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Sex-specific effects of maternal and postweaning high-fat diet on skeletal muscle mitochondrial respiration

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Original Article

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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 (HF) 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 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 (m/f), $n = 8/8$), Con/HF (m/f, $n = 7/4$), HF/Con (m/f, $n = 9/6$) and 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 ($P \leqslant 0.05$). Maximal adenosine diphosphate-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) *v.* 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.

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 *v.* 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 (HF) 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 HF diet, the 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 the muscle, which interferes 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

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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 three generations.[29] Muscle protein expression of respiratory chain complexes I–V is lower in offspring of mothers fed a HF diet, and bioinformatics revealed downregulation of pathways associated with oxidative phosphorylation (OXPHOS), electron transfer system (ETS) and adenosine triphosphate (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 HF 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 HF 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 HF-fed dams and then weaned to a HF diet into middle age.

Methods

Animals and design

This investigation was a substudy 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 HF 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 Con females (HF 25.0 ± 1.5 *v.* Con 19.1 ± 1.1 g, $P \leqslant 0.05$). Pregnancy was confirmed in $n = 10$ HF females and $n = 12$ Con females. The respective diets were maintained during pregnancy and lactation. Following spontaneous delivery, litter size was standardized to three males and three females (to normalize nursing). At 3 weeks of age, one male and one female per litter were weaned to an HF diet and two males and two females to a Con diet, resulting in four study groups based on maternal/offspring diet: Con/Con, Con/HF, HF/Con and HF/HF (Fig. 1). At 3 weeks of age, male and female offspring of HF dams had an average ~3 g/day greater food intake than offspring of Con dams; this increased to ~5 g/day greater food intake at 1 year. At 1 year of age, one male and one female offspring from each litter were euthanized by isoflurane overdose. Body composition and fasting glucose were assessed in $n = 6$ from each group. All mitochondrial function assays were performed within 4 h of euthanasia, leaving four to eight 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 dithiothreitol and 50 mM 4-morpholineethanesulfonic acid 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, USA). Body mass, lean body mass and body fat were determined using small animal software program. Each scan lasted ~1 min.

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, USA).

Mitochondrial respiration

Mitochondrial respiration was measured in a total of 110 fiber bundles from the medial gastrocnemius at 37°C in the oxygen

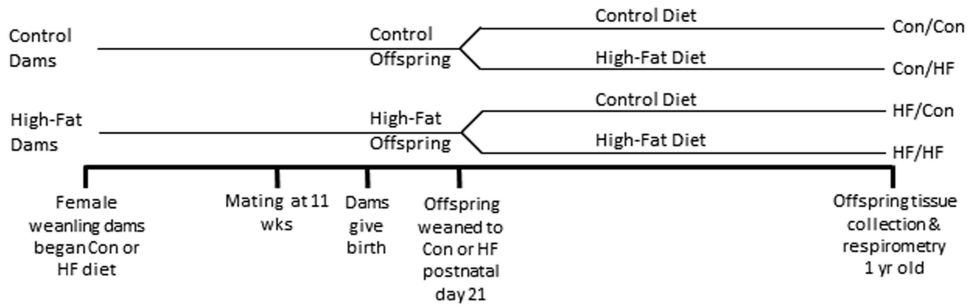

Fig. 1. Overview of the experiment. Con, control; HF, high-fat.

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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 ethylene glycol tetraacetic acid, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 1 g/l bovine serum albumin, pH 7.1). After washing, samples were blotted dry on filter paper and weighed before being placed into the respirometer chambers. OXPHOS and 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 adenosine diphosphate (ADP) to achieve maximal ADP-stimulated respiration from maximal electron flux through complex I, that is, complex I OXPHOS; (2) 10 mM succinate to saturate complex II and achieve maximal convergent electron flux through both complexes I and II, that is, OXPHOS capacity or complex I + II OXPHOS; (3) 10 µM cytochrome c to assess the integrity of the outer mitochondrial membrane, that is, quality of sample preparation (duplicate samples were rejected when OXPHOS increased by >15% during this step[36]; a total of two 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). The 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 two-way ANOVA with factors of maternal diet (Con, HF) and offspring postweaning diet (Con, HF). Significant interactions were followed up with Tukey's HSD or *t*-test. Pearson's correlation coefficient (*r*) was determined for selected variables.

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 100 g food and fat type is a percentage of total kcal.

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, $P \leqslant 0.05$). Postweaning HF diet had a similar effect (main effect of postweaning diet, $P \leqslant 0.05$). Maternal HF diet resulted in a greater fasting glucose in male offspring (main effect of maternal diet $P \leqslant 0.05$), while postweaning HF diet increased fasting glucose in both male and female offspring (main effect of postweaning diet

Table 2. Phenotype of 1-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#

Con, control; HF, high fat.
After *in utero* exposure to maternal Con or HF diet, offspring were weaned to Con or HF, creating four study groups: Con/Con, Con/HF, HF/Con, HF/HF. Six males and six females were measured from six separate litters per group. Data were analyzed by two-way ANOVA (maternal diet × postweaning diet).
*$P \leqslant 0.05$ main effect of maternal diet, maternal HF *v.* maternal Con.
#$P \leqslant 0.05$ main effect of postweaning diet, postweaning HF *v.* postweaning Con.

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Table 3. Muscle weights of 1-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

Con, control; HF, high fat.
After *in utero* exposure to maternal Con or 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 $n = 6$) and HF/HF (male $n = 4$, female $n = 4$). Muscle weights were averaged from both hindlimbs. Data were analyzed by 2-way ANOVA (maternal diet × postweaning diet).
#$P \leqslant 0.05$ and ^$P = 0.069$ main effect of postweaning diet, postweaning HF *v.* postweaning Con.

$P \leqslant 0.05$). 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 $P \leqslant 0.05$) 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 the 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 $P \leqslant 0.05$) (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 two-way ANOVAs were conducted on the respiration data within each maternal diet condition (maternal Con, maternal HF) using sex and postweaning diet as factors. Interactions of postweaning diet and sex were not significant within the maternal Con diet condition ($P > 0.05$), but were significant for maternal HF diet ($P \leqslant 0.05$). Complex I OXPHOS was greater in HF/Con females *v.* HF/Con males (+28%, $P = 0.046$) (Fig. 2a and 2b). Postweaning HF diet resulted in lower complex I OXPHOS in female offspring of HF dams (HF/HF *v.* HF/Con, −28%, $P = 0.041$), but did not affect complex I OXPHOS in males (HF/HF *v.* HF/Con, +27%, $P = 0.110$) (Fig. 2a and 2b). 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 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 *v.* HF/Con males (+29%, $P = 0.052$) (Fig. 2a and 2b). Complex I + II OXPHOS tended to be less in female HF/HF *v.* HF/Con (−24%, $P = 0.084$) but was not different in male HF/HF *v.* HF/Con (+25%, $P = 0.144$) (Fig. 2a and 2b). There were no significant interaction or main effects for ETS capacity ($P > 0.05$) (Fig. 2a and 2b).

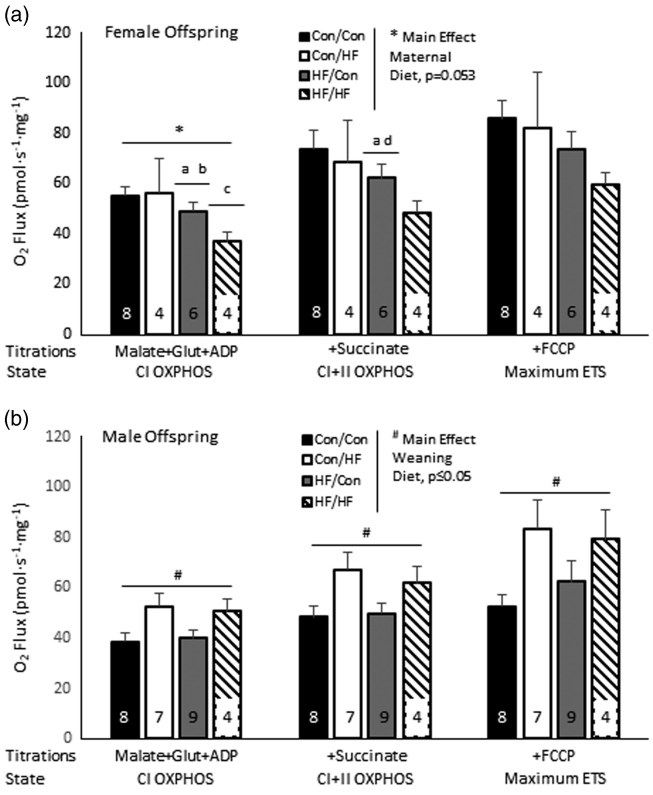

Fig. 2. Mitochondrial respiration in the medial gastrocnemius of 1-year-old female (*a*) and male (*b*) offspring. After *in utero* exposure to maternal control (Con) or high-fat (HF) diet, offspring were weaned to Con or HF, creating four groups for each sex: Con/Con (male $n = 8$, female $n = 8$), 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 oxidative phosphorylation (OXPHOS)]. Maximal convergent electron flux (Complex I + II OXPHOS). Maximal electron transfer system (ETS) capacity. Values are mean ± SE. Differences initially determined for each sex separately by two-way ANOVA with factors of maternal diet (Con, HF) and offspring postweaning diet (Con, HF). Initial analyses revealed maternal diet to affect respiration in female but not male offspring. Follow-up two-way ANOVAs were then conducted separately on the respiration data for each maternal diet condition (Con, HF) using sex and postweaning diet as factors. *Main effect ($P \leqslant 0.05$) of material diet in female offspring. #Main effect ($P \leqslant 0.05$) of weaning diet in male offspring. [a]$P \leqslant 0.05$ *v.* HF/Con males. [b]$P \leqslant 0.05$ *v.* HF/HF within sex. [c]$P = 0.081$ *v.* HF/HF males. [d]$P = 0.084$ *v.* HF/HF within sex. Numbers within each bar indicate the *n* for that group.

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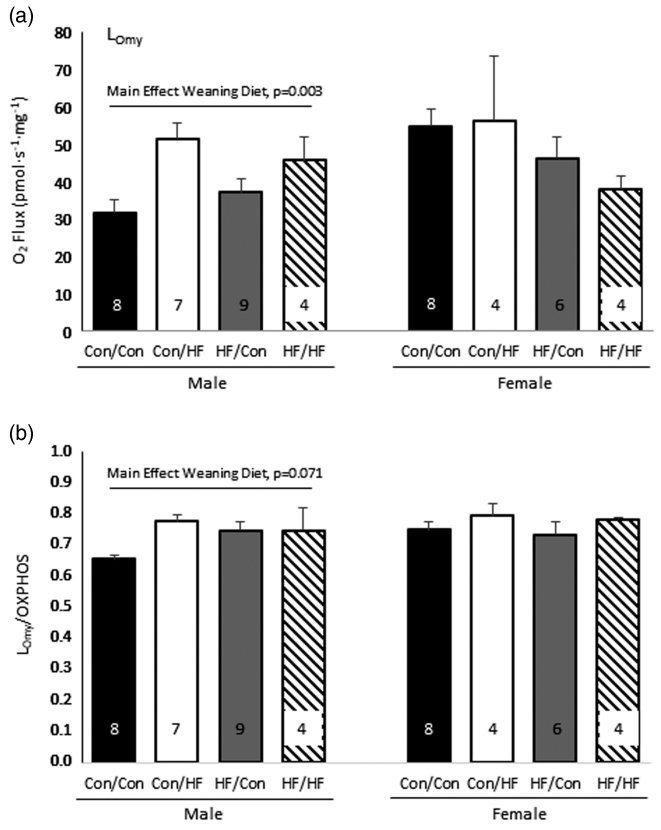

Fig. 3. Non-phosphorylating LEAK respiration induced by the ATP synthase inhibitor oligomycin (L_{Omy}) (*a*) and L_{Omy} expressed as a fraction of maximum oxidative phosphorylation (OXPHOS) capacity (L_{Omy}/OXPHOS) (*b*) in 1-year-old offspring. The four offspring groups for each sex were based on 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 $n=6$) and HF/HF (male $n=4$, female $n=4$). Values are mean ± SE. Mean differences were determined for each sex separately using two-way ANOVA with factors of maternal diet (Con, HF) and offspring postweaning diet (Con, HF). Numbers within each bar indicate the *n* for that group.

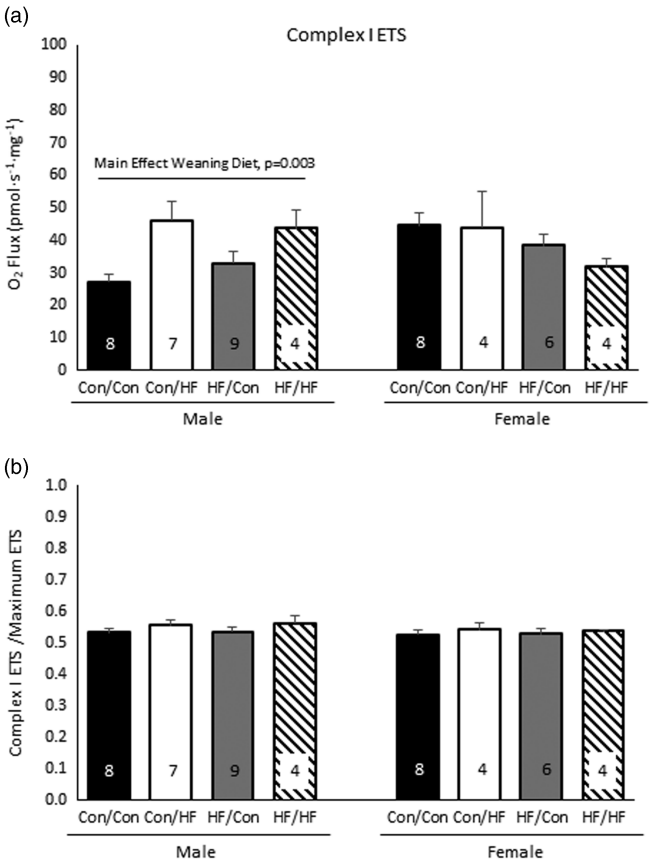

Fig. 4. Contribution of complex I to electron transfer system capacity [Complex I electron transfer system (ETS); *a*]. Complex I ETS was also expressed relative to maximum ETS obtained by titration with carbonylcyanide p-trifluoromethoxy-phenylhydrazone (FCCP) (*b*). The four offspring groups for each 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 $n=6$) and HF/HF (male $n=4$, female $n=4$). Values are mean ± SE. Mean differences were determined for each sex separately using two-way ANOVA with factors of maternal diet (Con, HF) and offspring postweaning diet (Con, HF). Numbers within each bar indicate the *n* for that group.

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/mg 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 difference 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 HF-fed *v.* Con-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 HF 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.*[29] showed that female mice fed a HF 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. The effect on mitochondrial dynamic proteins could be detected in the oocytes of the female

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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 HF 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 by-products 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 the 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 postpubertal 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 oxidations are lower in young women compared to men,[45,46] whereas this is reversed during physical activity.[47] Thus, whether the programmed loss of mitochondrial respiration that we found in the female offspring obese dams can be ameliorated by offspring exercise is a key future step to better understand these sexually dimorphic findings.

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

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

In summary, maternal and postweaning HF diet differentially affected mitochondrial respiration in skeletal muscle of male and female offspring. Females exposed to a HF diet *in utero* had greater adiposity and lower muscle respiratory capacity, effects that were exacerbated by continuing HF diet exposure for 1 year postweaning. In contrast, muscle respiration in male offspring was not affected by maternal HF diet and was actually greater when weaned to an 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 HF 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 HF diet.

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Conflicts of Interest. None.

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 have been approved by the institutional committee at Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

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