Four weeks of exercise early in life reprograms adult skeletal muscle insulin resistance caused by a paternal high-fat diet

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Key points

- A paternal high-fat diet/obesity before mating can negatively influence the metabolism of offspring.
- Exercise only early in life has a remarkable effect with respect to reprogramming adult rat offspring exposed to detrimental insults before conception.
- Exercise only early in life normalized adult whole body and muscle insulin resistance as a result of having a high-fat fed/obese father.
- Unlike the effects on the muscle, early exercise did not normalize the reduced adult pancreatic beta cell mass as a result of having a high-fat fed/obese father.
- Early-life exercise training may be able to reprogram an individual whose father was obese, inducing long-lasting beneficial effects on health.

Abstract  A paternal high-fat diet (HFD) impairs female rat offspring glucose tolerance, pancreatic morphology and insulin secretion. We examined whether only 4 weeks of exercise early in life could reprogram these negative effects. Male Sprague–Dawley rats consumed a HFD for 10 weeks before mating with chow-fed dams. Female offspring remained sedentary or performed moderate intensity treadmill exercise (5 days week−1, 60 min day−1, 20 m min−1) from 5 to 9 weeks of age. Paternal HFD impaired (P < 0.05) adult offspring whole body insulin sensitivity (I.P. insulin sensitivity test), as well as skeletal muscle ex vivo insulin sensitivity and TBC1D4 phosphorylation. It also lowered β-cell mass and reduced in vivo insulin secretion in response to

Filipe Falcão-Tebas obtained his Master’s degree in Nutrition at the Federal University of Pernambuco (Brazil) and completed his PhD at the Institute for Health and Sport, Victoria University (Australia). Filippe is interested in the role of exercise and diet during developmental stages and their potential to reprogram metabolic diseases later in life.
Introduction

The dramatic rise in type 2 diabetes (T2D) can partially be explained in terms of phenotypic plasticity (Hanson & Gluckman, 2014), when diverse environmental conditions modify the expression of a phenotype characteristic from a single genotype (West-Eberhard, 1989). The developmental origins of health and disease paradigm uses this concept to interpret how environmental cues early in life (gestation, lactation and early childhood) impact on long-term disease susceptibility, such as T2D (Bateson et al. 2004; McMullen & Mostyn, 2009; Wells, 2014). In humans, obese mothers have a greater probability of having obese offspring (Gale et al. 2007). Similarly, a maternal high-fat diet (HFD) in rodents causes offspring insulin resistance (Nivoit et al. 2009), hyperphagia and hypertension (Samuelsson et al. 2008) in adulthood, as well as reduced skeletal muscle mitochondrial transcription factors and oxidative phosphorylation (Latouche et al. 2014; Pileggi et al. 2016).

In addition, paternal HFD/obesity before mating can negatively influence the phenotype and metabolism of offspring (Soubry, 2015). Indeed, a father’s body fat (percentage or total) is predictive of long-term changes in body fat in premenarcheal girls (Figueroa-Colon et al. 2000) and a prospective epidemiological study observed that obesity in fathers before conception is linked with obesity in his offspring (Loomba et al. 2008). In rodents, studies are controversial with regards to effects on offspring body weight (Ng et al. 2010; Fullston et al. 2013; Cropley et al. 2016). Nevertheless, a paternal HFD appears to reduce glucose tolerance, islet size and function, as well as insulin secretion, in female offspring (Ng et al. 2010). Similarly, obesity induced by a paternal HFD results in offspring with impaired glucose tolerance and insulin resistance (Fullston et al. 2013), suggestive of reduced insulin sensitivity.

With regard to possible interventions that overcome the effects of a paternal HFD, there is an increased window of opportunity of adaptation from gestation up to infancy (Hanson & Gluckman, 2014). Higher physical activity in youth was shown to be associated with lower rates of T2D (and hypertension) in adulthood, independent of current physical activity (Fernandes & Zanesco, 2010).

It has been proposed that early-life exercise training is a positive stimulus for reprogramming offspring exposed to detrimental insults in utero, such as placental restriction (Laker et al. 2011; Laker et al. 2012; Gatford et al. 2014; Street et al. 2015). We found that exercise training early in life in rats (from 5 to 9 weeks of age only, treadmill running) normalized the 50% lower relative islet surface area and β-cell mass in adulthood in rats born small for gestational age (Laker et al. 2011). This was remarkable especially because early exercise did not have a sustained effect on skeletal muscle mitochondrial biogenesis in adult offspring using this design (Laker et al. 2012). No study has investigated whether exercise early in life can overcome the negative metabolic consequences of a paternal HFD before conception. The ‘classical’ insulin-stimulated glucose uptake pathway involves a series of signals to recruit serine/threonine protein kinases. Once protein kinase B (also known as Akt) is activated, the signal then propagates to activate an Akt substrate of 160 kDa (AS160, more recently referred to as TBC1 domain family member 4; TBC1D4; as well as TBC1D1) (Middelbeek et al. 2013). Next, TBC1D4 activates a Rab GTPase protein (Tan et al. 2012), which releases glucose transporter (GLUT)4 and allows it to be translocated to the plasma membrane. Some phosphorylation sites were found to be stimulus-specific. For example, phospho-Akt Ser308 is a specific site for insulin only, whereas phospho-Akt Ser473 and phospho-TBC1D4 Thr642 are responsive to both insulin and contraction (exercise) (Treebak et al. 2014). The long-term effects of paternal HFD and exercise early in life have not been investigated in terms of insulin signalling in the skeletal muscle.

In the present study, we aimed to determine whether paternal HFD impairs insulin sensitivity and mitochondrial function in skeletal muscle and pancreatic morphology in adult rat offspring and whether exercise early in life can attenuate these negative effects. Based on our previous studies in rats born small for gestational age, we hypothesized that early-life exercise would normalize
Methods

Ethical approval and animals

All procedures were performed according to the Australian Code for the Care and Use of Animals for Scientific Purposes (2013), after approval by Victoria University Animal Ethics Committee (#13/008). The authors understand the ethical principles under which The Journal of Physiology operates and confirm that this work meets the standards of the journal’s animal ethics checklist.

Sprague–Dawley rats were obtained from the Animal Research Centre (Perth, WA, Australia). Male rats were fed control chow diet (Rat and Mouse Cubes, 12.0% energy as fat; Specialty Feeds, Glen Forest, WA, Australia) or a HFD (combined SF01-025 and SF03-020, with 40.7% and 43.0% energy as fat; Specialty Feeds) from 4 to 14 weeks of age (Ng et al. 2010). All female breeders were chow fed and sedentary (Fig. 1). The rats were exposed to a 12:12 h light/dark cycle (lights on 07.00 h) and standard environmental conditions of 18–22°C and ~50% relative humidity. All animals had access to food and water ad libitum, as well as nesting materials and enrichment items. Mating was performed between 08.00 and 17.00 h (during the light cycle) when rats were 12 weeks old (one male to one female) and on control chow diet. Fourteen male breeders were obtained at 3 weeks of age and equally allocated into control diet or HFD groups randomly. All male rats were housed with female rats and, out of the seven rats in each group, five control diet and five HFD rats mated successfully.

Offspring

The study only included litter sizes of between nine and 15 pups. Birth was considered the postnatal day (PND)1. At PND21, offspring were weaned and only female pups remained in the study as described previously (Ng et al. 2010). Five dams were used to generate the female offspring; hence, two pups from each breeding pair were used in the present study. There were no interventions in offspring from birth until 4 weeks of age. Female offspring were randomized into sedentary or exercised group. All offspring performed acclimatization to the treadmill. Trained rats were subjected to a protocol of moderate intensity treadmill exercise training (5 days week−1, 60 min day−1 at ~65–75% VO2 max) from 5 to 9 weeks of

A

Fathers
• Control diet
• High-fat diet

Mothers
Sedentary and control diet

Female offspring
• Sedentary
• Exercised training

Four experimental groups were then generated:
• NS
• HS
• NE
• HE

B

Female offspring
• Sedentary
• Exercised training

Gestation

5wk
11wk
12wk
23wk
24wk
25wk

14wk
Birth

4wk

Glucose Tolerance Test
Ex vivo experiments and Tissues collection

Insulin Sensitivity Test

Figure 1. Experimental groups and timelines
A, experimental groups based on paternal diet and offspring exercise interventions. Male breeders were mated with only one female (1:1). B, experimental design with timelines for main experiments. NS, pups sired by control diet fathers that remained sedentary in life. HS, pups sired by high-fat diet (HFD) fathers that remained sedentary in life. NE, pups sired by normal diet fathers and exercised early in life. HE, pups sired by HFD fathers and exercised early in life.
life (Fig. 1), whereas sedentary rats were kept in their cages. This protocol was based on previous studies (Bedford et al. 1979; Laker et al. 2011). All offspring had body weight and food intake measured from 3 weeks up to 25 weeks of life. We have defined adolescent offspring as 11–12 weeks of age and adult offspring as 23–24 weeks of age. All analysis was conducted under blinded conditions using coded samples.

**Insulin sensitivity tests and glucose tolerance tests**

I.P. insulin sensitivity tests (IPIST) (1 U kg$^{-1}$ body weight) were performed after 2 h of fasting at 12 and 23 weeks of age, whereas I.P. glucose tolerance tests (IPGTT) (1.0 g kg$^{-1}$ body weight) (Fig. 1) were performed after an overnight fast 1 week later, as described previously (Ng et al. 2010; Laker et al. 2011). Tail vein blood glucose was measured using a glucometer (Accu-Chek Performa Nano; Roche Diagnostics, Mannheim, Germany). Insulin was analysed by a commercial radioimmunoassay (SRI-13K RI-13K; Linco Research, St Charles, Missouri, USA).

From the IPGTT at 24 weeks of age, the homeostatic model assessment of insulin resistance and the insulinogenic indices were obtained. The insulinogenic index provided an estimate of the early insulin response to glucose (a measure of $\beta$-cell function *in vivo*) (Singh & Saxena, 2010) and has been used previously in female Sprague–Dawley rats (Ng et al. 2010).

**Insulin-stimulated glucose uptake in skeletal muscle**

At 25 weeks of age, rats were deeply anaesthetized (60 mg kg$^{-1}$ I.P.; pentobarbitone; Virbac, Milperra, NSW, Australia) and anaesthesia was checked every 10 min by performing a tail pinch and observing no change in the respiratory rate. Epitrochlearis (EPI) and soleus (SOL) muscles were dissected, longitudinally split in half (Sharma et al. 2015) and incubated in chambers with Krebs Henseleit solution (in mM): 118.5 NaCl, 24.7 NaHCO$_3$, 4.74 KCl, 1.18 MgSO$_4$, 1.18 KH$_2$PO$_4$, 147.02 CaCl$_2$, 32 mannitol, 7.5% BSA and MilliQ H$_2$O, pH 7.4, maintained at 30°C and continuously oxygenated with 95% O$_2$ and 5% CO$_2$. Muscles were incubated for 20 min in Krebs Henseleit solution with 8 mM glucose. Muscles were then transferred to another Krebs Henseleit solution for 30 min, with 36 mM mannitol and 4 mM pyruvate. This second buffer also contained either 0 or 1.2 nM of insulin, which is considered to be a physiological concentration of insulin (Sharma et al. 2011). The third incubation was in Krebs Henseleit solution with the addition of 8 mM 2-deoxyglucose, 0.75 $\mu$Ci ml$^{-1}$ 2-[$^{1,2-}$$^3$H] deoxy-D-glucose and 0.225 $\mu$Ci ml$^{-1}$ [1,14$^C$] mannitol, and 0 or 1.2 nM insulin for 10 min.

Approximately 30 mg of EPI and 35 mg of SOL muscle were homogenized in ice-cold buffer, and lysates were prepared as described previously (Betteridge et al. 2016). Brieﬂy, western blots were carried out using 7.5–12% hand-cast TGX Stain-Free gels (Bio-Rad, Hercules, CA, USA) loaded with 4 $\mu$g of skeletal muscle lysate. The polyvinylidene ﬂuoride membranes (Bio-Rad) were imaged to quantify total protein using Imagelab 4.1 (Bio-Rad) (Murphy, 2011). After overnight incubation with primary antibodies, the membranes were incubated for 1 h with secondary antibodies (anti-mouse or rabbit IgG, HRP-linked antibody; dilution 1:10,000) and exposed to SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientiﬁc Inc., Waltham, MA, USA). Raw signal was normalized by total protein content and bands were analysed with ImageLab, version 5.2.1 (Bio-Rad).

The antibodies used were anti-GLUT4 (#ab37445; Abcam, Cambridge, MA, USA), anti-GLUT1 (#ab652; 2018 The Authors. The Journal of Physiology C © 2018 The Physiological Society
Pancreas morphology

After dissection of skeletal muscles, rats were killed by cardiac puncture and the pancreases were weighed and stored in 10% neutral buffered formalin at room temperature for up to 1 week, and then transferred to 70% ethanol and kept at 4°C until processed. Five sections per pancreas were immunostained to identify and localize insulin–positive β-cells (n = 8–10 per group). Fixed tissue was sent to Anatomical Pathology, Department of Medicine, University of Melbourne (Parkville, VIC, Australia) to be paraffin embedded, sectioned at 100 μm and stained for insulin using a guinea-pig polyclonal anti-porcine insulin antibody (Dako Corp., Glostrup, Denmark), diluted 1:100 and counterstained with haematoxylin. Digital images of microscopic sections were obtained via the Austin Health, Victorian Cancer Biobank Slide Scanning service (Heidelberg, VIC, Australia). Following standard protocols, whole slide sections were line scanned using an Aperio ScanScope XT (Aperio Technologies, Vista, CA, USA) at 40× magnification at a resolution of 0.5 μm pixel⁻¹. Digital images were analysed using ImageScope, version 12.2.2 (Aperio Technologies). Measurements were performed as described previously (Laker et al. 2011).

Five random cross-sections from different parts of the pancreas (head, body and tail) were analysed. Sampling from various parts of the pancreas is important because islet localization, composition and architecture may vary depending on the physiological and pathological states (Kharouta et al. 2009). Relative islet surface area, β-cell area, number of islets, β-cell mass and islet number were obtained as described previously (Tikellis et al. 2004; Laker et al. 2011).

Statistical analysis

Data are reported as the mean ± SEM. Data were checked for normality using the Shapiro–Wilk test. If the test was significant, data were log transformed and reanalysed. Statistical analyses were performed with SPSS, version 22.0 (IBM Corp., Armonk, NY, USA) and Prism, version 5.00 (GraphPad Software Inc., La Jolla, CA, USA). Student’s t test was used to investigate differences in paternal HFD in body weight and fat mass, as well as newborn offspring, with regard to organ mass. Two-way ANOVA was used with ‘Paternal diet’ and ‘Exercise early in life’ as the main factors for offspring phenotype, protein expression, enzyme activities, mitochondrial function and pancreas morphology. Three-way ANOVA with repeated measures was used for experiments that included the two factors previously noted, as well as different time points or treatment (e.g. IPIST, IPGTT, insulin incubation during 2DG uptake). If an interaction was found, a two-way ANOVA was applied in cases of three main factors for each time-point. For two-way ANOVA, if an interaction was found, a post hoc analysis using the least significant difference test was used. P < 0.05 was set a priori as the α-level of statistical significance.

Results

High-fat diet, fat mass and insulin resistance in fathers

The HFD intervention from 4 to 14 weeks of age prior to mating in male rats was successful for achieving breeder rats with a higher body and fat mass and insulin resistance (P < 0.05, data not shown).

Paternal HFD and offspring phenotype from birth until weaning

Paternal HFD did not change the number of male or female rats born, birth weight, or body weight from birth until PND 21 (P > 0.05). At PND 21, there was no significant difference between pups from HFD or chow fed fathers for soleus or EDL muscle mass (P = 0.07 and 0.09, respectively) or the mass of the liver, spleen, heart, kidneys and pancreas (P > 0.05).

Effect of paternal HFD and early-life exercise on body weight, food intake and glucose tolerance in adolescent and adult offspring

Offspring sired by HFD fathers had a lower body weight and food intake after 12 and 16 weeks of age, respectively (P < 0.05, data not shown). In adolescent offspring, paternal HFD did not affect insulin sensitivity, although it impaired glucose tolerance, increasing the glucose AUC. Exercise early in life normalized body weight after 16 weeks of age and normalized food intake from 18 weeks until 24 weeks of age. In adolescent rats, exercise early in life did not alter glucose tolerance in offspring of normal diet fathers. However, when offspring sired by high-fat eating fathers performed early exercise, the negative metabolic effects on glucose tolerance were attenuated (P < 0.05, data not shown). In adult offspring (24 weeks of age), insulin sensitivity was impaired by paternal HFD, whereas glucose tolerance was unaffected. Insulin levels during the
IPGTT increased less in offspring sired by HFD fathers (Fig. 2). During our experiments, one animal from the NE group (i.e. early-life exercised offspring sired by control diet fathers) suddenly died. An autopsy was conducted by the university animal welfare officer and the cause of death was not identified in the samples examined.

**Effect of paternal HFD and early-life exercise on offspring phenotype at 25 weeks of age (post mortem)**

Body weight, soleus, plantaris and gastrocnemius skeletal muscle weights were lower in offspring of paternal

![Graphs showing glucose levels and area under the curve (AUC) during insulin sensitivity tests (IPIST) and glucose tolerance tests (IPGTT)](image)

**Figure 2.** Exercise early in life protects adult female offspring sired by HFD fathers to develop impaired glucose tolerance

Glucose levels (A) and glucose area under the curve (AUC) (B) during the i.e. insulin sensitivity tests (IPIST, 23 weeks of age). Glucose (C) and insulin (E) levels during a glucose tolerance test (GTT, 24 weeks of age), and their respective areas under the curves (D and F). NS, offspring sired by control diet fathers (n = 10); HS, offspring sired by HFD fathers (n = 10); NE, early-life exercised offspring sired by control diet fathers (n = 9); HE, early-life exercised offspring sired by HFD fathers (n = 10). Values are the mean ± SEM. *Paternal HFD effect, P < 0.05. †Paternal HFD vs. Offspring exercise interaction, P < 0.05, followed by a least significant difference test (HS vs. HE).

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HFD with no changes in body length (Table 1). When offspring sired by HFD father exercised early in life, their body weight, soleus and plantaris muscle weights were normalized, although there were no effects on tibialis anterior, liver and heart.

**Effect of paternal HFD and early-life exercise on offspring skeletal muscle insulin-stimulated glucose uptake**

There were no differences in basal and insulin-stimulated glucose uptake ex vivo in soleus muscle among groups (Fig. 3). However, offspring sired by HFD fathers had reduced basal and insulin-stimulated glucose uptake ex vivo in the EPI muscle with no difference in the delta glucose uptake between basal and insulin-stimulated glucose uptake. In the EPI muscle, exercise early in life had no effect in offspring from chow fed fathers but normalized both basal and insulin-stimulated 2DG uptake in rats sired by HFD fathers (Fig. 3).

**Effect of paternal HFD and early-life exercise on skeletal muscle insulin signalling in offspring of HFD fathers**

Because glucose uptake was impaired in EPI muscle but not the SOL muscle, immunoblotting for insulin signalling was performed only in the EPI muscle (Figs. 3 and 4). Basal (0 nM), p-AktThr308, p-AktSer473 and p-TBC1D4Thr642 at 25 weeks were not affected by paternal HFD and exercise early in life (Fig. 4). Insulin-stimulated (1.2 nM) p-AktThr308 was unaffected but, unexpectedly, p-AktSer473 was increased in offspring of paternal HFD irrespective of early exercise (Fig. 4). Importantly, insulin-stimulated p-TBC1D4Thr642 was lower in offspring of paternal HFD and early-life exercise normalized this (Fig. 4). Paternal HFD did not reduce skeletal muscle GLUT1 and GLUT4 protein content in adult offspring. Early-life exercise increased both GLUT1 and GLUT4 in adult offspring above the control group irrespective of paternal diet (Fig. 3). Because there was no change in the total proteins that were measured, the phosphorylated protein and the ratio of phosphorylated protein to total protein were similar.

## Table 1. Effects of exercise early in life on adult (25 weeks of age, post mortem) female offspring sired by HFD fathers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NS (n = 10)</th>
<th>HS (n = 10)</th>
<th>NE (n = 9)</th>
<th>HE (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>333.5 ± 13.9</td>
<td>299.1 ± 6.9*</td>
<td>352.8 ± 11.8*</td>
<td>322.0 ± 9.3*</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>24.3 ± 0.2</td>
<td>24.0 ± 0.1</td>
<td>25.1 ± 0.1*</td>
<td>24.6 ± 0.2*</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>18.33 ± 0.45</td>
<td>18.18 ± 0.20</td>
<td>19.12 ± 0.69</td>
<td>18.73 ± 0.24</td>
</tr>
<tr>
<td>EDL (g)</td>
<td>0.16 ± 0.07</td>
<td>0.14 ± 0.05</td>
<td>0.17 ± 0.03*</td>
<td>0.16 ± 0.03*</td>
</tr>
<tr>
<td>Soleus (g)</td>
<td>0.15 ± 0.05</td>
<td>0.13 ± 0.06*</td>
<td>0.16 ± 0.05*</td>
<td>0.15 ± 0.04*</td>
</tr>
<tr>
<td>Gastrocnemius (g)</td>
<td>1.75 ± 0.05</td>
<td>1.62 ± 0.02</td>
<td>1.70 ± 0.4</td>
<td>1.72 ± 0.04</td>
</tr>
<tr>
<td>Tibialis anterior (g)</td>
<td>0.67 ± 0.02</td>
<td>0.63 ± 0.01</td>
<td>0.69 ± 0.01*</td>
<td>0.68 ± 0.12*</td>
</tr>
<tr>
<td>Plantaris (g)</td>
<td>0.33 ± 0.01</td>
<td>0.31 ± 0.07*</td>
<td>0.35 ± 0.01*</td>
<td>0.33 ± 0.03*</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>8.52 ± 0.31</td>
<td>8.15 ± 0.28</td>
<td>9.28 ± 0.31*</td>
<td>9.22 ± 0.46*</td>
</tr>
<tr>
<td>Pancreas (g)</td>
<td>1.36 ± 0.07</td>
<td>1.20 ± 0.09</td>
<td>1.46 ± 0.13</td>
<td>1.27 ± 0.11</td>
</tr>
<tr>
<td>Retroperitoneal fat (g)</td>
<td>6.17 ± 0.35</td>
<td>6.98 ± 0.59</td>
<td>7.46 ± 1.05</td>
<td>6.95 ± 0.91</td>
</tr>
<tr>
<td>Kidneys (g)*</td>
<td>0.98 ± 0.03</td>
<td>0.90 ± 0.03*</td>
<td>0.98 ± 0.03</td>
<td>1.00 ± 0.04*</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>1.03 ± 0.03</td>
<td>0.95 ± 0.02</td>
<td>1.08 ± 0.03*</td>
<td>1.04 ± 0.04*</td>
</tr>
<tr>
<td>Fasting glucose (mmol L⁻¹)</td>
<td>5.66 ± 0.09</td>
<td>5.63 ± 0.28</td>
<td>5.64 ± 0.12</td>
<td>5.59 ± 0.23</td>
</tr>
<tr>
<td>Fasting insulin (ng mL⁻¹)</td>
<td>0.15 ± 0.01</td>
<td>0.13 ± 0.02</td>
<td>0.11 ± 0.01*</td>
<td>0.12 ± 0.01*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.035 ± 0.006</td>
<td>0.034 ± 0.008</td>
<td>0.026 ± 0.005*</td>
<td>0.029 ± 0.003*</td>
</tr>
</tbody>
</table>

EDL, extensor digitorum longus; HFD, high-fat diet. HOMA-IR, homeostatic model assessment of insulin resistance. The equation was: HOMA-IR = [(fasting plasma glucose (mg dL⁻¹) × fasting plasma insulin (µU mL⁻¹))/2,430 (Cacho et al. 2008)]. NS, offspring sired by control diet fathers; HS, offspring sired by HFD fathers; NE, early-life exercised offspring sired by control diet fathers; HE, early-life exercised offspring sired by HFD fathers. * The left and right kidneys were combined because there was no statistical difference between them. Values are reported as the mean ± SEM. *Paternal HFD effect, P < 0.05, 0.01. #Exercise early in life effect, P < 0.05. †Paternal HFD vs. Exercise early in life interaction, P < 0.05.
paternal HFD or exercise early in life. Given that adult offspring CS activity was unaffected by paternal diet or early-life exercise in offspring, no normalization of respiration to CS activity was undertaken to avoid the introduction of variation during a normalization process. Mitochondrial H$_2$O$_2$ production was not altered by either paternal HFD or exercise early in life during any stages of respiration ($P > 0.05$, data not shown). Offspring sired from HFD fathers had lower PHF20, although there was no difference in Tfam protein expression ($P = 0.07$). PGC1α was also unaffected. Exercise early in life did not affect the expression of these proteins (Fig. 6).

**Figure 3. Paternal HFD before conception**

Paternal HFD before conception impairs basal and insulin-stimulated glucose uptake in epitrochlearis (B) but not soleus (A) muscle, whereas exercise early in life protects adult female offspring. Early-life exercise increases GLUT1 (C) and GLUT4 (D) in adult offspring epitrochlearis muscle. For GLUT1, two bands were identified and used to measure protein expression. Representative western blots show the quality and signal obtained with the respective antibodies; because they represent one animal, they do not necessarily represent an exact mean of their experimental group. NS, offspring sired by control diet fathers ($n = 6$); HS, offspring sired by HFD fathers ($n = 6$); NE, early-life exercised offspring sired by control diet fathers ($n = 5$); HE, early-life exercised offspring sired by HFD fathers ($n = 5$). Values are the mean ± SEM. & Insulin effect, $P < 0.05$. ∗ Paternal HFD effect, $P < 0.05$, 0.01. #Exercise early in life effect, $P < 0.05$. + Paternal HFD vs. Offspring exercise interaction, $P < 0.05$, followed by a least significant difference test (HS vs. HE).
although it did not change the number of $< 5000 \mu\text{m}^2$ and 10,000–20,000 $\mu\text{m}^2$ islets (Fig. 7).

**Discussion**

In the present study, we found that adult offspring sired by HFD fed fathers had reduced whole body and *ex vivo* skeletal muscle insulin sensitivity and reduced glucose-stimulated insulin secretion (with lower pancreatic $\beta$-cell mass). Only 4 weeks of exercise early in life normalized whole body and *ex vivo* insulin sensitivity in these adult offspring of HFD fed fathers. The benefits of the early exercise appeared to be mainly at the level of skeletal muscle because lower glucose-stimulated insulin secretion and lower pancreatic $\beta$-cell mass were not normalized by early exercise training.

Similar to previous studies (Fullston *et al.* 2013; Masuyama *et al.* 2016), paternal HFD before mating did not affect the number of pups born, the ratio between males and females, or the birth weight of the litter. In humans, paternal body mass index (BMI) is positively associated with offspring total and central fatness in youth (Labayen *et al.* 2010) but, in rodents, body weights tend to be higher or not different after HFD in fathers (Ng *et al.* 2010). However, we found that body weight was lower in female adult offspring fathered by rats fed with HFD, which was also reported in male offspring (Lecomte *et al.* 2017). Ng *et al.* (2010) found no difference in body weight

![Figure 4](image_url)

**Figure 4.** Basal and insulin signalling in epitrochlearis muscle of adult female offspring that exercised early in life or not, sired by HFD fathers or control diet fathers

Akt Thr308 (A), Ser473 (B) and TBC1D4 Thr642 (C) phosphorylation. For p-TBC1D4 Thr672 in insulin-stimulated condition (1.2 mM), two bands were identified and used to measure protein expression. NS, offspring sired by control diet fathers ($n = 6$); HS, offspring sired by HFD fathers ($n = 6$); NE, early-life exercised offspring sired by control diet fathers ($n = 5$); HE, early-life exercised offspring sired by HFD fathers ($n = 5$). Values are the mean ± SEM. &, && Insulin effect, $P < 0.05$, 0.01. *Paternal HFD effect, $P < 0.05$. *Paternal HFD vs. Offspring exercise interaction, $P < 0.05$, followed by a least significant difference test (HS vs. HE).
of their female offspring sired by HFD father at 12 weeks of age, although it is possible that the differences in older animals (e.g. 25 weeks of age) may have been missed in their study, as was examined in the present study. It is also possible that their offspring were not lighter because the fathers in that study (Ng et al. 2010) were fed standard chow in addition to the HFD, whereas our fathers were fed only the HFD (Ng, 2011). Although not mentioned in the study by Ng et al. (2010), it was subsequently stated in Ng’s PhD thesis that, in addition to high-fat pellets ‘a small amount of standard laboratory chow was also available in the cage’ (Ng, 2011).

Exercise early in life increased food intake and normalized the reduced body weight in offspring of HFD

![Graphs showing mitochondrial respiration parameters](image)

**Figure 5.** Mass-specific mitochondrial respiration in adult offspring that performed exercise early in life or not, sired by control diet or HFD fathers before conception

The following parameters were measured: leak respiration through complex I (ClL) (A), Maximum oxidative phosphorylation capacity (P) through Cl (ClP) (B), P through Cl+II combined (Cl+IIp) (C), electron transport system capacity (E) through Cl+II (Cl+IIE) (D) and E through Cl (E) (E). Citrate synthase activity was also measured in the plantaris muscle (F). NS, offspring sired by control diet fathers (n = 9); HS, offspring sired by HFD fathers (n = 8); NE, early-life exercised offspring sired by control diet fathers (n = 7); HE, early-life exercised offspring sired by HFD fathers (n = 8). F, paternal HFD and exercise early in life does not affect citrate synthase activity. NS, offspring sired by control diet fathers (n = 10); HS, offspring sired by HFD fathers (n = 10); NE, early-life exercised offspring sired by control diet fathers (n = 9); HE, early-life exercised offspring sired by HFD fathers (n = 10). Values are the mean ± SEM. #Exercise early in life effect, P < 0.05. +Paternal HFD vs. Offspring exercise interaction, P < 0.05, followed by a least significant difference test (HS vs. HE).
fed fathers by increases non-fat mass, including skeletal muscles, liver and heart weights. It is very interesting that only 4 weeks of exercise early in life is able to result in these changes 4 months later when the rats were 25 weeks of age.

*In vivo* insulin sensitivity, observed from the IPIST, was lower in offspring at 23 weeks of age after paternal HFD, which is in agreement with previous findings at 16, 26 and 39 weeks of age (Fullston *et al.* 2013). The lower insulin sensitivity was probably a result of reduced skeletal muscle insulin sensitivity because *ex vivo* insulin-stimulated glucose uptake and TBC1D4 Thr642 phosphorylation were also lower in the EPI muscle of offspring of HFD fed fathers. Only 4 weeks of exercise early in life in offspring sired by fathers fed a HFD was found to normalize insulin sensitivity, *ex vivo* insulin-stimulated glucose uptake and TBC1D4 Thr642 phosphorylation in adulthood. The early-life exercise also resulted in greater GLUT4 protein levels in adulthood, which may have contributed to the normalizing of insulin sensitivity. The effects of HFD fathers and early-life exercise on skeletal muscle insulin sensitivity appeared to be at the level of TBC1D4 because insulin-stimulated was not reduced by having a HFD fed father and also there was no effect of early-life exercise training on pAkt. It is not clear why offspring sired by fathers fed a HFD had higher insulin-stimulated pAktSer473. Although the results of the present study suggest that the effects of HFD fed fathers and early-life exercise on insulin sensitivity were at the level of the skeletal muscle, further studies are needed using tracer and clamp techniques to determine whether hepatic insulin resistance might also play a role.

We also observed lower basal *ex vivo* EPI glucose uptake in the adult offspring of a HFD fed father. Basal glucose uptake in skeletal muscle is considered to be related to GLUT1 protein, an insulin independent glucose transporter found in the sarcolemmal membrane (Ebeling *et al.* 1998). We found that GLUT1 protein content was 23% lower in HFD offspring, although this was not significant (*P* = 0.11). Early exercise training normalized the reduced basal *ex vivo* EPI glucose uptake and increased GLUT1 protein.

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**Figure 6.** Protein expression in plantaris muscle of adult offspring that performed exercise early in life or not, sired by control diet or HFD fathers before conception

PCG1α (*A*), Tfam (*B*) and PHF20 (*C*) protein expressions. For TFAM and PHF20, two bands were identified and used to measure protein expression. NS, offspring sired by control diet fathers (*n* = 10); HS, offspring sired by HFD fathers (*n* = 10); NE, early-life exercised offspring sired by control diet fathers (*n* = 9); HE, early-life exercised offspring sired by HFD fathers (*n* = 10). Representative western blots show the quality and signal obtained with the respective antibodies; because they represent one animal, they do not necessarily represent an exact mean of their experimental group. Values are the mean ± SEM. *Paternal HFD effect, *P* < 0.05.
Figure 7. Pancreas morphology in adult offspring sired by fathers fed a HFD or a control diet that did or did not exercise early in life

The parameters measured were: relative islet surface area expressed as a percentage of total pancreas surface area (A), number of islets (B), β-cell mass (C) calculated as the product of whole pancreas weight before fixation and the ratio of insulin positive/total pancreas cross-sectional area, β-cell area (D) and β-cell proportion per islet (E).
protein content in adulthood. It is without precedent that these changes in basal and insulin-stimulated glucose uptake are observed 4 months after the exercise ceased, suggesting that epigenetic changes may have occurred. We normally state ‘use it or lose it’ when it comes to exercise because adaptations to exercise are lost within days to weeks after exercise training ceases. Yet, as for short-term exercise early in life in our previous study in rats born small for gestational age, the effects are observed 4 months later (Laker et al. 2011). It will be important for future studies to examine possible epigenetic changes in this regard.

Some studies have indicated that skeletal muscle mitochondrial function can influence insulin sensitivity (Szendroedi et al. 2012; Holloszy, 2013; Martin & McGee, 2014). Skeletal muscle mitochondrial function of all the measured complexes was lower (20–30%) in offspring sired by HFD fed fathers, although there was no indication of an effect on respiratory ratios, reactive oxygen species production or citrate synthase activity. Exercise early in life in rats from chow fed fathers did not affect mitochondrial respiration but, when offspring sired by HFD fed fathers performed exercise early in life, their mitochondrial respiration was improved in adulthood. This remarkable effect of early-life exercise on mitochondrial function months later in offspring of HFD/obese fathers may have contributed to the normalization of insulin-stimulated glucose uptake in skeletal muscle. Similarly, the positive effects of maternal exercise are only observed in offspring of mothers on a HFD (Laker et al. 2014) or a low-protein diet (Falcao-Tebas et al. 2012), although maternal exercise in chow fed mums has no effects on the offspring. Similar effects might apply to exercise early in life, where positive effects are only observed when there was a prior negative event on the offspring.

Given the reprogramming effects of early-life exercise on skeletal muscle, we were surprised that early-life exercise had no effect on the reduced β-cell mass and reduced insulin secretion in adult offspring from fathers fed a HFD. However, we found changes in islet numbers and distribution in offspring of HFD fed fathers, and these alterations were only partially normalized by early-life exercise. We had previously demonstrated that exercise early in life fully restored the large decrease in β-cell mass (~65%) and pancreatic islet surface area in rats born small for gestational age (Laker et al. 2011). This suggests that the mechanisms causing decreases in β-cell mass with intrauterine growth restriction are different from those that involve the father’s sperm. It will be important to determine which genes are associated with the lower β-cell mass in the two models. In addition, the disparate effects of exercise early in life in the two models may be related to sex differences because Laker et al. (2011) used males, whereas only females were used in the present study, or they may be a result of rat strain differences between the Wistar–Kyoto rats used in the small for gestational age study (Laker et al. 2011) and the Sprague–Dawley rats used in the present study.

The underlying mechanism of how offspring exercise may modulate an offspring’s phenotype later in life might be related to epigenetic adaptations. Considering exercise early in life as an environmental factor, it would be possible to modify cellular and physiological phenotypic traits in the offspring by switching genes on and off (Handy et al. 2011). Different epigenetic markers, such as microRNAs, as well as acetylation and methylation of histones, might be involved in the long-term responses observed in the offspring. Although other studies have provided evidence to support TBC1D4Thr642 phosphorylation increasing during clamp, 5 h into exercise recovery (healthy men, 25–28 years) (Pehmoller et al. 2012) or 2 days after the last bout of exercise training (Frosig et al. 2007), in the present study, we have demonstrated its long-term effects (many weeks after exercise training had ceased) for the first time. Sixteen weeks after the last exercise session, the positive effects of exercise early in life were still observed in adult offspring sired by obese fathers fed with a HFD before conception. Further experiments investigating epigenetic markers in these offspring should provide useful insights that would help explain these findings.

In conclusion, exercise early in life attenuated the negative effects of paternal HFD whole body and ex vivo insulin sensitivity in adulthood. Exercise early in life increased GLUT4 protein and normalized the reduced skeletal muscle insulin-stimulated TBC1D4 phosphorylation, and also increased mitochondrial respiration. Surprisingly, early-life exercise did not have any positive effects on the reduced pancreatic β-cell mass in offspring from HFD fed fathers, which probably explains why exercise had no effects on insulin secretion in adulthood. Taken together with our previous findings in small for gestational age offspring, these results provide strong support for the importance of exercise early in life, especially in children who suffered some type of developmental insult.
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Exercise in offspring with a high-fat fed father


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**Additional information**

**Competing interests**

The authors declare that they have no competing interests.

**Author contributions**

FFT and GKM conceived and designed the work. FFT, JK, CA, JPK, SA and ECM conducted the acquisition of data. FFT, ECM and GKM performed the analysis and interpretation of data. FFT, ECM and GKM drafted the manuscript. FFT, JK, CA, JPK, SA and ECM and GKM revised it critically for important intellectual content. All authors approved the final version of the manuscript submitted for publication.
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