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Sex-Specific Effects of Maternal and Postweaning High-Fat Diet on Skeletal Muscle Mitochondrial Respiration

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Abstract

Exposure to maternal over-nutrition in utero is linked with developmental programming of obesity, metabolic syndrome, and cardiovascular disease in offspring, which may be exacerbated by postnatal high-fat diet. Skeletal muscle mitochondrial function contributes to substrate metabolism, and is impaired in metabolic disease. We examined muscle mitochondrial respiration in male and female mice exposed to maternal high-fat (HF) diet in utero, followed by postweaning HF diet until middle-age. After in utero exposure to maternal control (Con) or HF diet (45% kcal fat; 39.4% lard, 5.5% soybean oil), offspring were weaned to Con or HF, creating four groups: Con/Con (male/female, n=8/8), Con/HF (m/f, n=7/4), HF/Con (m/f, n=9/6), HF/HF (m/f, n=4/4). Oxidative phosphorylation (OXPHOS) and electron transfer system (ETS) capacity were measured in permeabilized gastrocnemius bundles. Maternal HF diet increased fasting glucose and lean body mass in males, and body fat percentage in both sexes ($p \leq 0.05$). Maximal ADP-stimulated respiration (complex I OXPHOS) was decreased by maternal HF diet in female offspring (-21%, $p=0.053$), but not in male (-0%, $p>0.05$). Sexually divergent responses were exacerbated in offspring weaned to HF diet. In females, OXPHOS capacity was lower (-28%, $p=0.041$) when weaned to high-fat (HF/HF) vs. control diet (HF/Con). In males, OXPHOS (+33%, $p=0.009$) and ETS (+42%, $p=0.016$) capacity increased. Our data suggest that maternal lard-based HF diet, rich in saturated fat, affects offspring skeletal muscle respiration in a sex-dependent manner, and these differences are exacerbated by HF diet in adulthood.

Keywords: fetal programming, developmental programming, oxidative phosphorylation, respirometry, sexual dimorphism

Introduction

The worldwide prevalence of obesity has nearly doubled since 1980 [1], making it a global public health concern. Among the world's obese adults, women account for a greater proportion of cases (15% vs. 11% in men) [1] and this trend is projected to continue [2, 3]. In the United States, one-third of adult women are obese [4] and approximately one in five women are obese during pregnancy [5]. Obesity at conception and throughout pregnancy not only increases the risk of adverse events during labor and delivery [6], but also programs long-term consequences on offspring health [7, 8]. The developmental programming hypothesis proposes that the intrauterine environment modulates fetal development, thereby affecting offspring healthspan [9]. In animal models and human studies, in utero exposure to maternal over-nutrition is linked to a greater propensity for obesity, metabolic syndrome, and cardiovascular disease in the offspring [10-13]. Rodent studies also demonstrate that exposure to a high-fat diet during postweaning exacerbates these programmed disease phenotypes [11, 14-16].

A primary feature of metabolic disease is impairment of mitochondrial function. The extent to which maternal obesity programs offspring mitochondrial function has been studied in several tissues important to fetal growth, reproduction, and metabolism including the placenta [17-19], ovaries [20], heart [14, 21], liver [22], and skeletal muscle [23, 24]. Skeletal muscle, comprising the majority of body mass in healthy adults and the tissue compartment with the widest span of metabolic activity, is a key contributor to substrate metabolism. When challenged with a high-fat diet, healthy skeletal muscle will preferentially oxidize fatty acids [25]. Adaptation to lipid overload through enhanced oxidation minimizes lipid peroxidation and accumulation of ectopic lipids within muscle, which interfere with insulin signaling and mitochondrial function [26]. The flexibility that enables this adaptation to substrate availability is mediated to a significant degree by mitochondria [27]. Specifically, skeletal muscles expressing high mitochondrial oxidative capacity, as seen in physically active or endurance trained individuals, are associated with an enhanced

ability to increase lipid oxidation use when challenged by lipid overload [28]. In offspring of obese mothers on the other hand, maternal programming of metabolic disease can be passed through aberrant oocyte mitochondria, to express in muscles across at least 3 generations [29]. Muscle protein expression of respiratory chain complexes I-V are lower in offspring of mothers fed a high-fat diet, and bioinformatics revealed downregulation of pathways associated with oxidative phosphorylation (OXPHOS), electron transport system (ETS), and ATP synthesis [24, 30]. Under these conditions, it is a strong possibility that OXPHOS capacity could be compromised. However, there is limited data on the impact of maternal obesity and postnatal diet on skeletal muscle mitochondrial function. We are aware of only a single report that examined maternal and postweaning high-fat diet effects on in situ muscle mitochondrial respiration [31], which found no effect of maternal diet in male offspring at postnatal day 70. However, the impact on offspring of either sex exposed to longer-term high-fat diet was not explored.

Recently, sex has received renewed attention as a biological variable of importance [32]. Evidence suggests that the programming effect of maternal obesity on cardiovascular impairments in the offspring depends on sex [33]. Given that inheritance of the mitochondrial genome is exclusively via the female parent, maternal mitochondrial dysfunction may translate to programmed alteration in mitochondrial ETS expression [24, 29] or mitochondrial function. We therefore aimed to evaluate skeletal muscle mitochondrial function in male and female mice born to high-fat fed dams and then weaned to a high-fat diet into middle-age.

Methods

Animals and design

This investigation was a sub-study of a larger experiment on the effects of maternal diet and postweaning on obesity in male and female mice. Female C57BL/6J weanling mice from

Jackson Laboratory were fed either a high-fat diet (HF, 45% kcal fat; 39.4% lard, 5.5% soybean oil; Research Diet D12451; N=12) or a control diet (Con, 10% kcal fat, D12450H; N=12) (Fig. 1). The nutrient composition of the diets is shown in Table 1. The HF diet contains lard rich in saturated fat to promote obesity and metabolic disease. At 11 weeks of age when mating occurred, HF females were significantly heavier than control females (HF 25.0±1.5 vs. Con 19.1±1.1 g, $p \leq 0.05$). Pregnancy was confirmed in N=10 HF females and N=12 control females. The respective diets were maintained during pregnancy and lactation. Following spontaneous delivery, litter size was standardized to 3 males and 3 females (to normalize nursing). At 3 weeks of age, 1 male and 1 female per litter were weaned to a HF diet and 2 males and 2 females to a control diet, resulting in four study groups based on maternal/offspring diet: Con/Con, Con/HF, HF/Con, HF/HF (Fig. 1). At 3 weeks of age, male and female offspring of HF dams had an average ~3 g/d greater food intake than offspring of Con dams; this increased to ~5 g/d greater food intake at 1 year. At one-year of age, 1 male and 1 female offspring from each litter were euthanized by isoflurane overdose. Body composition and fasting glucose was assessed in N=6 from each group. All mitochondrial function assays were performed within 4 hours of euthanasia, leaving 4-8 viable muscle samples in each group at the time of assay. One male Con/Con mouse was not assessed due to disease, and two other mice (one HF/Con male and one HF/Con female) were excluded due to quality control of the mitochondrial preparation.

Following removal of the vital organs, hindlimb skeletal muscles were isolated and the medial gastrocnemius placed immediately into ice-cold preservation buffer (BIOPS: 2.77 mM CaK_2EGTA, 7.23 mM K_2EGTA, 5.77 mM Na_2ATP, 6.56 mM $MgCl_2 \cdot 6H_2O$, 20 mM Taurine, 15 mM Na_2PCr, 20 mM Imidazole, 0.5 mM DTT, 50 mM MES hydrate) for in situ analysis of mitochondrial function. This muscle contains a mixed fiber type composition and has been previously used to investigate mitochondrial respiratory function in mouse studies of HF diet and

metabolic disease [34]. All procedures were approved by the Animal Care and Use Committee at Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

Body composition

Body composition was assessed under anesthesia (ketamine 100 mg/kg body mass and xylazine 10 mg/kg body mass) in 1-year old offspring by dual x-ray absorptiometry (DXA, QDR 4500A, Hologic, Bedford, MA). Body mass, lean body mass, and body fat was determined using small animal software program. Each scan lasted approximately one minute.

Fasting blood glucose

After an overnight fast, blood was collected from 1-year old offspring at sacrifice via cardiac puncture and blood glucose was measured using a Hemocue B-glucose analyzer (HemoCue Inc., Mission Viejo, CA).

Mitochondrial respiration

Mitochondrial respiration was measured in a total of 110 fiber bundles from the medial gastrocnemius at 37℃ in the oxygen concentration range of 550-350 nmol/ml using high-resolution respirometry (O2k, Oroboros, AT). After isolation from the hindlimb, the medial gastrocnemius was placed in a petri dish containing ice-cold BIOPS media and mechanically separated into duplicate fiber bundles (~4-6 mg each) using sharp forceps under a dissecting microscope. Fiber bundles were then permeabilized in BIOPS containing saponin (50 µg/ml) for 20 min and subsequently washed in respiration medium (MiR05) on ice for 10 min (MiR05: 0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM Sucrose, and 1g/l BSA, pH 7.1). After washing, samples were blotted dry on filter paper and weighed before being placed into the respirometer chambers. OXPHOS and electron transport system (ETS) capacity were assessed using a substrate-uncoupler-inhibitor-titration protocol [35] that consisted of the following sequential injections at saturating

concentrations: 1) 2 mM malate, 10 mM glutamate, and 2.5 mM ADP to achieve maximal ADP-stimulated respiration from maximal electron flux through complex I i.e. complex I OXPHOS; 2) 10 mM succinate to saturate complex II and achieve maximal convergent electron flux through both complexes I and II i.e. OXPHOS capacity or complex I+II OXPHOS; 3) 10 µM cytochrome c to assess the integrity of the outer mitochondrial membrane i.e. quality of sample preparation (duplicate samples were rejected when OXPHOS increased by >15% during this step [36]; a total of 2 duplicate samples were rejected); 4) 2.5 µM oligomycin to inhibit ATP synthase and evaluate non-phosphorylating LEAK respiration in the presence of high adenylates (L_{Omy}); 5) 0.5 µM carbonylcyanide p-trifluoromethoxy-phenylhydrazone (FCCP) to assess ETS capacity; 6) 0.5 µM rotenone to inhibit complex I and calculate the complex I contribution to ETS capacity; and 7) 2.5 µM Antimycin A to inhibit complex III and obtain residual oxygen consumption (non-mitochondrial respiration). Oxygen concentration in the respirometer chambers was maintained within the linear calibrated range (550-350 nmol/ml) using injections of 100% O_2 as necessary.

Oxygen flux for each respiratory state was expressed relative to sample weight and corrected by subtracting the residual O_2 consumption. Oxygen fluxes from each duplicate measurement were averaged and used for subsequent analysis. To determine the fraction of OXPHOS capacity serving LEAK respiration, the O_2 flux after oligomycin injection (L_{Omy}; step 4) was divided by complex I+II OXPHOS (step 2). To calculate the contribution of complex I to maximal ETS flux, O_2 flux after rotenone injection (step 6) was subtracted from the maximum uncoupled respiration induced by FCCP (step 5). To calculate complex I supported ETS flux as a fraction of ETS capacity, oxidation after rotenone injection (step 6) was divided by maximum uncoupled oxidation (step 5) and subtracted from one.

Statistical analysis

Data are presented as mean ± SE. Differences were determined for each sex separately using 2-way ANOVA with factors of maternal diet (control, high-fat) and offspring

postweaning diet (control, high-fat). Significant interactions were followed-up with Tukey's HSD or t-test. Pearson's correlation coefficient (r) was determined for selected variables.

Results

Phenotype of male and female offspring

The characteristics of 1-year old offspring are shown in Table 2. Male and female offspring of HF diet fed dams had greater body weight, increased adiposity, and lower lean mass compared to offspring of control-fed dams (main effect of maternal diet, $p \leq 0.05$). Postweaning HF diet had a similar effect (main effect of postweaning diet, $p \leq 0.05$). Maternal HF diet resulted in a greater fasting glucose in male offspring (main effect of maternal diet $p \leq 0.05$), while postweaning HF diet increased fasting glucose in both male and female offspring (main effect of postweaning diet $p \leq 0.05$). Maternal diet did not affect gastrocnemius or soleus weight ($p > 0.05$), but postweaning HF diet increased gastrocnemius in males (main effect of postweaning diet $p \leq 0.05$) and tended to reduce it in females ($p = 0.069$) (Table 3).

Maternal HF diet impaired muscle mitochondrial function in female but not male offspring

There tended to be a main effect of maternal diet in female, but not male offspring, with ~20% lower ADP-stimulated respiration (complex I OXPHOS) ($p = 0.053$) in female offspring of HF dams compared with offspring of Con dams (Fig. 2A). Complex I+II OXPHOS and maximal ETS capacity were also ~20% lower in female offspring of HF dams, although this did not reach significance ($p = 0.101$-0.129) (Fig. 2A). In male offspring, mitochondrial respiration was not affected by maternal diet (Fig. 2B). In males, postweaning HF diet increased maximal complex I OXPHOS (+33%), complex I+II OXPHOS (+33%), and ETS capacity (+42%) independently of maternal diet (main effects of postweaning diet $p \leq 0.05$) (Fig. 2B).

Gastrocnemius weight correlated significantly with complex I+II OXPHOS (r=0.454, p=0.030) and ETS capacity (r=0.471, p=0.023) in females, but there were no associations between mitochondrial function and gastrocnemius weight in males.

Combined maternal and postweaning HF diet impaired muscle mitochondrial function in female but not male offspring

Initial analyses revealed maternal diet to affect respiration in female but not male offspring. Therefore, follow-up 2-way ANOVAs were conducted on the respiration data within each maternal diet condition (maternal control, maternal high-fat) using sex and postweaning diet as factors. Interactions of postweaning diet and sex were not significant within the maternal control diet condition ($p>0.05$), but were significant for maternal HF diet ($p\leq0.05$). Complex I OXPHOS was greater in HF/Con females vs. HF/Con males (+28%, p=0.046) (Fig. 2A, B). Postweaning HF diet resulted in lower complex I OXPHOS in female offspring of HF dams (HF/HF vs. HF/Con, -28%, p=0.041), but did not affect complex I OXPHOS in males (HF/HF vs. HF/Con, +27%, p=0.110) (Fig. 2A, B). Together, complex I OXPHOS tended to be lower in HF/HF females compared to HF/HF males (-27%, p=0.081). Similar patterns were seen in for complex I+II OXPHOS and ETS capacity, although these did not consistently reach statistical significance (p=0.035 and p=0.110 respectively). The post hoc removal of a single outlier in the Con/HF female group increased the occurrence of statistical significance in these other respiratory states. Nonetheless, complex I+II OXPHOS tended to be greater in HF/Con females vs. HF/Con males (+29%, p=0.052) (Fig. 2A, B). Complex I+II OXPHOS tended to be less in female HF/HF vs. HF/Con (-24%, p=0.084) but was not different in male HF/HF vs. HF/Con (+25%, p=0.144) (Fig. 2A, B). There were no significant interaction or main effects for ETS capacity ($p>0.05$) (Fig. 2A, B).

Postweaning HF diet increased LEAK respiration and complex I supported ETS capacity in male offspring

Oligomycin-induced LEAK respiration (L_{Omy}) was greater with postweaning HF diet in male offspring only (+43%, main effect of postweaning diet, p=0.003) (Fig. 3A). LEAK respiration expressed as a fraction of OXPHOS (L_{Omy}/OXPHOS) tended to be greater with postweaning HF diet in male offspring (+9%, main effect of postweaning diet, p=0.071). On the other hand L_{Omy} was 54.8±12.7 pmol.s^{-1}.mg^{-1} in female Con/Con and lower in HF/Con and HF/HF (Fig. 3A), but not different across conditions as a fraction of OXPHOS (Fig. 3B). The contribution of complex I to maximum ETS capacity was increased by postweaning HF diet in male offspring only (+49%, main effect of postweaning diet p=0.003) (Fig. 4A). Within the maternal HF diet condition, there was a tendency for an interaction between sex and postweaning diet (p=0.057) on complex I supported ETS capacity (a decrease in oxidation in females and an increase in males with postweaning HF diet) in a similar pattern to that observed in complex I OXPHOS (Fig. 4A). When normalized to ETS capacity, there were no differences in complex I supported OXPHOS among all groups (Fig. 4B).

Discussion

We report that maternal HF diet resulted in lower rates of mitochondrial respiration in skeletal muscle of female but not of male offspring. The degree of respiratory impairment was consistent across a range of respiratory states: maximal complex I OXPHOS, complex I+II OXPHOS, and ETS capacity were each ~20% less in female offspring of high-fat-fed vs. control-fed dams. This was exacerbated by a postweaning HF diet maintained into adulthood (at 1 year), where postweaning HF diet resulted in further decline in muscle OXPHOS and ETS capacity in females, but increased these variables in males. These findings suggest that maternal and postweaning high-fat diet differentially affect muscle mitochondrial respiration in male and female offspring.

Some precedence for sexually dimorphic effects of developmental programming on mitochondrial function exists in the literature. Saben et al. showed that female mice fed a high fat and high sucrose diet gave birth to offspring that developed abnormal muscle mitochondrial morphology, a deranged ratio of the mitochondrial dynamic proteins Drp-1 and Opa-1 and reduced expression of ETS complex proteins [29]. The effect on mitochondrial dynamic proteins could be detected in the oocytes of the female F1 and F2 generation offspring, suggesting that the maternal derangement could be passed down the germline. On the other hand, Shelley et al. [37] showed no effect of maternal HF diet on respiratory chain enzyme activity in female offspring. The difference may be that their study did not exacerbate the mitochondrial dysfunction by long-term postweaning HF diet, as our study did. Further, our significant positive correlations between gastrocnemius mass and muscle respiration in the females suggest that loss of muscle mass in female HF fed offspring might be associated with an energetic impairment. A similar association was not observed in male muscles. Together these data suggest that maternal HF diet results in sexually dimorphic mitochondrial programming, which becomes most apparent when muscle is challenged by HF diet well into middle age.

The absence of a maternal HF diet effect on muscle respiration in male offspring was somewhat surprising. Previous investigations that focused on skeletal muscle mitochondria were conducted almost exclusively in male offspring [23, 24, 30, 31, 38]. Several genes and proteins regulating mitochondrial health (e.g. impaired mitochondrial dynamics, decreased PGC-1α, reduced complex I-V) were differentially expressed in males after in utero exposure to maternal high-fat diet [23, 24, 30]. These modifications strongly point to a corresponding alteration of mitochondrial function; however, our data do not support this inference, as muscle respiration in male offspring was affected principally by postweaning HF diet alone, at least when indexed to muscle mass rather than a marker of mitochondrial mass.

Although proton leak contributes to the inefficiency of OXPHOS by uncoupling oxidation from ATP production, dissipation of the proton gradient provides protection against oxidative stress generated as byproducts of oxidative metabolism [39]. High LEAK respiration may be a compensatory adaptation to alleviate increased production of reactive oxygen species or oxidative stress. LEAK respiration was not altered by maternal diet in offspring of either sex, but was increased with weaning HF diet in male offspring only, suggesting a possible protective response to oxidative stress. In females, however, the absolute rate of LEAK respiration was high even in controls, which may reduce the capacity for compensation to oxidative stress by uncoupling, and increase oxidative damage of mitochondrial membranes, proteins and/or mtDNA, and ultimately reduce respiratory capacity. These suggestions remain to be verified.

Our data showed that additive postweaning HF diet increased fasting glucose in both males and females, though the effect appeared more marked in males. Notably, the increase in percentage body fat is greater in the females, suggesting that perhaps there is less glucose uptake by adipose tissue in males than females. A dyshomeostasis in female triglyceride handing may help explain the reduced mitochondrial function in female muscle, as the ability to adapt to lipid overload through enhanced oxidation minimizes lipid peroxidation and the accumulation of ectopic lipids, which interfere with mitochondrial function [26]. Therefore, females appeared to better regulate glucose, perhaps at the expense of lipid metabolism in contrast to males where lipid control appears preferred. This may help explain increased plasma glucose concentration in males and provide evidence for programming of metabolic dysfunction despite unaffected muscle respiration. Further work is needed to explore these suggestions.

In human studies, insulin sensitivity is reduced in post-pubertal males, but increased in females [40]. Circulating estradiol concentration has been implicated in mediating this effect [41-43], and is subject to programming by maternal obesity [44]. In addition, prandial and postprandial fat oxidation is lower in young women compared to men [45, 46], whereas this is reversed during

294 physical activity [47]. Thus, whether the programmed loss of mitochondrial respiration that we
295 found in the female offspring obese dams can be ameliorated by offspring exercise is a key
296 future step to better understand these sexually dimorphic findings.

297 Our use of a lard-based HF diet to induce obesity merits further discussion as dietary
298 lipid composition can generate diverse metabolic effects with implications for human health. For
299 instance, short-term (8 weeks) HF diet based on either lard (enriched in saturated fat) or corn oil
300 (concentrated in omega-6 polyunsaturated fatty acids) results in similar weight gain and insulin
301 resistance but lard-based HF diet causes greater fatty liver and increased enzyme activity of
302 stearoyl-CoA desaturase-1 [48]. Although we did not examine the liver, hepatic mitochondrial
303 dysfunction is an important feature of fatty liver, and could be subject to maternal programming
304 and weaning diet effects in the offspring.

305 In this study we aimed to minimize the impact of litter specific effects by using only one
306 offspring of each sex per litter. In addition, mitochondrial function assays require viable tissue,
307 with viability being maintained for ~8-10 hours after euthanasia. These experimental constraints,
308 limited the number of animals and muscles available for study, and some groups suffer from a
309 low number of samples (e.g. n=4 in 3 of the 8 experimental conditions). Although posthoc
310 analysis reveals low statistical power (1-β) for interactions between maternal and postweaning
311 diet (ranging 0.20-0.45), we note that the primary conclusion of sexually dimorphic responses in
312 mitochondrial variables in maternal HF diet groups carries an observed power of 0.70-0.80.

313 In summary, maternal and postweaning high-fat diet differentially affected mitochondrial
314 respiration in skeletal muscle of male and female offspring. Females exposed to a high-fat diet
315 in utero had greater adiposity and lower muscle respiratory capacity; effects that were
316 exacerbated by continuing HF diet exposure for 1 year postweaning. In contrast, muscle
317 respiration in male offspring was not affected by maternal HF diet, and was actually greater
318 when weaned to a HF diet. Unlike females, there was an increase in relative LEAK respiration

with postweaning HF diet, consistent with the proposal that male offspring compensated for the effects of high-fat overload via mitochondrial uncoupling (possibly to alleviate oxidative stress). Overall, the most deleterious effects on muscle mitochondrial function occurred in female mice exposed to maternal and postweaning high-fat diet.

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Conflicts of Interest

The authors declare no conflicts of interest

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (Animal Welfare Act, USDA), and has been approved by the institutional committee at Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

References

1. WHO. Chapter 7. Global Target: 7: Halt the rise in diabetes and obesity. *Global Status Report on Noncommunicable Diseases 2014*.
2. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)*. 2008;32(9), 1431-1437.
3. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. *Obesity (Silver Spring)*. 2008;16(10), 2323-2330.
4. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012;307(5), 491-497.
5. Fisher SC, Kim SY, Sharma AJ, Rochat R, Morrow B. Is obesity still increasing among pregnant women? Prepregnancy obesity trends in 20 states, 2003-2009. *Prev Med*. 2013;56(6), 372-378.
6. Baeten JM, Bukusi EA, Lambe M. Pregnancy complications and outcomes among overweight and obese nulliparous women. *Am J Public Health*. 2001;91(3), 436-440.
7. White CL, Purpera MN, Morrison CD. Maternal obesity is necessary for programming effect of high-fat diet on offspring. *Am J Physiol Regul Integr Comp Physiol*. 2009;296(5), R1464-1472.
8. Srinivasan M, Katewa SD, Palaniyappan A, Pandya JD, Patel MS. Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. *Am J Physiol Endocrinol Metab*. 2006;291(4), E792-799.
9. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr*. 2004;23(6 Suppl), 588S-595S.
10. Desai M, Jellyman JK, Han G, Beall M, Lane RH, Ross MG. Maternal obesity and high-fat diet program offspring metabolic syndrome. *Am J Obstet Gynecol*. 2014;211(3), 237 e231-237 e213.
11. Guberman C, Jellyman JK, Han G, Ross MG, Desai M. Maternal high-fat diet programs rat offspring hypertension and activates the adipose renin-angiotensin system. *Am J Obstet Gynecol*. 2013;209(3), 262 e261-268.
12. Lee KK, Raja EA, Lee AJ, Bhattacharya S, Norman JE, Reynolds RM. Maternal Obesity During Pregnancy Associates With Premature Mortality and Major Cardiovascular Events in Later Life. *Hypertension*. 2015;66(5), 938-944.
13. Reynolds RM, Allan KM, Raja EA, et al. Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1 323 275 person years. *BMJ*. 2013;347, f4539.
14. Turdi S, Ge W, Hu N, Bradley KM, Wang X, Ren J. Interaction between maternal and postnatal high fat diet leads to a greater risk of myocardial dysfunction in offspring via enhanced lipotoxicity, IRS-1 serine phosphorylation and mitochondrial defects. *J Mol Cell Cardiol*. 2013;55, 117-129.
15. Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJ, Badger TM. Maternal obesity at conception programs obesity in the offspring. *Am J Physiol Regul Integr Comp Physiol*. 2008;294(2), R528-538.
16. Page KC, Malik RE, Ripple JA, Anday EK. Maternal and postweaning diet interaction alters hypothalamic gene expression and modulates response to a high-fat diet in male offspring. *Am J Physiol Regul Integr Comp Physiol*. 2009;297(4), R1049-1057.
17. Muralimanoharan S, Gao X, Weintraub S, Myatt L, Maloyan A. Sexual dimorphism in activation of placental autophagy in obese women with evidence for fetal programming from a placenta-specific mouse model. *Autophagy*. 2016;12(5), 752-769.

18. Muralimanoharan S, Guo C, Myatt L, Maloyan A. Sexual dimorphism in miR-210 expression and mitochondrial dysfunction in the placenta with maternal obesity. *Int J Obes (Lond)*. 2015;39(8), 1274-1281.
19. Mele J, Muralimanoharan S, Maloyan A, Myatt L. Impaired mitochondrial function in human placenta with increased maternal adiposity. *Am J Physiol Endocrinol Metab*. 2014;307(5), E419-425.
20. Aiken CE, Tarry-Adkins JL, Penfold NC, Dearden L, Ozanne SE. Decreased ovarian reserve, dysregulation of mitochondrial biogenesis, and increased lipid peroxidation in female mouse offspring exposed to an obesogenic maternal diet. *FASEB J*. 2016;30(4), 1548-1556.
21. Fernandez-Twinn DS, Blackmore HL, Siggens L, et al. The programming of cardiac hypertrophy in the offspring by maternal obesity is associated with hyperinsulinemia, AKT, ERK, and mTOR activation. *Endocrinology*. 2012;153(12), 5961-5971.
22. Alfaradhi MZ, Fernandez-Twinn DS, Martin-Gronert MS, Musial B, Fowden A, Ozanne SE. Oxidative stress and altered lipid homeostasis in the programming of offspring fatty liver by maternal obesity. *Am J Physiol Regul Integr Comp Physiol*. 2014;307(1), R26-34.
23. Borengasser SJ, Faske J, Kang P, Blackburn ML, Badger TM, Shankar K. In utero exposure to prepregnancy maternal obesity and postweaning high-fat diet impair regulators of mitochondrial dynamics in rat placenta and offspring. *Physiol Genomics*. 2014;46(23), 841-850.
24. Latouche C, Heywood SE, Henry SL, et al. Maternal overnutrition programs changes in the expression of skeletal muscle genes that are associated with insulin resistance and defects of oxidative phosphorylation in adult male rat offspring. *J Nutr*. 2014;144(3), 237-244.
25. Battaglia GM, Zheng D, Hickner RC, Houmard JA. Effect of exercise training on metabolic flexibility in response to a high-fat diet in obese individuals. *Am J Physiol Endocrinol Metab*. 2012;303(12), E1440-1445.
26. Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. *Am J Physiol Endocrinol Metab*. 2008;295(5), E1009-1017.
27. Chomentowski P, Coen PM, Radikova Z, Goodpaster BH, Toledo FG. Skeletal muscle mitochondria in insulin resistance: differences in intermyofibrillar versus subsarcolemmal subpopulations and relationship to metabolic flexibility. *J Clin Endocrinol Metab*. 2011;96(2), 494-503.
28. Dube JJ, Coen PM, DiStefano G, et al. Effects of acute lipid overload on skeletal muscle insulin resistance, metabolic flexibility, and mitochondrial performance. *Am J Physiol Endocrinol Metab*. 2014;307(12), E1117-1124.
29. Saben JL, Boudoures AL, Asghar Z, et al. Maternal Metabolic Syndrome Programs Mitochondrial Dysfunction via Germline Changes across Three Generations. *Cell Rep*. 2016;16(1), 1-8.
30. Pileggi CA, Hedges CP, Segovia SA, et al. Maternal High Fat Diet Alters Skeletal Muscle Mitochondrial Catalytic Activity in Adult Male Rat Offspring. *Front Physiol*. 2016;7, 546.
31. Hellgren LI, Jensen RI, Waterstradt MS, Quistorff B, Lauritzen L. Acute and perinatal programming effects of a fat-rich diet on rat muscle mitochondrial function and hepatic lipid accumulation. *Acta Obstet Gynecol Scand*. 2014;93(11), 1170-1180.
32. Miller VM, Reckelhoff JF. Sex as a Biological Variable: Now What?! *Physiology (Bethesda)*. 2016;31(2), 78-80.
33. Khan IY, Taylor PD, Dekou V, et al. Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension*. 2003;41(1), 168-175.
34. Morrow RM, Picard M, Derbeneva O, et al. Mitochondrial energy deficiency leads to hyperproliferation of skeletal muscle mitochondria and enhanced insulin sensitivity. *Proc Natl Acad Sci U S A*. 2017;114(10), 2705-2710.

35. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods Mol Biol*. 2012;810, 25-58.
36. Kuznetsov AV, Veksler V, Gellerich FN, Saks V, Margreiter R, Kunz WS. Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells. *Nat Protoc*. 2008;3(6), 965-976.
37. Shelley P, Martin-Gronert MS, Rowlerson A, et al. Altered skeletal muscle insulin signaling and mitochondrial complex II-III linked activity in adult offspring of obese mice. *Am J Physiol Regul Integr Comp Physiol*. 2009;297(3), R675-681.
38. Simar D, Chen H, Lambert K, Mercier J, Morris MJ. Interaction between maternal obesity and post-natal over-nutrition on skeletal muscle metabolism. *Nutr Metab Cardiovasc Dis*. 2012;22(3), 269-276.
39. Divakaruni AS, Brand MD. The regulation and physiology of mitochondrial proton leak. *Physiology (Bethesda)*. 2011;26(3), 192-205.
40. Moran A, Jacobs DR, Jr., Steinberger J, et al. Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females. *Circulation*. 2008;117(18), 2361-2368.
41. Newbold RR, Padilla-Banks E, Snyder RJ, Jefferson WN. Perinatal exposure to environmental estrogens and the development of obesity. *Mol Nutr Food Res*. 2007;51(7), 912-917.
42. Newbold RR, Padilla-Banks E, Jefferson WN. Environmental estrogens and obesity. *Mol Cell Endocrinol*. 2009;304(1-2), 84-89.
43. Van Pelt RE, Gozansky WS, Schwartz RS, Kohrt WM. Intravenous estrogens increase insulin clearance and action in postmenopausal women. *American journal of physiology Endocrinology and metabolism*. 2003;285(2), E311-317.
44. Grantham JP, Henneberg M. The estrogen hypothesis of obesity. *PLoS One*. 2014;9(6), e99776.
45. Levadoux E, Morio B, Montaurier C, et al. Reduced whole-body fat oxidation in women and in the elderly. *Int J Obes Relat Metab Disord*. 2001;25(1), 39-44.
46. Uranga AP, Levine J, Jensen M. Isotope tracer measures of meal fatty acid metabolism: reproducibility and effects of the menstrual cycle. *American journal of physiology Endocrinology and metabolism*. 2005;288(3), E547-555.
47. Tarnopolsky MA. Sex differences in exercise metabolism and the role of 17-beta estradiol. *Med Sci Sports Exerc*. 2008;40(4), 648-654.
48. Pavlisova J, Bardova K, Stankova B, Tvrzicka E, Kopecky J, Rossmeisl M. Corn oil versus lard: Metabolic effects of omega-3 fatty acids in mice fed obesogenic diets with different fatty acid composition. *Biochimie*. 2016;124, 150-162.

Table 1. Nutrient composition of diets.

	Purified Diet D12450H (10% kcal fat)	Purified Diet D12451 (45% kcal fat)
Nutrients (%)		
Carbohydrate	70	35
Protein	20	20
Fat	10	45
Fat Type		
Lard	4.4	39.4
Soybean oil	2.4	5.5

Nutrient values are percentage per 100g food and fat type is percentage of total kcal.

Table 2. Phenotype of one-year old male and female offspring.

	Male				Female			
	Con/Con	**Con/HF**	**HF/Con**	**HF/HF**	**Con/Con**	**Con/HF**	**HF/Con**	**HF/HF**
Body weight (g)	39.7±2.4	53.8±2.0$^{\#}$	54.6±1.7*	60.0±1.7*$^{\#}$	30.4±1.5	44.4±1.2$^{\#}$	42.5±3.3*	62.1±1.8*$^{\#}$
Lean body weight (g)	23.0±0.6	24.9±0.3$^{\#}$	23.5±0.5*	26.2±0.4*$^{\#}$	17.7±0.3	16.1±0.4	18.2±0.3	18.5±0.6
Lean body weight (%)	59.9±2.8	50.6±2.2$^{\#}$	46.2±1.6*	41.1±2.3*$^{\#}$	57.7±1.6	36.9±2.8$^{\#}$	48.9±1.6*	32.9±1.5*$^{\#}$
Body fat (%)	37.7±2.9	47.4±2.3$^{\#}$	51.8±1.7*	56.1±2.3*$^{\#}$	39.8±1.7	60.8±2.9$^{\#}$	48.7±1.5*	65.9±1.4*$^{\#}$
Fasting glucose (mg/dl)	124±7.1	179±7.3$^{\#}$	186±7.3*	212±7.8*$^{\#}$	123±5.8	134±5.3$^{\#}$	128±5.6	141±5.5$^{\#}$

After in utero exposure to maternal control (Con) or high-fat (HF) diet, offspring were weaned to Con or HF, creating four study groups: Con/Con, Con/HF, HF/Con, HF/HF. Six males and 6 females were measured from 6 separate litters per group. Data was analyzed by 2-way ANOVA (maternal diet x postweaning diet). *p≤0.05 main effect of maternal diet, maternal HF vs. maternal Con. #p≤0.05 main effect of postweaning diet, postweaning HF vs. postweaning Con.

Table 3. Muscle weights of one-year old male and female offspring.

	Male				Female			
	Con/Con	**Con/HF**	**HF/Con**	**HF/HF**	**Con/Con**	**Con/HF**	**HF/Con**	**HF/HF**
Gastrocnemius (mg)	127.3±3.0	138.8±1.2#	133.8±1.5	140.6±3.3#	107.7±2.8	99.3±5.6^	108.9±1.9	104.3±3.7^
Soleus (mg)	7.9±0.3	8.6±0.5	8.7±0.3	8.6±0.3	6.6±0.3	7.0±0.7	6.4±0.2	6.9±0.4

After in utero exposure to maternal control (Con) or high-fat (HF) diet, offspring were weaned to Con or HF, creating four groups for each sex: Con/Con (male n=8, female n=8), Con/HF (male n=7, female n=4), HF/Con (male n=9, female n=6), HF/HF (male n=4, female n=4). Muscle weights were averaged from both hindlimbs. Data was analyzed by 2-way ANOVA (maternal diet x postweaning diet). #p≤0.05 and ^p=0.069 main effect of postweaning diet, postweaning HF vs. postweaning Con.

Figure Legends

Figure 1. Overview of experiment. Con, control diet. HF, high-fat diet.

Figure 2. Mitochondrial respiration in the medial gastrocnemius of one-year old female (A) and male (B) offspring. After in utero exposure to maternal control (Con) or high-fat (HF) diet, offspring were weaned to Con or HF, creating four groups for each sex: Con/Con (male n=8, female n=8), Con/HF (male n=7, female n=4), HF/Con (male n=9, female n=6), HF/HF (male n=4, female n=4). Maximal ADP-stimulated respiration (CI OXPHOS). Maximal convergent electron flux (Complex I+II OXPHOS). Maximal electron transfer system capacity (ETS). Values are mean ± SE. Differences initially determined for each sex separately by 2-way ANOVA with factors of maternal diet (control, high-fat) and offspring postweaning diet (control, high-fat). Initial analyses revealed maternal diet to affect respiration in female but not male offspring. Follow-up 2-way ANOVAs were then conducted separately on the respiration data for each maternal diet condition (control, high-fat) using sex and postweaning diet as factors. * Main effect ($p \leq 0.05$) of material diet in female offspring. $^{\#}$ Main effect ($p \leq 0.05$) of weaning diet in male offspring. ^{a} $p \leq 0.05$ vs. HF/Con males. ^{b} $p \leq 0.05$ vs. HF/HF within sex. ^{c} $p=0.081$ vs. HF/HF males. ^{d} $p=0.084$ vs. HF/HF within sex. Numbers within each bar indicates the n for that group.

Figure 3. Non-phosphorylating LEAK respiration induced by the ATP synthase inhibitor oligomycin (L_{Omy}) (A), and L_{Omy} expressed as a fraction of maximum oxidative phosphorylation (OXPHOS) capacity (L_{Omy}/OXPHOS) (B) in one-year old offspring. The four offspring groups for each sex were based on maternal control (Con) or high-fat (HF) diet, and postweaning Con or HF: Con/Con (male n=8, female n=8), Con/HF (male n=7, female n=4), HF/Con (male n=9, female n=6), HF/HF (male n=4, female n=4). Values are mean ± SE. Mean differences were determined for each sex separately using 2-way ANOVA with factors of maternal diet (control,

high-fat) and offspring postweaning diet (control, high-fat). Numbers within each bar indicates the n for that group.

Figure 4. Contribution of complex I to electron transfer system capacity (Complex I ETS; A). Complex I ETS was also expressed relative to maximum ETS obtained by titration with FCCP (B). The four offspring groups for each sex were based on maternal control (Con) or high-fat (HF) diet, and postweaning Con or HF: Con/Con (male n=8, female n=8), Con/HF (male n=7, female n=4), HF/Con (male n=9, female n=6), HF/HF (male n=4, female n=4). Values are mean ± SE. Mean differences were determined for each sex separately using 2-way ANOVA with factors of maternal diet (control, high-fat) and offspring postweaning diet (control, high-fat). Numbers within each bar indicates the n for that group.

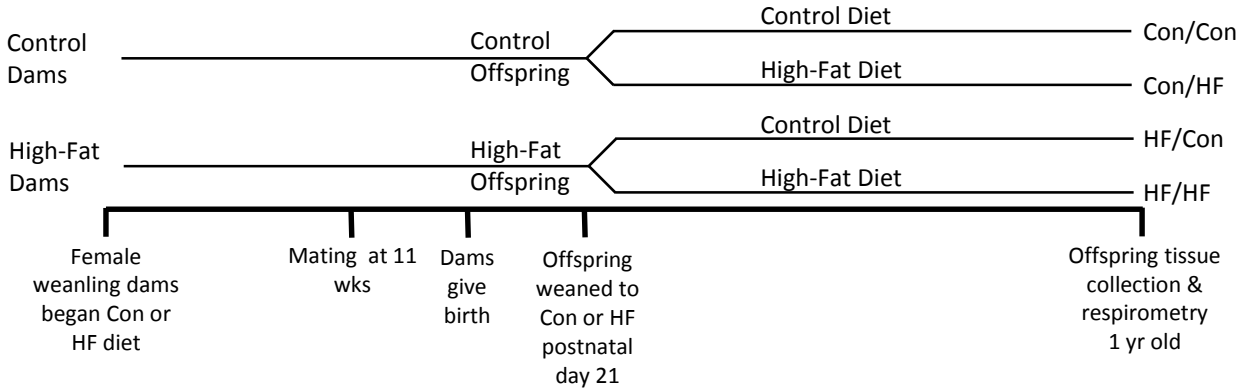
Control Dams
Control Offspring
Control Diet
High-Fat Diet
Con/Con
Con/HF
High-Fat Dams
High-Fat Offspring
Control Diet
High-Fat Diet
HF/Con
HF/HF
Female weanling dams began Con or HF diet
Mating at 11 wks
Dams give birth
Offspring weaned to Con or HF postnatal day 21
Offspring tissue collection & respirometry 1 yr old

Figure 1.

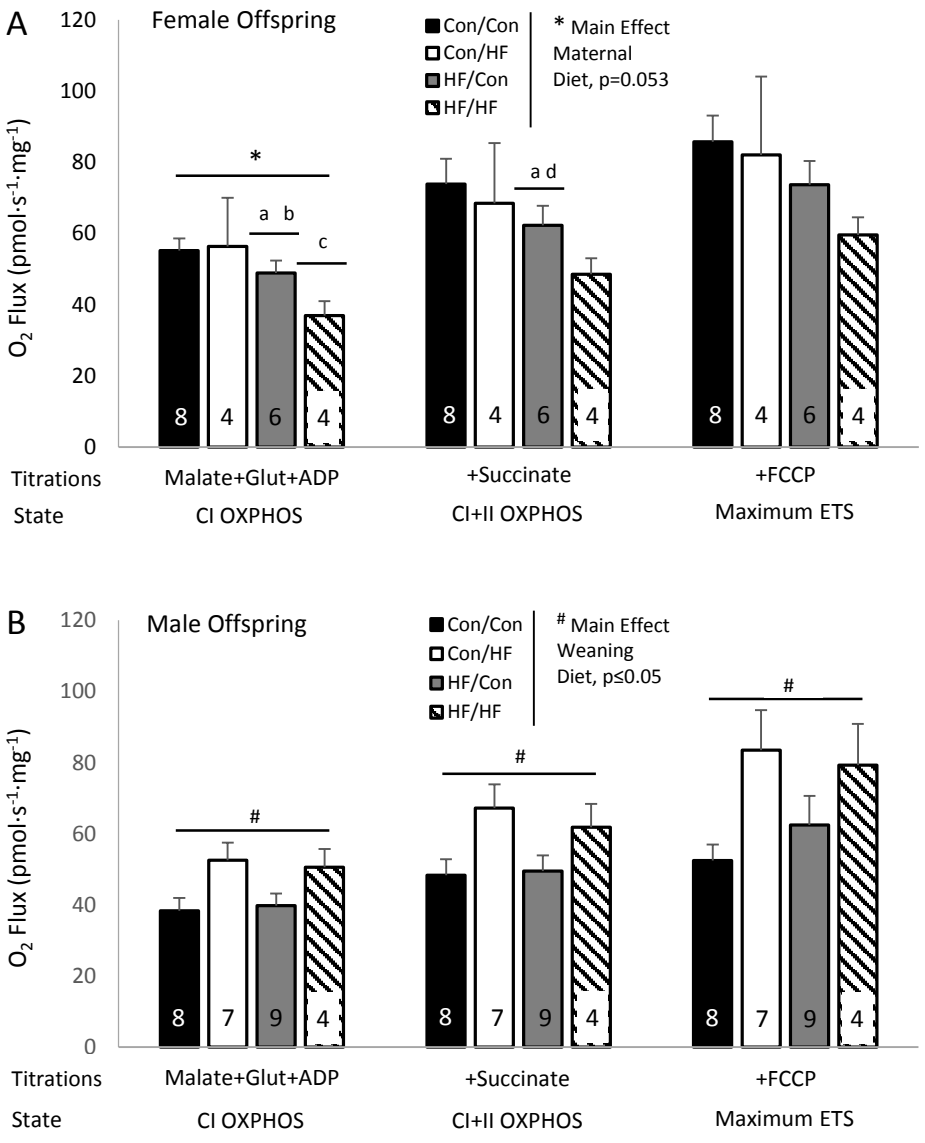
A
Female Offspring
Con/Con
Con/HF
HF/Con
HF/HF
* Main Effect
Maternal
Diet, p=0.053
O2 Flux (pmol·s-1·mg-1)
120
100
80
60
40
20
0
*
a b
c
a d
8
4
6
4
8
4
6
4
8
4
6
4
Titrations
Malate+Glut+ADP
+Succinate
+FCCP
State
CI OXPHOS
CI+II OXPHOS
Maximum ETS
B
Male Offspring
Con/Con
Con/HF
HF/Con
HF/HF
Main Effect
Weaning
Diet, p≤0.05
O2 Flux (pmol·s-1·mg-1)
120
100
80
60
40
20
0
#
#
#
8
7
9
4
8
7
9
4
8
7
9
4
Titrations
Malate+Glut+ADP
+Succinate
+FCCP
State
CI OXPHOS
CI+II OXPHOS
Maximum ETS

Figure 2.

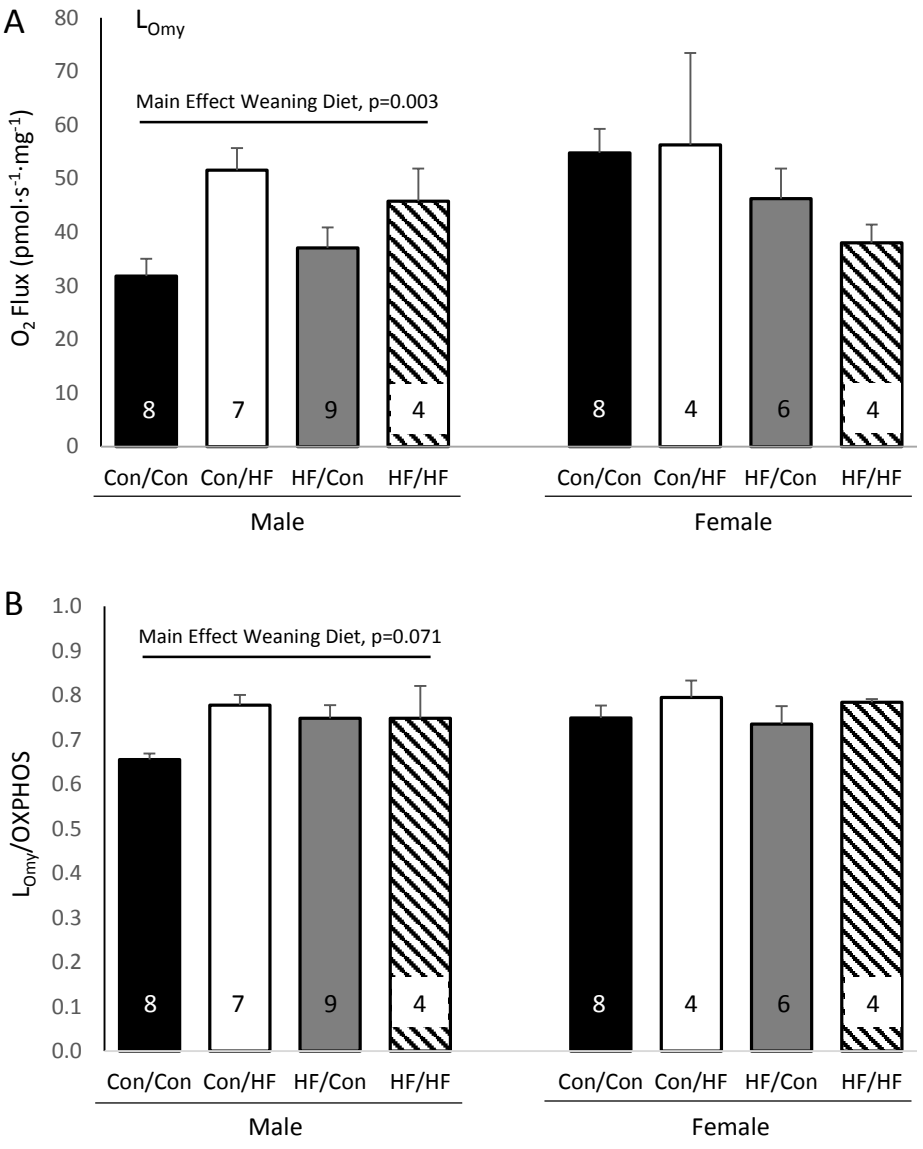
A
L_{Omy}
Main Effect Weaning Diet, p=0.003
O_2 Flux (pmol·s^{-1}·mg^{-1})
80
70
60
50
40
30
20
10
0
8
7
9
4
8
4
6
4
Con/Con
Con/HF
HF/Con
HF/HF
Con/Con
Con/HF
HF/Con
HF/HF
Male
Female
B
Main Effect Weaning Diet, p=0.071
L_{Omy}/OXPHOS
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
8
7
9
4
8
4
6
4
Con/Con
Con/HF
HF/Con
HF/HF
Con/Con
Con/HF
HF/Con
HF/HF
Male
Female

Figure 3.

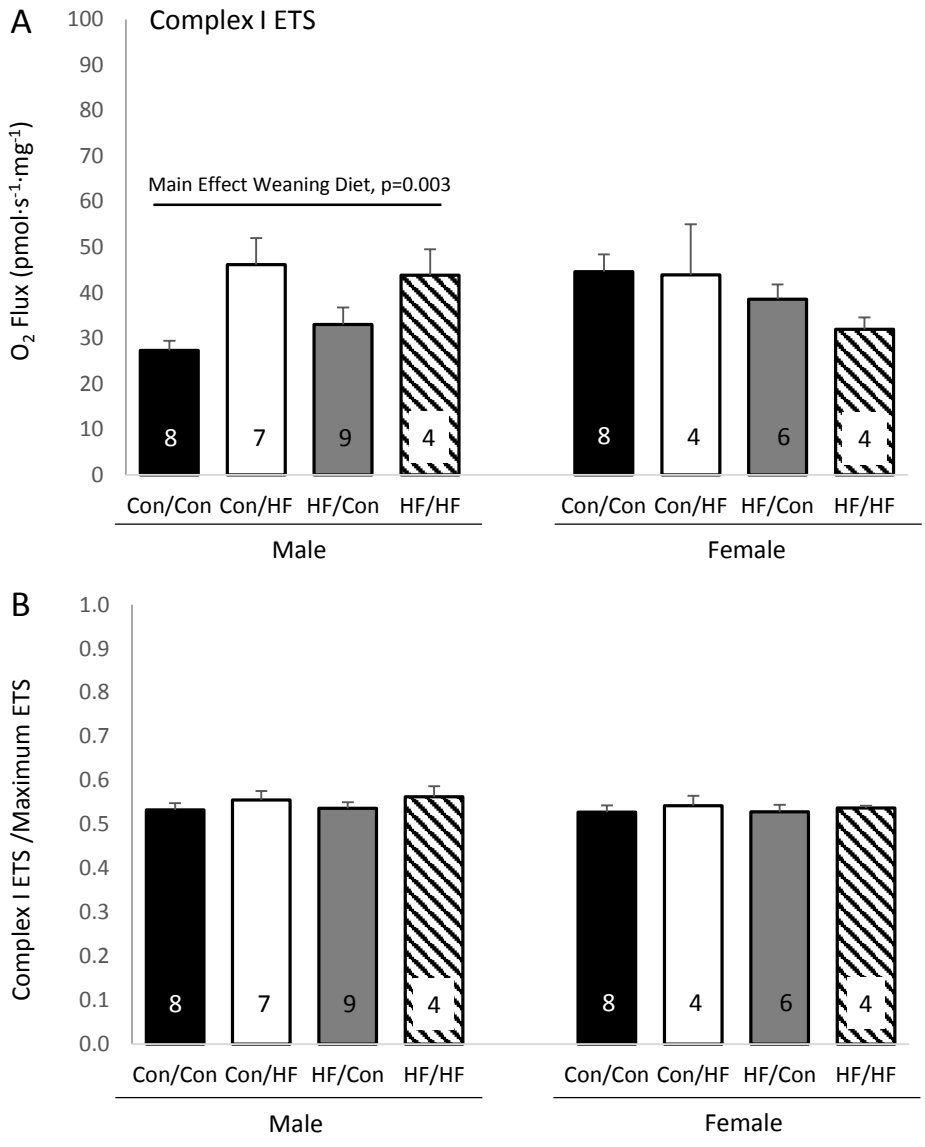
A
Complex I ETS
O_2 Flux (pmol·s^{-1}·mg^{-1})
100
90
80
70
60
50
40
30
20
10
0
Main Effect Weaning Diet, p=0.003
8
7
9
4
8
4
6
4
Con/Con Con/HF HF/Con HF/HF
Con/Con Con/HF HF/Con HF/HF
Male
Female
B
Complex I ETS /Maximum ETS
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
8
7
9
4
8
4
6
4
Con/Con Con/HF HF/Con HF/HF
Con/Con Con/HF HF/Con HF/HF
Male
Female

Figure 4.