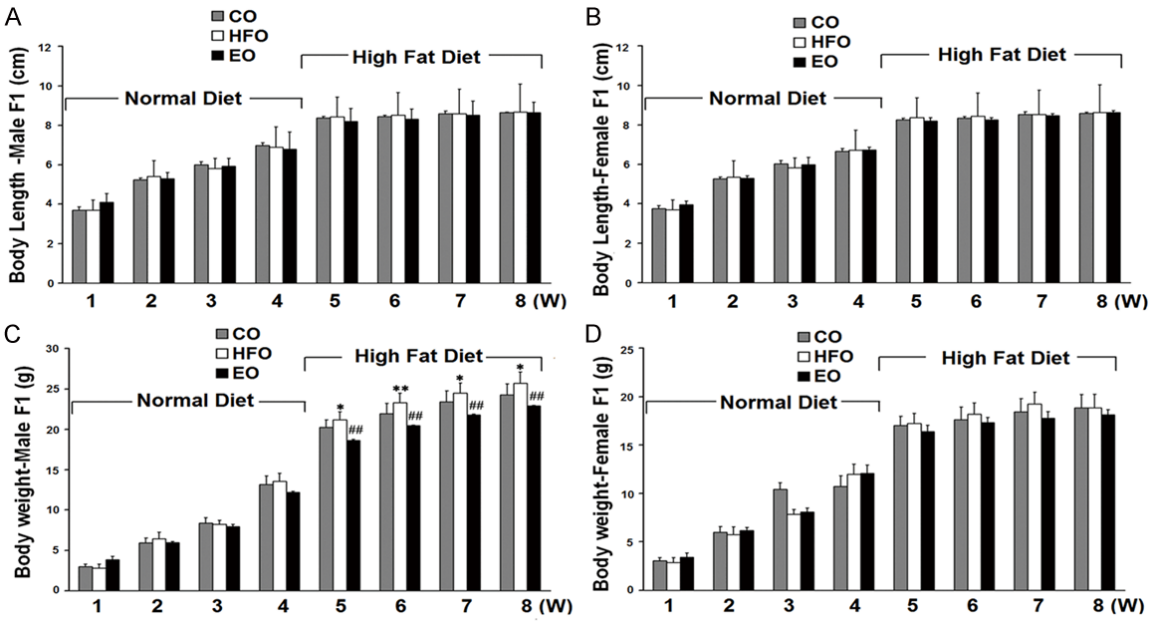

Figure 2. Effects of paternal HF and exercise on offspring weight and length. A and C. Body length and weight in female pups. B and D. Body length and weight in male pups. Results are presented as means ± SE (CO: n = 7 male, n = 8 female; EO: n = 9 males, n = 8 female; HFO: n = 8 male, n = 6 female). *indicates HFO vs CO: *P < 0.05, and **P < 0.01. #EO vs CO: #P < 0.05, and ##P < 0.01.

(Invitrogen, USA) and oligo (dT). Gene expression was assessed by real-time quantitative PCR using SYBR green and gene-specific primers in a Bio-Rad iQ5 real-time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). In addition, β-actin quantification was used as an internal control. The following sets of primers were used: Pgc-1α, 5'-AGCCGTGACCACTGACAACGAG-3' (forward) and 5'-GCTGCATGGTTCTGAGTGCTAAG-3' (reverse); Glut4, 5'-TGGCTCCCTTCAGTTTGG-3' (forward) and 5'-TGCCTTGT GGGATGGAAT-3' (reverse); Nrf1, 5'-TTTGGCGCA-

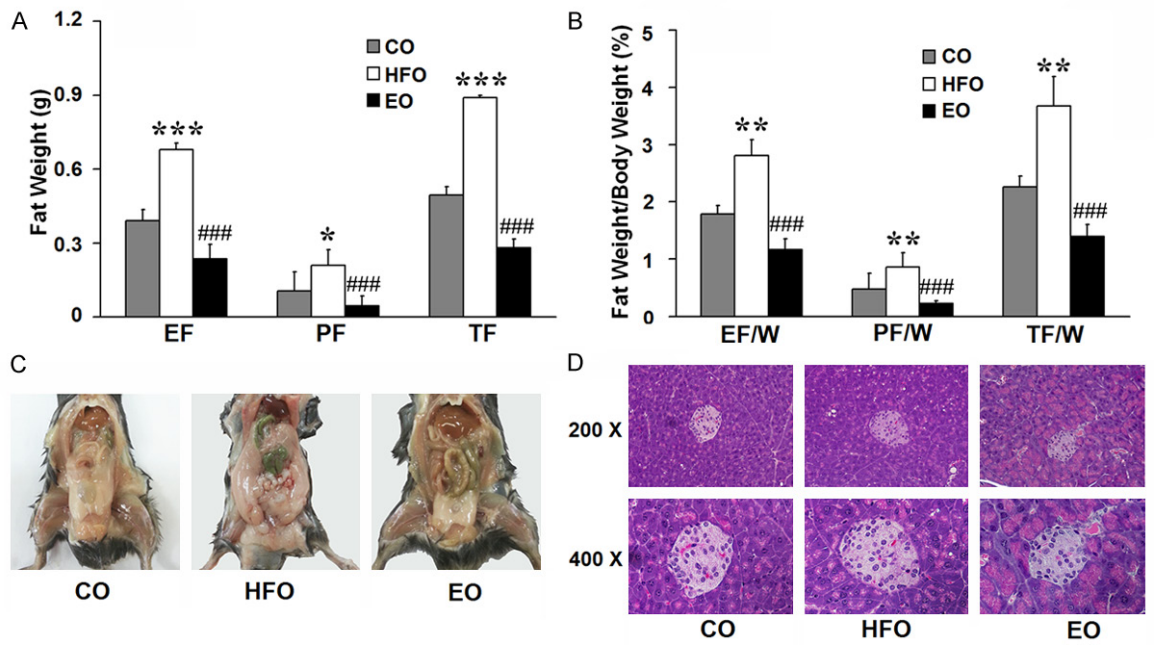

Figure 3. Effects of paternal HFD and exercise on offspring visceral fat and histology of pancreas in male offspring at 8 weeks of age. A and B. The EFT, PFT, TFT, and F/W in male pups. C. Representative images of visceral fat from CO, EO, and HFO mice. D. Pancreatic tissue in male offspring stained with HE. Results are presented as means ± SE (CO n = 7; EO n = 9; HFO n = 8). *indicates HFO vs CO: *P < 0.05, **P < 0.01, and ***P < 0.001. #EO vs CO: ###P < 0.001.

GCACCTTTGGAGAAT-3' (forward) and 5'-CC CCCGACCTGTGG AATACTTGAG-3' (reverse); Cytochrome C oxidase subunit 4 (Cox4), 5'-TGGCCCCATCCCTCATACTTTCGATC-3' (forward) and 5'-AGCGGGCTCTCACTTCTTCCACTC-3' (reverse); β-actin 5'-CGGTGAAGGCGACAGCAG-TTGG-3' (forward) and 5'-GGACGCGACCA-TCCTCCTCTTAG-3' (reverse). Relative expression level of target gene was determined as $2^{-\Delta\Delta CT}$.

Western blotting assay

Total proteins were extracted from tissue by homogenization in NP-40 lysis buffer, and protein concentration was measured using the Bradford assay. The following primary antibodies were used in this study: β-actin (Sigma, St. Louis, MO); PGC-1α (Cell Signaling Technology, USA). Western blot was analyzed by scanning with a scanner and digitalized using image analysis software (Quantity One, Hercules, CA, USA).

Histological analysis

The specimens were fixed in 4% paraformaldehyde for two weeks, conventionally washed, and processed for conventional paraffin embedding. Sections (7 μm) were mounted on glass slides, de-waxed in xylene, rehydrated through graded alcohols, washed in distilled water, and stained with hematoxylin and eosin (HE). All slides were examined under a microscope.

Statistical analysis

All data are expressed as means ± SD. Statistical significance was defined as $P < 0.05$ and determined by one-way analysis of variance analysis (ANOVA) following by Dunnett multiple comparisons test between different groups with the SPSS software (SPSS for Windows 18.0, SPSS Inc., USA).

Results

Effects of Paternal HF and exercise on offspring body weight, length and visceral fat pad weights

There were no significant effect of paternal HF and exercise on offspring body length was observed (**Figure 2A** and **2B**). No difference was seen in the body weight of all offspring from birth up to 4 weeks of age. However, after 4 weeks of HFD exposure (8 weeks of age),

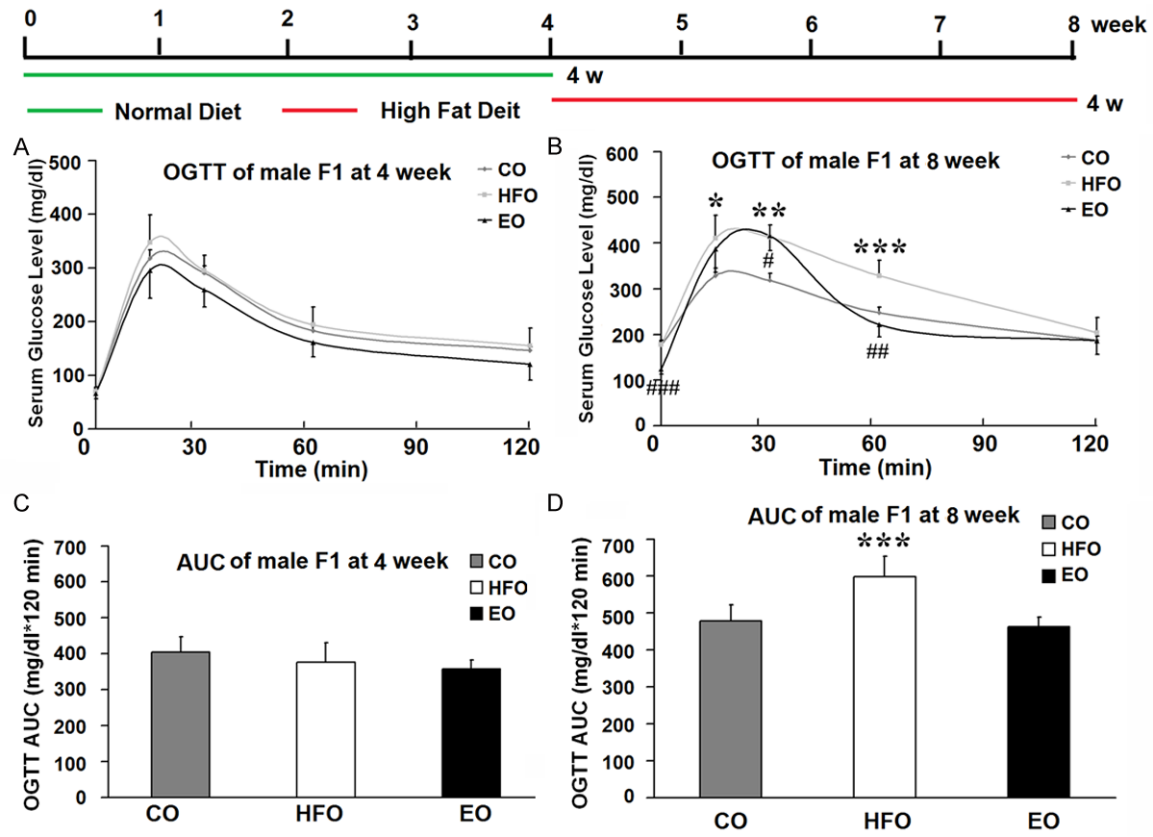

Figure 4. Effects of paternal HFD and exercise on HFD fed male offspring OGTT. OGTT was assessed in male offspring, respectively, by measuring blood glucose over time. Blood glucose level during OGTT and AUC at 4 (A and C, respectively), 8 (B and D, respectively) weeks of age. Results are presented as means ± SE (CO n = 7; EO n = 9; HFO n = 8). *indicates HFO vs CO: *P < 0.05, **P < 0.01, and ***P < 0.001. #EO vs CO: #P < 0.05, ##P < 0.01, and ###P < 0.001.

paternal HFD and exercise had sex-specific effects on offspring phenotype. The males from obese fathers were significantly heavier (HFO vs CO, $P < 0.05$ **Figure 2C**), whereas the male pups from exercise fathers were lighter (EO vs CO, $P < 0.01$ **Figure 2C**), with no significant difference in female pups (**Figure 2D**). The male HFO had an increase in epididymal (70%, $P < 0.001$) and perirenal (100%, $P < 0.05$) fat pad mass compared with the CO (**Figure 3A-C**). The male EO group presented a decrease in epididymal and perirenal fat pad mass compared with the CO (62%, 228%, respectively, $P < 0.001$) as shown in **Figure 4A-C**.

Effects of Paternal HF and exercise on offspring OGTT, blood lipid and pancreas

Paternal obesity and exercise had sex-specific effects on the OGTT of offspring. In male offspring, there was no difference in OGTT analyses and area under the curve (AUC) among the three groups at 4 weeks (**Figure 4A** and **4C**). After 4 weeks, HFD- induced, HF male offspring displayed glucose intolerance with greater AUC compared with C group offspring, whereas paternal exercise had moderate beneficial effects on male EO mice, which had lower fasting blood glucose and at 60 min later than CO (**Figure 4B** and **4D**). Conversely, analysis of OGTT from female offspring revealed no significant difference among the three groups either at 4 weeks age or after induction of HFD. Lipid profile, plasma TG, T-CHO, LDL-C, and FFA were significantly lower in male pups from exercised fathers, whereas they were all significantly higher in pups from obese fathers. High-density lipoprotein cholesterol (HDL-C) showed the opposite (EO vs CO, HFO vs CO, **Table 1**). Fasting insulin (Fins) concentrations showed no significant difference in male pups (**Table 1**). Histologic examination of male offspring pancreatic tissue revealed no significant pathologic differences among the three groups (**Figure 3D**). In sum, these data show that paternal exercise or obesity has marked effects on the

Table 1. Effect of paternal exercise and HFD on lipid profile and Fins of male offspring

	CO (*n* = 7)	HFO (*n* = 8)	EO (*n* = 9)	*P*-value	*P*-value
TG (mmol/L)	1.16 ± 0.29	1.71 ± 0.28	0.38 ± 0.12	0.018*	0.000$^{\#\#\#}$
CHO (mmol/l)	4.14 ± 0.46	4.87 ± 0.44	3.41 ± 0.40	0.018*	0.018$^{\#}$
HDL-C (mmol/l)	2.03 ± 0.16	1.71 ± 0.30	2.53 ± 0.29	0.05*	0.003$^{\#\#}$
LDL-C (mmol/l)	1.09 ± 0.23	1.38 ± 0.22	0.39 ± 0.23	0.145	0.034$^{\#}$
FFA (μmol/l)	451.90 ± 12.6	522.01 ± 22.13	406.00 ± 17.12	0.000***	0.000$^{\#\#\#}$
Fins (ng/ml)	1.24 ± 0.31	1.28 ± 0.31	1.74 ± 0.22	0.807	0.097

Values are presented as means ± SE. *HFO vs CO: $^{*}P < 0.05$, and $^{***}P < 0.001$. $^{\#}$EO vs CO: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$.

metabolic health of male offspring. Glucose intolerance in male HFO was caused neither by increasing insulin production via an increase in β cell mass nor a decrease in β cell mass leading to impaired insulin secretion: It resulted from insulin resistance in peripheral tissue.

Expression and function of PGC-1α in skeletal muscle and adipose tissue of male offspring

Paternal exercise significantly increased Pgc-1α mRNA and protein levels in skeletal muscle ($P < 0.05$ **Figure 5A** and **5C**) and epididymal adipose tissue ($P < 0.01$ **Figure 5B** and **5D**) compared with control male pups. Paternal HFD significantly decreased mRNA and protein contents in epididymal fat of offspring relative to the control male pups ($P < 0.001$ **Figure 5B** and **5D**) with no significant difference in skeletal muscle (**Figure 5A** and **5C**). In skeletal muscle, expression of Nrf1 and Cox4 mRNAs, but not Glut4 mRNA, was significantly higher in E offspring. No significant differences were found in HF offspring (**Figure 5E**). In epididymal adipose tissue, Nrf1, Cox4, and Glut4 mRNAs exhibited a similar expression pattern to that of PGC-1α in skeletal muscle; namely, expression was significantly higher in E offspring. In addition, Nrf1 and Glut4, but not Cox4 mRNAs were lower ($P < 0.05$) in HF offspring (**Figure 5F**).

Discussion

Increasing evidences revealed that parental lifestyle and environmental exposures (such as exercise, HFD, and pathogenic and chemical exposure during embryogenesis) lead to variations in offspring and grand-offspring development [15, 16]. Thus far, most researches in this area have only focused on maternal contributions, whereas very little is known regarding the influence of paternal lifestyle on offspring's phenotype. In this study, we investigated the effects of paternal exercise or HFD on the adiposity, glucose tolerance, and insulin secretion in HFD offspring. Our results indicated that paternal exercise and obesity influence the metabolic health outcomes of male pups. We also found that the PGC-1α expressions were increased in skeletal muscle and epididymal fat from fathers of exercise. Obese fathers significantly decreased the mRNA and protein expressions of Pgc-1α in epididymal fat of offspring, and paternal lifestyle may have a greater effect in males than females. For the first time, we demonstrated that paternal exercise or HFD may alter the offspring's HFD-induced metabolic risk via altering the expressions and functions of PGC-1α in male offspring epididymal adipose tissues and skeletal muscle.

Exercise training is an environmental stimulus that affects many systems throughout the body, and could also induce trans-generational modifications. Clinical and laboratory studies have revealed that maternal exercises exert beneficial effects for the mother and fetus, but the potential impact of paternal exercise has not been investigated. In this study, our results revealed that there was no difference in glucose tolerance and body weight among the EO, HFO, and CO mice at 4 weeks of age. However, when mice were exposed to HFD for 4 weeks, male offspring of HF had an impaired glucose tolerance, hyperlipidemia, and higher visceral fat weight and body weight compared with the control male pups. Paternal exercise ameliorated the HFD-induced metabolic disorders in male offspring. These findings are similar to results obtained by Stanford *et al.* The study of Stanford *et al* found that maternal exercise has marked effects on the metabolic health of male offspring, and could ameliorate the detrimental effect of maternal high-fat diet [17]. Our results

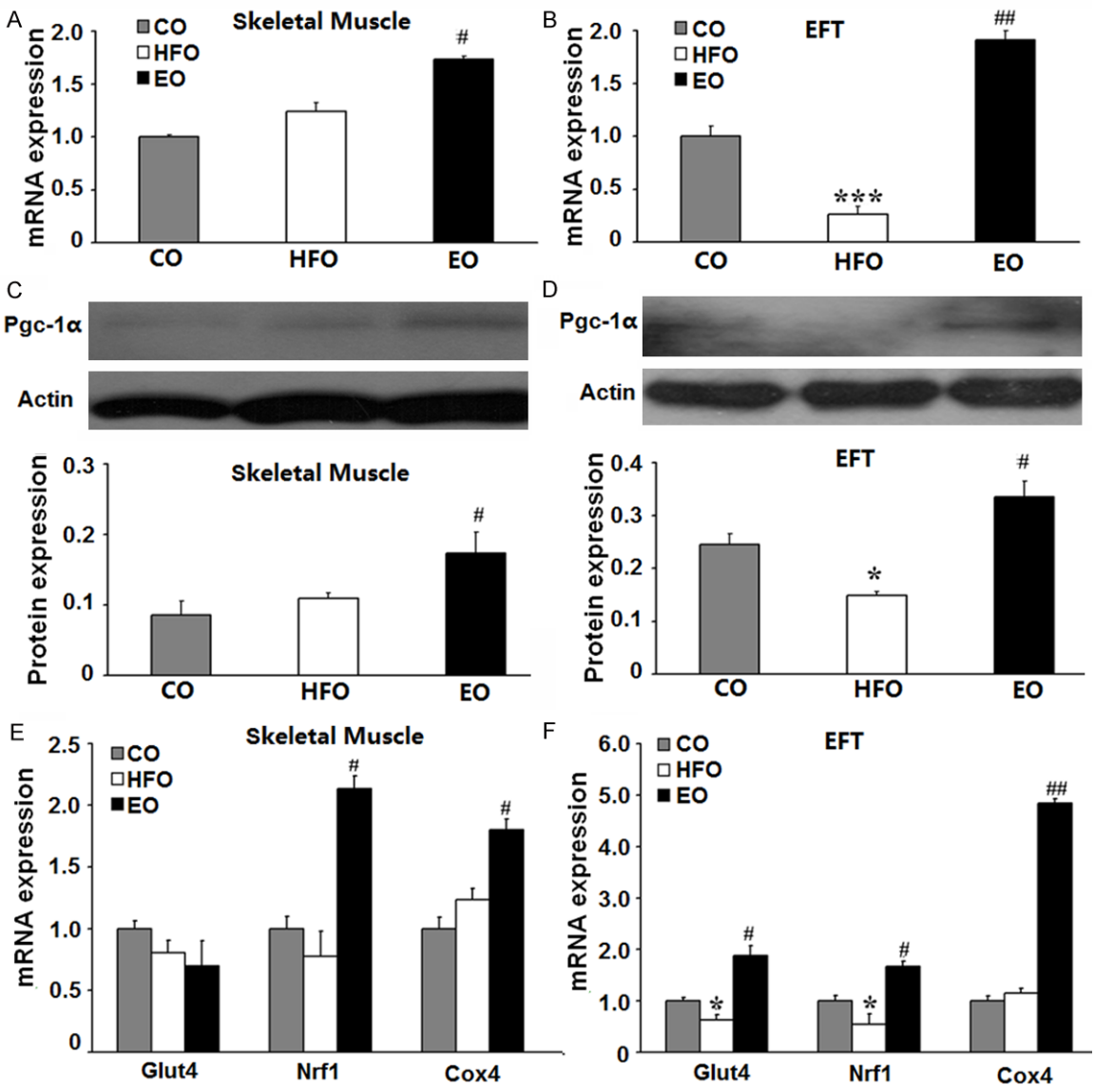

Figure 5. Paternal HFD and exercise significantly affect Pgc-1α and downstream target gene expression in male offspring. Pgc-1α mRNA and protein expression were assessed by real-time PCR and western blot, respectively, in male offspring skeletal muscle and EFT at 8 weeks of age. Pgc-1α mRNA (A) and protein expression (C) in skeletal muscle. Pgc-1α mRNA (B) and protein expression (D) in EFT. Glut4, Nrf1, Cox4 mRNA expression in the skeletal muscle (E) and epididymal adipose tissue (F). Results are presented as means ± SE (n = 6, each group). *indicates HFO vs CO: *P < 0.05, **P < 0.01, and ***P < 0.001. #EO vs CO: #P < 0.05, and ##P < 0.01.

indicated that paternal exercise can ameliorate the metabolic disturbance by 4- week HFD, and this effect only occurs among male offspring. The long-term effects of exercise have been demonstrated in both male and female offspring, but were greater in males [18]. In the maternal study across generations, sex differences in programming effects have been previously shown in both humans and animal models [19], and may be mediated by the expression of placental genes [19, 20].

PGC-1α is recognized as a key regulator of mitochondrial biogenesis and function [21]. Patti *et al* found that PGC-1α is reduced by about 50% in skeletal muscle in prediabetic and diabetic humans [22]. Schöttl *et al* discovered that the combined reduction of PGC-1α and insulin-signaling molecules in adipose tissue could result in adipose tissue dysfunction, leading to the impairments of systemic insulin response in insulin-resistant subjects [23]. Pgc-1α has recognized as a potential candidate gene mediat-

ing diabetes-related metabolic phenotypes. In skeletal muscle and white adipose tissue, Pgc-1α has been extensively shown to increase in response to physical activity and caloric restriction in obese subjects, which related to improved insulin sensitivity [24-26]. Furthermore, palmitate treatment of skeletal muscle cells from healthy human subjects could result in the down-regulation of Pgc-1α and mitochondrial gene expression [27]. Therefore, there might be a link among the exercise, obesity, over-nutrition, and PGC-1α expression in muscle and adipose tissue. Laker *et al* showed that maternal HFD during pregnancy resulted in significantly reduced PGC-1α in skeletal muscle and can induce metabolic dysfunction [5]. However, the PGC-1α expression in offspring white adipose tissue and skeletal muscle exposed to paternal exercise has been unknown to date. In the present study, we proved that paternal treadmill exercise can significantly increase PGC-1α expression in skeletal muscle and epididymal adipose tissue of male pups, which is associated with decreased obesity and improved glucose intolerance induced by HFD in male offspring. Furthermore, mRNA expressions of Glut4, Nrf1 and Cox4 in epididymal adipose tissue and mRNA expressions of Nrf1 and Cox4 in skeletal muscle exhibited a similar expression pattern to that of Pgc-1α. Paternal HFD can significantly decrease Pgc-1α and its downstream target genes (Glut4 and Nrf1) in epididymal adipose tissue, but no significant difference was seen in the skeletal muscle of male pups. PGC-1α initiates the process of mitochondrial biogenesis, and the interaction between PGC-1α and NRF-1 constitutes the first step of this process [28]. Thus, it has been speculated that exercise by lean fathers increased mitochondrial biogenesis and limited excess visceral fat accumulation with reduced circulating triglycerides in offspring. Paternal obesity is linked to down-regulated PGC-1α and mitochondrial biogenesis in WAT, which leads to harmful, persistent effects in offspring, including predisposition to obesity and disorders of glucose and fatty acids metabolism. The precise mechanisms of trans-generational programming through paternal lineage remains unknown, but inherited aberrant epigenetic profiles transmitted by sperm as potential mediators are clearly implicated [29, 30]. HFD could alter global methylation in mature sperm cells and sperm microRNA content [31]. Exercise epigenetics is a nascent area of research with vast implications for global health. Evidence is accumulating that exercise also can modify the sperm epigenetic profiles via DNA methylation [32] or microRNA and improves the metabolic health of offspring [33]. It has not yet been investigated whether sperm epigenetic profiles by the sperm of HFD or exercise fathers are faithfully replicated in offspring and result in a different phenotype in offspring.

Conclusions

Collectively, our results showed that paternal exercise could up-regulate the Pgc-1α expression in both epididymal adipose tissue and skeletal muscle of male offspring, which are closely associated with the decreased postnatal risk of HFD induced obesity and metabolic dysfunction. Further studies are needed to elucidate the mechanisms behind the beneficial effects of parental exercise on offspring, which may help to diminish the growing incidence of obesity, T2DM, and other metabolic disorders.

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Disclosure of conflict of interest

None.

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