Published in final edited form as:

J Strength Cond Res. 2018 May ; 32(5): 1391–1403. doi:10.1519/JSC.0000000000002416.

AEROBIC EXERCISE PRECONCEPTION AND DURING PREGNANCY ENHANCES OXIDATIVE CAPACITY IN THE HINDLIMB MUSCLES OF MICE OFFSPRING

Jenney Liu¹, Icksoo Lee², Han-Zhong Feng³, Sujay S. Galen⁴, Philipp P. Hüttemann¹, Guy A. Perkins⁵, J.-P. Jin³, Maik Hüttemann^{1,7}, and Moh H. Malek^{4,6,7}

¹Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, USA

²College of Medicine, College of Medicine, Dankook University, Cheonan-si, Chungcheongnam-do, 31116, Republic of Korea

³Department of Physiology, School of Medicine, Wayne State University, Detroit, MI 48201, USA

⁴Physical Therapy Program, Eugene Applebaum College of Pharmacy & Health Sciences, Wayne State University, Detroit, MI 48201, USA

⁵National Center for Microscopy and Imaging Research, University of California, San Diego, La Jolla, CA 92093

⁶Integrative Physiology of Exercise Laboratory, Department of Health Care Sciences, Eugene Applebaum College of Pharmacy & Health Sciences, Wayne State University, Detroit, MI 48201, USA

⁷Cardiovascular Research Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA

Abstract

Little is known about the effect of maternal exercise on offspring skeletal muscle health. The purpose of this study, therefore, was to determine whether maternal exercise (preconception and during pregnancy) alters offspring skeletal muscle capillarity and mitochondrial biogenesis. We hypothesized that offspring from exercised dams would have higher capillarity and mitochondrial density in the hindlimb muscles compared to offspring from sedentary dams. Female mice in the exercise condition had access to a running wheel in their individual cage 30 days prior to mating and throughout pregnancy, whereas the sedentary group did not have access to the running wheel prior to mating and during pregnancy. Male offspring from both groups were sacrificed at 2 months old and tissues analyzed. The results indicated no significant ($p > 0.05$) mean differences for capillarity density, capillarity-to-fiber ratio or regulators of angiogenesis such as VEGF-A and TSP-1. Compared to offspring from sedentary dams, however, offspring from exercised dams had an increase in protein expression of myosin heavy chain type I (MHC I) (~134%; $p = 0.009$), but

no change in MHC II. For mitochondrial morphology, we found significant (all p -values < 0.0124) increases in mitochondrial volume density (~55%) and length (~18%) as well as mitochondria per unit area (~19%). For mitochondrial enzymes, there were also significant (all p -values < 0.0058) increases in basal citrate synthase (~79%) and cytochrome c oxidase (~67%) in the non-oxidative muscle fibers as well as increases in basal [ATP] (~52%). Lastly, there were also significant mean differences in protein expression for regulators (FIS1, Lon protease, and TFAM) of mitochondrial biogenesis. These findings suggest that maternal exercise before and during pregnancy enhances offspring skeletal muscle mitochondria functionality, but not capillarity.

Keywords

Exercise physiology; *in utero* environment; rodent model; wheel running

INTRODUCTION

Epidemiological studies have suggested that prenatal exposure to various perturbations may influence development of the offspring in either a positive or negative manner. This concept is called the Fetal Programming Hypothesis or the Developmental Origins of Health and Disease which was initially known as the Barker Hypothesis (6, 30). Gluckman and Hanson (20) suggest maintaining homeostasis during fetal development is critical, and therefore, various physiological adjustments occur in response to the external perturbation. The emergence of maternal-fetal interaction during pregnancy in response to exercise and resultant manifestation in the offspring is a growing area of research (7, 14).

There is increasing evidence that preconception exposure to environmental stimuli influences the germ cells and, therefore may influence the offspring health (19, 48). For example, as part of the Longitudinal Investigation of Fertility and the Environment (LIFE) study, Robledo et al. (47) reported that maternal and paternal preconception exposure to persistent organic pollutants resulted in significant birth size differences in the offspring. In an epidemiological study, Zhu et al. (60) found an association between maternal preconception exposure to air pollution exposure and increased odds of cleft palate in the offspring. The studies that have examined the effect of maternal exercise on the offspring have focused on bone composition (19, 48) and neurodevelopmental (12, 31). In a rodent model, Gaeini and colleagues (19) examined the effect of maternal endurance training prior to conception, during pregnancy, or a combination of both on markers of offspring bone formation and resorption. The investigators reported that the preconception exercise period was critical to the changes observed in the regulators of bone formation and resorption rather than exercise during pregnancy only, or the combination of exercise prior to conception and during pregnancy (19). In humans studies show a positive association between maternal exercise during pregnancy and fetoplacental growth (12), whereas in rodent studies (31) maternal exercise during pregnancy indicate significant increases in brain-derived neurotrophic factor mRNA expression and enhanced hippocampal neurogenesis which functionally translated to improved short-term memory. Taken together, the results of these studies suggest that maternal exercise may have significant impact on the offspring.

Two vital components of the oxygen transport pathway are capillary beds, which are the interface between oxygen in the red blood cells and its diffusion into muscle, and mitochondria in which oxygen is used to produce energy through oxidative phosphorylation (32, 55). Human and rodent studies indicate an interplay between growth factors that stimulate angiogenesis (vascular endothelial growth factor, VEGF) and inhibit angiogenesis (thrombospondin, TSP-1) which, in turn, influences mitochondrial regulatory factors such as peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α) (41, 42). Our laboratory previously examined this relationship in a murine model of mild mitochondrial myopathy by deleting cytochrome *c* oxidase subunit 7a1 (32). The significant increase (~60%) in basal TSP-1 protein expression and the significant decrease (~40%) in VEGF receptor phosphorylation in the hindlimb muscles of these transgenic animals compared with controls corresponded to significant reductions in capillary density (32). When mitochondrial function was measured, we found that basal ATP levels to be nearly 70% reduced compared to controls. These molecular changes within the skeletal muscle manifested in a ~38% reduction in endurance capacity as measured via treadmill exercise.

To date, the effect of maternal endurance exercise during pregnancy on capillarity and mitochondrial function in the skeletal muscle of the offspring has not yet been established. The purpose of this study, therefore, was to determine whether maternal exercise (preconception and during pregnancy) alters offspring skeletal muscle capillarity and mitochondrial biogenesis. We hypothesized that offspring from exercised dams would have higher capillarity and mitochondrial density in the hindlimb muscles compared to offspring from sedentary dams.

METHODS

Experimental Approach

A between-subjects design was used where virgin female mice were randomized to either a sedentary or exercise group. Female mice in the exercise group had stainless steel running wheel introduced into their cage 30 days prior to pregnancy and during their pregnancy. Offspring from both sedentary and exercise dams were weaned 21 days after birth. When the offspring in each group were 2 months old, the males were randomly selected to be sacrificed and hindlimb muscles harvested to determine capillarity and mitochondrial characteristics.

Animals and Breeding Protocol

Five separate sets of trio breeding were used, therefore, each virgin sire was mated with one sedentary and one exercised dam. Both male (n=5) and female mice (n=10) (C57BL/6N, Harlan Laboratories, Inc.) were 2 months old when purchased. After 30 days of wheel running or sedentary living mating was initiated. Mating was confirmed by visualization of a semen plug initially, but then palpation of the dam's belly at gestational day 10 with visual confirmation. Thereafter, the sires were removed from the cage. The sedentary dams were placed in cages without a running wheel, whereas exercised dams were placed in cages with access to a running wheel. All dams had access to food and water *ad libitum*. Sires were sacrificed via CO₂ inhalation followed by cervical dislocation. All animal care and

experimental procedures were approved by the University Institutional Animal Care and Use Committee (IACUC).

Voluntary Wheel Running

Exercised dams were housed individually in cages ($35.3 \times 23.5 \times 20$ cm) equipped with running wheels (12.7 cm in diameter; Lafayette Instrument, Lafayette, IN), and allowed access to the wheel during the 30 days prior to breeding. This approach allowed us to identify female mice who did not engage the running wheel activity on a daily basis. In our sample, all female mice maintained at least 1 h of exercise for every 24 h period. Once confirmation of pregnancy was established (see above), females in the exercise group were given access to the running wheel throughout the pregnancy. Once the dams delivered their pups, the wheel was removed from the cage. The wheel was electronically connected to a computer (Dell Inspiron E1705, Dell Inc., Round Rock, TX) and running activity was monitored hourly with custom LabVIEW programming software (version 2014, National Instruments, Austin, TX). This software records the exercise distance and duration of each dam for each hour during a 24-h period.

Tissue Collection

Male offspring (2 months old) from sedentary and exercised dams were euthanized with an overdose of sodium pentobarbital (200 mg/kg i.p.) and the soleus, plantaris, and quadriceps femoris muscles were removed as routinely performed in our laboratory (23, 24, 32, 35-37, 39). Transverse 8- μ m serial sections, from the left lateral gastrocnemius muscle, were cut on a cryotome (Leica CM 1950; Leica Microsystems, Buffalo Grove, IL, USA) at -30°C and mounted on slides for histochemical analysis of capillary number.

Capillary Density

Section were stained for capillarity using the method of Sillau and Bachero (51). Muscle sections from the lateral gastrocnemius muscle were viewed under a digital microscope (10 $\times$ magnification, Leica DMD108, Buffalo Grove, IL) and randomly selected for animals in each group corresponding to ~70–80% of the entire muscle being analyzed for each animal. Quantification of capillaries were performed using the recommended method of Hepple (21, 22) by measuring the following: 1) the number of capillaries around a fiber (N_{CAF}), 2) the capillary-to-fiber ratio on an individual-fiber basis (C/F_i), and 3) capillary density (CD) which was calculated by using the fiber area as the reference space. Capillary-to-fiber perimeter exchange index (CFPE) was estimated from the capillary-to-fiber surface area. Fiber cross-sectional area (FCSA) and perimeter (FP) was measured with the image analysis system and commercial software (SigmaScan Pro v. 5.0, Systat Software, Inc., Point Richmond, CA) as routinely done in our laboratory (24, 25, 32, 35).

Determination of Slow Skeletal Muscle Isoform of Troponin T (TnT)

Consistent with our previous work (17), frozen lateral gastrocnemius muscles were homogenized in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer containing 50 mM Tris-HCl, pH 8.8, 2% SDS, 3% β -mercaptoethanol, 0.1% bromophenol blue and 10% glycerol using a high speed mechanical tissue homogenizer (PRO Scientific,

Monroe, CT) to extract total proteins. After heating at 80°C for 5 min and clarification by centrifugation at top speed in a microcentrifuge, the samples were loaded on 14% gel with an acrylamide-to-bisacrylamide ratio of 180:1 prepared in a modified Laemmli discontinuous buffer system, in which the stacking and resolving gels are both at pH 8.8. After electrophoresis, the gels were fixed and stained with Coomassie Blue R250 to reveal the protein bands. The amounts of sample loading were normalized to the actin band quantified using 2-D densitometry. The resolved protein bands in duplicate gels were electrophoretically transferred to a nitrocellulose membrane using a Bio-Rad semidry transfer apparatus at 5 mA/cm² for 15 min. The blotted membranes were blocked with 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS, 150 mM NaCl, 50mM Tris-HCl, pH 7.5) and incubated with primary monoclonal antibodies (mAb). An anti-TnI mouse antibody TnI-1 (28), anti-slow TnT mouse antibody CT3, and anti-fast TnT mouse antibody T12 (27) were used in the present study. Following washes with TBS containing 0.05% Tween-20, the membranes were further incubated with alkaline phosphatase-labeled anti-mouse IgG second antibody (Santa Cruz Biotechnology). After final washes as above, the expression of calponin isoforms and α -smooth muscle actin was revealed by developing the blots in 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium chromogenic substrate solution.

Determination of Myosin Heavy Chain Isoform

Myosin heavy chain (MHC) isoforms expressed in the lateral gastrocnemius muscle was examined using glycerol-SDS-PAGE (16, 17). Briefly, SDS-PAGE samples equivalent to 5 μ g of muscle tissue (wet weight) were resolved on 8% polyacrylamide gel with acrylamide:bis-acrylamide ratio of 50:1, prepared in 200 mM Tris base, 100 mM glycine, pH 8.8, containing 0.4% SDS and 30% glycerol. The stacking gel contained 4% polyacrylamide with acrylamide:bis-acrylamide ratio of 50:1, 70 mM Tris-HCl (pH 6.7), 4 mM EDTA, 0.4% SDS, and 30% glycerol. The upper cathode running buffer consists of 100 mM Tris base, 150 mM glycine, 0.1% SDS, and 10 mM β -mercaptoethanol. The lower anode running buffer was 50% dilution of the upper running buffer without β -mercaptoethanol. The 0.75-mm-thick Bio-Rad minigels were run at 100 V in an icebox for 24 h. The resolved protein bands were visualized after staining with Coomassie blue R250.

Western Blot

Western blot analyses were performed as previously described (24, 32). Briefly, the entire left plantaris muscle was homogenized in a glass tissue grinder with RIPA buffer (Sigma-Aldrich, St. Louis, MO) and protease and phosphatase inhibitor cocktails (PhosSTOP Phosphatase and Complete Protease Inhibitor Cocktail, Sigma Aldrich, St. Louis, MO). Homogenates were passed through an insulin syringe five times, centrifuged for 10 min at 4 °C, and the supernatant was collected, aliquoted, and stored at -80 °C. The BCA protein assay kit (Bio-Rad Laboratories) was used to quantify protein concentration for each sample.

Protein samples (30 μ g) loaded onto 7.5% (TSP-1 and VEGFR2) or 12% TGX pre-cast gels (Bio-Rad) for 1 h at 190 and transferred to polyvinylidene fluoride membranes (PVDF-FL, Immobilon transfer membranes; Millipore, Billerica, MA) with a semi-dry blotting apparatus (9 V, 51 min, Bio-Rad). After 1 h of blocking (Li-Cor Biosciences, Lincoln, NE),

the primary antibody was added and the membrane stored at 4 °C on a rocker. The secondary antibody was added and the membrane was incubated at room temperature for 1 h on the second day. Following secondary antibody incubation, the membrane was washed four more times (5-min/wash) in TBST at room temperature. Care was taken not to expose the membrane to light.

The monoclonal primary antibodies used were TSP-1 (1:500; sc-59886), anti-slow skeletal myosin heavy chain (NOQ7.5.4D, ab11083; Abcam), and anti-fast myosin skeletal heavy chain antibody (MY-32, ab51263; Abcam). The polyclonal primary antibodies used were VEGF (1:500, sc-507), VEGFR2 (1:500; 2479, Cell Signaling), TFAM (1:1000; 19998-l-AP; Proteintech), FIS1 (1:500; 10956-l-AP; Proteintech), LON protease (1:500; ab103809; Abcam) and α -tubulin (1:2,000, Cell Signaling 2144). The secondary antibodies used were goat anti-mouse IRDye (1:30,000) and goat anti-rabbit IRDye (1:30,000) purchased from Li-Cor Biosciences. Loading control for target proteins were normalized to α -tubulin. Quantification of bands was performed with the Odyssey software program (Li-Cor Biosciences).

Mitochondrial Morphology

Pieces of the right plantaris muscle were fixed in 2% paraformaldehyde plus 2.5% glutaraldehyde (Ted Pella, Redding, CA) in 0.1 M sodium cacodylate (pH 7.4) on ice for 24 hours (25, 39). Specific preparation of the tissues for electron microscopy were consistent with our previous studies (25, 39).

Citrate Synthase Activity

Consistent with our previous studies, basal citrate synthase activity was measured in the plantaris and soleus muscles of offspring for each group using the method of Srere (52). Samples were analyzed in a Beckman DU 730 spectrophotometer (Beckman, Fullerton, CA, USA) at 412 nm. All samples were tested in triplicate and measured at 37 °C.

Basal Cytochrome c Oxidase Activity and ATP Measurements

Mitochondrial function was analyzed in the harvested quadriceps femoris muscles with a micro-Clark-type oxygen electrode in a closed chamber (Oxygraph System; Hansatech, Norfolk, UK) at 32 °C as previously done (24, 32). Frozen tissue was solubilized in 10 mM K-HEPES (pH 7.4), 40 mM KCl, 1% Tween 20, 2 μ M oligomycin, 1 mM PMSF, 10 mM KF, 2 mM EGTA, and 1 mM sodium vanadate. Oxygen consumption of the mitochondria was measured in the presence of 20 mM ascorbate and saturating amounts of cytochrome c (30 μ M) from cow heart (Sigma-Aldrich, St. Louis, MO). Oxygen consumption was recorded on a computer and analyzed with the Oxygraph software. Protein concentration was determined with the DC protein assay kit (Bio-Rad, Hercules, CA, USA). Oxygen consumption was defined as consumed O₂ (nanomoles, nM) per minute per milligram total protein and data were normalized to the offspring from sedentary dams.

ATP levels were determined via the boiling method with flash-frozen quadriceps femoris tissues using the ATP bioluminescence assay kit HS II (Roche Applied Science) as described

(23, 33). Data were standardized to the protein concentration, which was determined as described above and data were normalized to the offspring from sedentary dams.

Statistical Analyses

The data were statistically analyzed using separate independent samples t-tests for each dependent variable of interest. Data were analyzed using the Statistical Package for the Social Sciences software (v. 22.0, IBM SPSS, Armonk, NY) with a $p < 0.05$.

RESULTS

Anthropometric and Muscle Mass

As shown in Table 1, there were no significant mean differences for body mass between the two groups of offspring. Moreover, the masses of the soleus, plantaris, and quadriceps femoris muscles were not different between groups. For the lateral gastrocnemius muscle, however, offspring from exercised dams has significantly smaller muscle mass than offspring from sedentary dams.

Habitual Exercise Profile

As shown in Figure 1, the volume of preconception exercise, measured as distance ran, shows the typical patterns of responses. That is, in the first week of introducing the wheel to the animal exercise activity increases day-to-day which then levels off in subsequent weeks. This profile is consistent with other studies using murine model voluntary wheel running (40, 57). During the conception phase (*not shown*), dams in the exercise group averaged 3567 m (SEM = 605 m) which corresponded to a similar exercise volume in the first week of the preconception phase.

Hindlimb muscle assessment

As shown in Table 2, when the lateral gastrocnemius muscle was assessed for various morphological characteristics there were no significant mean differences for fiber area ($p = 0.07$) and perimeter as well as estimated diffusion distance (R_{95}). As a result, we wanted to further explore this trend toward a significant difference in cross-sectional area for this specific muscle. Therefore, we calculated the frequency distribution of the fiber cross-sectional area for the lateral gastrocnemius muscle between the two groups (Figure 2). Moreover, to determine the relationship between group and frequency of muscle cross-sectional area we aggregated the frequency range into two sizes (small size: 1,000 to 2,999 μm^2 ; large size: 3,000 to 5500+) per group. Thereafter, we performed a Chi-square Goodness of Fit Test and found a significant interaction was found [$\chi^2 (1, N=267) = 23.66$, $p < 0.001$]. Offspring from exercised dams were more likely to have larger cross-sectional areas than offspring from sedentary dams.

To further determine whether or not there may be changes in the myofilament protein contents of the lateral gastrocnemius muscle, we examined expression of thin filament regulatory proteins (Figure 3). As such, we found no significant mean differences between the two groups for expression of slow skeletal muscle TnT and TnI isoforms (ssTnT and ssTnI; Figure 3, panels B and C) in this muscle. We did, however, find that MHC IIx was

significantly lower in the lateral gastrocnemius muscle of offspring from exercise dams compared to offspring from sedentary dams (Figure 3, panel D).

Angiogenic Response

When skeletal muscle capillarity was determined (Figure 4), we found no significant mean differences between the two groups. Next, we determined whether or not there were changes in protein expression for key angiogenic regulators and found no significant mean differences between groups. We did, however, find a significant increase in MHC I protein expression in the plantaris muscle of offspring from exercised dams (Figure 5). We did not observe any differences in MHC II protein expression between the two groups of offspring.

Mitochondrial Structure and Function

As shown in Figure 6, we conducted electron microscopy analysis to determine morphological characteristics of the mitochondria between the two offspring groups. As such, we found that mitochondrial volume density and length as well as mitochondria per unit area were significantly increased in the offspring from exercised dams compared to offspring from sedentary dams. There were, however, no significant mean differences for mitochondrial cross section and cristae abundance between the two groups. When activity of the mitochondrial enzyme citrate synthase was assessed, we found that there were significant mean increases for the plantaris muscle, but not for the soleus muscle (Figure 7). In addition, in the offspring from exercised dams cytochrome *c* oxidase activity as well as cellular energy levels were significantly increased by 67% and 55%, respectively (Figure 8). Lastly, we found significant mean increases for key mitochondrial regulators in the hindlimb muscles of offspring from exercised dams compared to offspring from sedentary dams (Figure 9).

DISCUSSION

A unique and surprising finding of the present investigation was that maternal exercise preconception and during pregnancy did not alter regulators of angiogenesis in the offspring skeletal muscle compared to controls. We did, however, find significant increases in mitochondrial morphology in predominantly glycolytic hindlimb muscles in the offspring from exercised dams compared to offspring from sedentary dams. Moreover, we observed increases in protein expression of mitochondrial regulators as well as enzymes indicating a significant improvement in oxidative capacity. It should be noted, that we did not measure the anthropometrics of the dams, because this was not an epigenetics study linking changes in the mother's skeletal muscle to changes in the offspring skeletal muscle. Rather, we focused on determining whether maternal exercise preconception and during pregnancy would enhance angiogenesis and mitochondrial proliferation in the offspring skeletal muscle.

Lack of angiogenic response in offspring of exercised dams

Typically, one of the main adaptations of skeletal muscle to endurance exercise is an increase in capillary proliferation, and it has been suggested that the potential mechanism underlying angiogenesis is due, in part, to a balance between pro- and anti-angiogenic

regulators (41-43). The two main genes attributed to capillary maintenance are pro-angiogenic VEGF-A and anti-angiogenic TSP-1 (41-43). An important distinction, therefore, needs to be addressed when examining exercise-induced angiogenesis in skeletal muscle. Specifically, the volume of exercise is a salient feature for modulating protein expression of VEGF-A and TSP-1 and thus capillary proliferation. Studies that use voluntary wheel running typically report that mice exercise, on average, for 2-4 h daily (2, 40), whereas most treadmill training protocols average between 30-60 min of daily exercise (24, 35). For example, Olenich et al. (40) reported significant increases in basal VEGF-A protein levels in the hindlimb muscle of mice after 7 days following voluntary wheel running compared to control, whereas Waters and colleagues (57) reported significant increases in basal VEGF protein levels in the plantaris muscle after 3 and 7 days of voluntary wheel running compared to control. Audet et al. (4) examined hindlimb muscle capillarity in mice selectively bred for high voluntary wheel running (called mini muscle). The authors reported that no change in capillary density in the soleus muscle between animals in the mini muscle group relative to control (4). For the gastrocnemius muscle, however, the authors reported that basal VEGF protein levels were significantly higher in the mini muscle group compared to control, but there was no difference between the groups for TSP-1 protein levels (4). In the plantaris muscle, however, the authors observed significantly higher VEGF-A protein levels with a concomitant decrease in TSP-1 protein levels in the mini muscle group compared to control (4).

Despite the high level of physical activity, we observed no changes in capillarity for the hindlimb muscle (Figure 4). In addition, there were no significant differences in regulators of angiogenesis between offspring from sedentary and exercised dams. As shown in Table 1, we observed significant mean differences for muscle mass between the two groups for the lateral gastrocnemius muscle, whereas in Table 2 we observed no significant mean differences for this muscle related to fiber area and perimeter. When we examined the distribution of cross-sectional area (small vs. large fibers) between the two groups, however, we found that offspring from exercised dams had significantly increased fiber cross-sectional area than offspring from sedentary dams. Furthermore, we also examined expression levels for slow skeletal muscle isoform of troponin T and troponin I which specifically express in the adult slow type muscle fibers to indicate the muscle fiber type composition (58). The results (Figure 4) showed a trend of increased level of slow skeletal muscle troponin T as normalized against actin and increased slow to fast troponin I isoform ratio in the normally fast fiber dominant lateral gastrocnemius muscle of the offspring from exercise dams as compared with that from sedentary dams, indicating a difference in fiber type contents. The contents of myosin isoforms as another indicator of muscle fiber type composition confirmed this observation by demonstrating that the lateral gastrocnemius muscle of offspring from exercise dams mainly contains MHC IIb with small amounts of IIa and IIx with significantly less MHC IIx than that of control for sedentary dam group. The results suggest that exercise during pregnancy may lead to a fast to slow fiber type switch in primarily glycolytic muscles of the offspring. This notion was further shown in the plantaris muscle which is primarily glycolytic. We found that for the plantaris muscle MHC I protein expression was significantly increased in skeletal muscle of offspring from exercised dams when compared to offspring from sedentary dams. Thus, in this study maternal exercise did

not increase capillarity in the offspring skeletal muscle, but increased protein expression of fiber types associated with high oxidative capacity. It should be noted, however, that we did not measure intramuscular fat in the present study which may provide further insight regarding potential differences in muscle density between the two offspring groups. There is a growing number of human model studies, examining different populations, which suggest that fatty infiltration may be associated with skeletal muscle dysfunction (5, 45).

Human model studies show that maternal anthropometrics and BMI (body mass index) influence offspring development (49, 50). For example, Chaparro et al. (11) reported that a high maternal pre-pregnancy BMI was associated with a higher offspring percentage of fat-mass and, therefore lower percentage of lean mass. In the current study, we did not measure maternal anthropometrics either prior to– or during pregnancy. This was because we used a common inbred mouse strain (C57BL/6) that were 2 months old and provided a standard chow as opposed to a high-fat diet. Inbred strains provide a unique advantage in rodent research, because they are genetically similar to one another. Moreover, as shown in Figure 1, dams in the exercise group performed a high volume of daily wheel running 30 days prior to mating. In addition, while pregnant these same dams continued their wheel running activity albeit at a lower volume than prior to pregnancy. The dams in the sedentary group did not have any excessive weight gain daily visual inspection by the investigator and the university animal care staff. Another consideration is that current models of measuring body composition in rodent models include the use of functional magnetic resonance imaging which require the rodent to undergo anesthesia using isoflurane. Studies indicate that *in utero* exposure to isoflurane may influence offspring development (29, 44). Nevertheless, in human model studies where differences in BMI and genotype are more pronounced from subject-to-subject it is critical to measure maternal anthropometrics throughout pregnancy to provide potential insight into offspring skeletal muscle structure and function.

Increased mitochondrial biogenesis in offspring of exercised dams

Mitochondria are referred to as the powerhouses of the cell, because they are responsible for generating the majority of energy in various tissues such as skeletal muscle (13). Moreover, mitochondria are adaptive and may increase with endurance training or decrease with detraining (24). The rate-limiting enzyme associated with oxidative phosphorylation is cytochrome *c* oxidase (CcO) (1, 53). Rodent studies suggest that deletion of a single subunit of CcO results in development of dilated cardiomyopathy (23) and severe reduction in skeletal muscle oxidative capacity (32). Other components that influence mitochondrial structure and function are fission 1 protein (Fis1), Lon protease (LonP), and mitochondrial transcription factor A (TFAM). Fis1 has been suggested to play a critical role in mitochondrial morphology such as length (46) and apoptosis (56). For example, Ding and colleagues (15) reported that Fis1 mRNA significantly increased after a single 45 min workout of high intensity treadmill running in the hindlimb muscle of rats compared to the control condition. For Fis1 protein expression, however, there was a significant increase after 120 and 150 min of the exercise workout compared to the resting condition (15). Ding et al. (15) also reported that both Fis1 mRNA and protein expression remained significantly higher than the resting condition 24 h after the single exercise workout. It should be noted,

however, that the investigators did not conduct morphological analyses on the tissues to determine potential changes in mitochondrial size and shape (15).

Another important regulator responsible for maintaining mitochondrial health is the LON protease which catalyzes mitochondrial degradation due to oxidative stress (10). Using a rodent model of microgravity, Wagatsuma and colleagues (54) reported that hindlimb suspension, which can induce oxidative stress in the muscle, significantly reduced LON protease mRNA expression compared to controls. Ngo et al. (38) reported that silencing LON induction in cell cultures followed by exposure to stressors increased mitochondrial dysfunction. In addition, aging studies indicate reduced basal LON content which may suggest increased susceptibility to oxidative stress within the skeletal muscle (10). Furthermore, Fu et al. (18) suggested that LON protease binds to a promoter region associated with TFAM. TFAM is also critical to mitochondrial maintenance, because studies have shown that TFAM null mice have impaired mitochondrial structure and function (59). In addition, human model studies indicate that basal TFAM content in skeletal muscle significantly increase with endurance training in healthy (34) and clinical populations (26).

In the current study, we examined offspring skeletal muscle morphology as it relates to mitochondrial structure and function in the plantaris and quadriceps femoris muscles, respectively. These muscles were purposefully selected, because they are either primarily glycolytic (plantaris) or mixed (quadriceps femoris) in their fiber type composition (9). Therefore, any potential changes in oxidative capacity of the muscle, as a result of our intervention, would be better detected in these muscles as opposed to a predominantly oxidative muscle such as the soleus. As shown in Figure 6, the electron microscopy shows an overall visual difference for mitochondria between the two groups. When the muscle was assessed for various mitochondrial indices, we found significant increases in mitochondrial volume density, length, and unit per area (Figure 6). We did not, however, find any significant mean differences for mitochondrial cross section and cristae abundance. Next, we examined basal concentrations of two key enzymes associated with increased oxidative capacity. As shown in Figure 7, citrate synthase activity in the plantaris muscle of offspring from exercised dams increased by ~67% compared to the plantaris muscle of offspring from sedentary dams. When the soleus muscle was examined there were no significant mean differences for basal citrate synthase concentrations between the two groups, likely because this muscle is already maxed out in terms of mitochondrial capacity. We also examined the specific activity of cytochrome *c* oxidase, which is the rate-limiting enzyme in the ETC, and ATP levels. As shown in Figure 8, we found that offspring from exercised dams had significantly improvement in cytochrome *c* oxidase and [ATP] compared to controls. When the basal protein expression of FIS1, LON protease, and TFAM in the plantaris muscle of the offspring from the two groups were examined, we observed a significant increase (from ~22% to ~52%) for all three regulators of mitochondria (Figure 9). Taken together, these studies indicate that exercise during pregnancy enhances skeletal muscle mitochondrial structure and function.

In summary, the results of our study indicate that maternal exercise preconception and during pregnancy influences offspring skeletal muscle morphology and function. Interestingly, we found no significant changes in capillary indices and protein expression for

regulators of angiogenesis in the skeletal muscle of offspring from exercised dams. For mitochondrial structure and function, however, we found that maternal exercise during pregnancy significantly increased various mitochondrial parameters including morphology, enzyme activities, and protein expressions for glycolytic and mixed muscles. Future studies may need to focus on whether or not the observed increase in oxidative capacity provides a protective mechanism against oxidative stress, for example in the context of aging or a high-fat diet.

Acknowledgments

This study was supported, in part, by the Faculty Research Awards Program (FRAP) awarded to MHM and NIH grant P41GM103412-24 (GAP).

Literature Cited

1. Acin-Perez R, Bayona-Bafaluy MP, Bueno M, Machicado C, Fernandez-Silva P, Perez-Martos A, Montoya J, Lopez-Perez MJ, Sancho J, Enriquez JA. An intragenic suppressor in the cytochrome c oxidase I gene of mouse mitochondrial DNA. *Hum Mol Genet.* 2003; 12:329–339. [PubMed: 12554686]
2. Allen DL, Harrison BC, Maass A, Bell ML, Byrnes WC, Leinwand LA. Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. *J Appl Physiol* (1985). 2001; 90:1900–1908. [PubMed: 11299284]
3. Artal R, O'Toole M. Guidelines of the American College of Obstetricians and Gynecologists for exercise during pregnancy and the postpartum period. *Br J Sports Med.* 2003; 37:6–12. discussion 12. [PubMed: 12547738]
4. Audet GN, Meek TH, Garland T Jr, Olfert IM. Expression of angiogenic regulators and skeletal muscle capillarity in selectively bred high aerobic capacity mice. *Exp Physiol.* 2011; 96:1138–1150. [PubMed: 21804139]
5. Baker JF, Von Feldt J, Mostoufi-Moab S, Noaiseh G, Taratuta E, Kim W, Leonard MB. Deficits in muscle mass, muscle density, and modified associations with fat in rheumatoid arthritis. *Arthritis Care Res (Hoboken).* 2014; 66:1612–1618. [PubMed: 24664868]
6. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet.* 1989; 2:577–580. [PubMed: 2570282]
7. Bisson M, Marc I, Brassard P. Cerebral blood flow regulation, exercise and pregnancy: why should we care? *Clin Sci (Lond).* 2016; 130:651–665. [PubMed: 26993053]
8. Blaize AN, Pearson KJ, Newcomer SC. Impact of Maternal Exercise during Pregnancy on Offspring Chronic Disease Susceptibility. *Exerc Sport Sci Rev.* 2015; 43:198–203. [PubMed: 26196867]
9. Bloemberg D, Quadrilatero J. Rapid determination of myosin heavy chain expression in rat, mouse, and human skeletal muscle using multicolor immunofluorescence analysis. *PLoS One.* 2012; 7:e35273. [PubMed: 22530000]
10. Bota DA, Davies KJ. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. *Nat Cell Biol.* 2002; 4:674–680. [PubMed: 12198491]
11. Chaparro MP, Koupil I, Byberg L. Maternal pre-pregnancy BMI and offspring body composition in young adulthood: the modifying role of offspring sex and birth order. *Public Health Nutr.* 2017:1–6.
12. Clapp JF 3rd, Lopez B, Harcar-Sevcik R. Neonatal behavioral profile of the offspring of women who continued to exercise regularly throughout pregnancy. *American journal of obstetrics and gynecology.* 1999; 180:91–94. [PubMed: 9914584]
13. Coburn, JW., Malek, MH., National Strength & Conditioning Association (U.S.). NSCA's essentials of personal training. 2nd. Champaign, IL: Human Kinetics; 2012.
14. Di Mascio D, Magro-Malosso ER, Saccone G, Marhefka GD, Berghella V. Exercise during pregnancy in normal-weight women and risk of preterm birth: a systematic review and meta-

- analysis of randomized controlled trials. American journal of obstetrics and gynecology. 2016; 215:561–571. [PubMed: 27319364]
15. Ding H, Jiang N, Liu H, Liu X, Liu D, Zhao F, Wen L, Liu S, Ji LL, Zhang Y. Response of mitochondrial fusion and fission protein gene expression to exercise in rat skeletal muscle. *Biochimica et biophysica acta*. 2010; 1800:250–256. [PubMed: 19716857]
 16. Feng HZ, Chen M, Weinstein LS, Jin JP. Improved fatigue resistance in Galpha-deficient and aging mouse skeletal muscles due to adaptive increases in slow fibers. *J Appl Physiol* (1985). 2011; 111:834–843. [PubMed: 21680879]
 17. Feng HZ, Chen X, Malek MH, Jin JP. Slow recovery of the impaired fatigue resistance in postunloading mouse soleus muscle corresponding to decreased mitochondrial function and a compensatory increase in type I slow fibers. *Am J Physiol Cell Physiol*. 2016; 310:C27–40. [PubMed: 26447205]
 18. Fu GK, Markovitz DM. The human LON protease binds to mitochondrial promoters in a single-stranded, site-specific, strand-specific manner. *Biochemistry*. 1998; 37:1905–1909. [PubMed: 9485316]
 19. Gaeini AA, Shafiei Neek L, Choobineh S, Baghaban Eslaminejad M, Satarifard S, Sayahpour FA, Mousavi SN. Preconception endurance training with voluntary exercise during pregnancy positively influences on remodeling markers in female offspring bone. *J Matern Fetal Neonatal Med*. 2016; 29:3634–3640. [PubMed: 26754877]
 20. Gluckman PD, Hanson MA. The developmental origins of the metabolic syndrome. *Trends in endocrinology and metabolism: TEM*. 2004; 15:183–187. [PubMed: 15109618]
 21. Hepple RT. A new measurement of tissue capillarity: the capillary-to-fibre perimeter exchange index. *Can J Appl Physiol*. 1997; 22:11–22. [PubMed: 9018404]
 22. Hepple RT, Mathieu-Costello O. Estimating the size of the capillary-to-fiber interface in skeletal muscle: a comparison of methods. *J Appl Physiol*. 2001; 91:2150–2156. [PubMed: 11641356]
 23. Hüttemann M, Klewer S, Lee I, Pecinova A, Pecina P, Liu J, Lee M, Doan JW, Larson D, Slack E, Maghsoodi B, Erickson RP, Grossman LI. Mice deleted for heart-type cytochrome *c* oxidase subunit 7a1 develop dilated cardiomyopathy. *Mitochondrion*. 2012; 12:294–304. [PubMed: 22119795]
 24. Hüttemann M, Lee I, Malek MH. (-)-Epicatechin maintains endurance training adaptation in mice after 14 days of detraining. *FASEB J*. 2012; 26:1413–1422. [PubMed: 22179525]
 25. Hüttemann M, Lee I, Perkins GA, Britton SL, Koch LG, Malek MH. (-)-Epicatechin is associated with increased angiogenic and mitochondrial signalling in the hindlimb of rats selectively bred for innate low running capacity. *Clin Sci (Lond)*. 2013; 124:663–674. [PubMed: 23252598]
 26. Jarosch I, Gehlert S, Jacko D, Koczulla RA, Wencker M, Welte T, Bloch W, Janciauskiene S, Kenn K. Different Training-Induced Skeletal Muscle Adaptations in COPD Patients with and without Alpha-1 Antitrypsin Deficiency. *Respiration; international review of thoracic diseases*. 2016; 92:339–347. [PubMed: 27686000]
 27. Jin JP, Chong SM. Localization of the two tropomyosin-binding sites of troponin T. *Arch Biochem Biophys*. 2010; 500:144–150. [PubMed: 20529660]
 28. Jin JP, Yang FW, Yu ZB, Ruse CI, Bond M, Chen A. The highly conserved COOH terminus of troponin I forms a Ca²⁺-modulated allosteric domain in the troponin complex. *Biochemistry*. 2001; 40:2623–2631. [PubMed: 11327886]
 29. Kong FJ, Tang YW, Lou AF, Chen H, Xu LH, Zhang XM, Lu HS. Effects of isoflurane exposure during pregnancy on postnatal memory and learning in offspring rats. *Mol Biol Rep*. 2012; 39:4849–4855. [PubMed: 21947886]
 30. Langley-Evans, SC. Fetal nutrition and adult disease : programming of chronic disease through fetal exposure to undernutrition. Wallingford, Oxfordshire, OX ; Cambridge, MA: CABI Pub; 2004.
 31. Lee HH, Kim H, Lee JW, Kim YS, Yang HY, Chang HK, Lee TH, Shin MC, Lee MH, Shin MS, Park S, Baek S, Kim CJ. Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. *Brain Dev*. 2006; 28:147–154. [PubMed: 16368211]

32. Lee I, Hüttemann M, Liu J, Grossman LI, Malek MH. Deletion of heart-type cytochrome *c* oxidase subunit 7A1 impairs skeletal muscle angiogenesis and oxidative phosphorylation. *J Physiol.* 2012; 590:5231–5243. [PubMed: 22869013]
33. Lee I, Salomon AR, Ficarro S, Mathes I, Lottspeich F, Grossman LI, Hüttemann M. cAMP-dependent tyrosine phosphorylation of subunit I inhibits cytochrome *c* oxidase activity. *J Biol Chem.* 2005; 280:6094–6100. [PubMed: 15557277]
34. Little JP, Safdar A, Wilkin GP, Tarnopolsky MA, Gibala MJ. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *J Physiol.* 2010; 588:1011–1022. [PubMed: 20100740]
35. Malek MH, Hüttemann M, Lee I, Coburn JW. Similar skeletal muscle angiogenic and mitochondrial signalling following 8 weeks of endurance exercise in mice: discontinuous versus continuous training. *Exp Physiol.* 2013; 98:807–818. [PubMed: 23180811]
36. Malek MH, Olfert IM. Global deletion of thrombospondin-1 increases cardiac and skeletal muscle capillarity and exercise capacity in mice. *Exp Physiol.* 2009; 94:749–760. [PubMed: 19297388]
37. Malek MH, Olfert IM, Esposito F. Detraining losses of skeletal muscle capillarization are associated with vascular endothelial growth factor protein expression in rats. *Exp Physiol.* 2010; 85:359–368.
38. Ngo JK, Davies KJ. Mitochondrial Lon protease is a human stress protein. *Free Radic Biol Med.* 2009; 46:1042–1048. [PubMed: 19439239]
39. Nogueira L, Ramirez-Sanchez I, Perkins GA, Murphy A, Taub PR, Ceballos G, Villarreal FJ, Hogan MC, Malek MH. (-)-Epicatechin enhances fatigue resistance and oxidative capacity in mouse muscle. *J Physiol.* 2011; 589:4615–4631. [PubMed: 21788351]
40. Olenich SA, Gutierrez-Reed N, Audet GN, Olfert IM. Temporal response of positive and negative regulators in response to acute and chronic exercise training in mice. *J Physiol.* 2013; 591:5157–5169. [PubMed: 23878369]
41. Olfert IM. Physiological capillary regression is not dependent on reducing VEGF expression. *Microcirculation.* 2015
42. Olfert IM, Baum O, Hellsten Y, Egginton S. Advances and challenges in skeletal muscle angiogenesis. *Am J Physiol Heart Circ Physiol.* 2015 ajpheart 00635 02015.
43. Olfert IM, Birot O. Importance of anti-angiogenic factors in the regulation of skeletal muscle angiogenesis. *Microcirculation.* 2011; 18:316–330. [PubMed: 21418382]
44. Palanisamy A, Baxter MG, Keel PK, Xie Z, Crosby G, Culley DJ. Rats exposed to isoflurane in utero during early gestation are behaviorally abnormal as adults. *Anesthesiology.* 2011; 114:521–528. [PubMed: 21307768]
45. Rahemi H, Nigam N, Wakeling JM. The effect of intramuscular fat on skeletal muscle mechanics: implications for the elderly and obese. *J R Soc Interface.* 2015; 12:20150365. [PubMed: 26156300]
46. Richter V, Singh AP, Kvensakul M, Ryan MT, Osellame LD. Splitting up the powerhouse: structural insights into the mechanism of mitochondrial fission. *Cell Mol Life Sci.* 2015; 72:3695–3707. [PubMed: 26059473]
47. Robledo CA, Mendola P, Yeung E, Mannisto T, Sundaram R, Liu D, Ying Q, Sherman S, Grantz KL. Preconception and early pregnancy air pollution exposures and risk of gestational diabetes mellitus. *Environmental research.* 2015; 137:316–322. [PubMed: 25601734]
48. Rosa BV, Blair HT, Vickers MH, Dittmer KE, Morel PC, Knight CG, Firth EC. Moderate exercise during pregnancy in Wistar rats alters bone and body composition of the adult offspring in a sex-dependent manner. *PLoS One.* 2013; 8:e82378. [PubMed: 24340022]
49. Segura MT, Demmelmair H, Krauss-Etschmann S, Nathan P, Dehmel S, Padilla MC, Rueda R, Koletzko B, Campoy C. Maternal BMI and gestational diabetes alter placental lipid transporters and fatty acid composition. *Placenta.* 2017; 57:144–151. [PubMed: 28864004]
50. Sharp GC, Salas LA, Monnereau C, Allard C, Yousefi P, Everson TM, Bohlin J, Xu Z, Huang RC, Reese SE, Xu CJ, Baiz N, Hoyo C, Agha G, Roy R, Holloway JW, Ghantous A, Merid SK, Bakulski KM, Kupers LK, Zhang H, Richmond RC, Page CM, Duijts L, Lie RT, Melton PE, Vonk JM, Nohr EA, Williams-DeVane C, Huen K, Rifas-Shiman SL, Ruiz-Arenas C, Gonseth S, Rezwan FI, Herceg Z, Ekstrom S, Croen L, Falahi F, Perron P, Karagas MR, Quraishi BM,

Suderman M, Magnus MC, Jaddoe VWV, Taylor JA, Anderson D, Zhao S, Smit HA, Josey MJ, Bradman A, Baccarelli AA, Bustamante M, Haberg SE, Pershagen G, Hertz-Picciotto I, Newschaffer C, Corpeleijn E, Bouchard L, Lawlor DA, Maguire RL, Barcellos LF, Davey Smith G, Eskenazi B, Karmaus W, Marsit CJ, Hivert MF, Snieder H, Fallin MD, Melen E, Munthe-Kaas MC, Arshad H, Wiemels JL, Annesi-Maesano I, Vrijheid M, Oken E, Holland N, Murphy SK, Sorensen TIA, Koppelman GH, Newnham JP, Wilcox AJ, Nystad W, London SJ, Felix JF, Relton CL. Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation: findings from the pregnancy and childhood epigenetics (PACE) consortium. *Hum Mol Genet*. 2017; 26:4067–4085. [PubMed: 29016858]

51. Sillau AH, Banchero N. Visualization of capillaries in skeletal muscle by the ATPase reaction. *Pflugers Arch*. 1977; 369:269–271. [PubMed: 142963]
52. Srere PA. Citrate Synthase. *Methods Enzymol*. 1969; 13:3–5.
53. Villani G, Greco M, Papa S, Attardi G. Low reserve of cytochrome c oxidase capacity in vivo in the respiratory chain of a variety of human cell types. *J Biol Chem*. 1998; 273:31829–31836. [PubMed: 9822650]
54. Wagatsuma A, Kotake N, Kawachi T, Shiozuka M, Yamada S, Matsuda R. Mitochondrial adaptations in skeletal muscle to hindlimb unloading. *Mol Cell Biochem*. 2011; 350:1–11. [PubMed: 21165677]
55. Wagner PD. Determinants of maximal oxygen transport and utilization. *Annu Rev Physiol*. 1996; 58:21–50. [PubMed: 8815793]
56. Wang K, Long B, Jiao JQ, Wang JX, Liu JP, Li Q, Li PF. miR-484 regulates mitochondrial network through targeting Fis1. *Nature communications*. 2012; 3:781.
57. Waters RE, Rotevatn S, Li P, Annex BH, Yan Z. Voluntary running induces fiber type-specific angiogenesis in mouse skeletal muscle. *Am J Physiol Cell Physiol*. 2004; 287:C1342–1348. [PubMed: 15253894]
58. Wei B, Jin JP. TNNT1, TNNT2, and TNNT3: Isoform genes, regulation, and structure-function relationships. *Gene*. 2016; 582:1–13. [PubMed: 26774798]
59. Wrendenberg A, Wilborn R, Wilhelmsson H, Graff C, Wiener HH, Burden SJ, Oldfors A, Westerblad H, Larsson NG. Increased mitochondrial mass in mitochondrial myopathy mice. *PNAS*. 2002; 99:15066–15071. [PubMed: 12417746]
60. Zhu Y, Zhang C, Liu D, Grantz KL, Wallace M, Mendola P. Maternal ambient air pollution exposure preconception and during early gestation and offspring congenital orofacial defects. *Environmental research*. 2015; 140:714–720. [PubMed: 26099933]

Practical Application

Typically, it is suggested that women engage in aerobic exercise during their pregnancy (3). For example, the American College of Obstetricians and Gynecologists (ACOG) provides guidelines for physical activity during pregnancy (3). Our data supported the Guidelines of the American College of Obstetricians (3) suggesting that exercise during non-complicated pregnancy generally has beneficial effects. These results may provide some insight into the benefits of maternal habitual exercise on the offspring skeletal muscle structure and function. That is, the mother's consistent exercise regimen may fortify the skeletal muscle of the offspring and potentially reduce the susceptibility of the newborn to chronic disease (8). Indeed, muscle regenerative potential following injury may be, in part, due to factors transmitted to the offspring from the habitual exercise regimen of the mother prior to and during pregnancy.

Figure 1.
Depiction of a mouse on the running wheel inside the cage and the daily preconception running distance (mean $\pm$ SEM).

Figure 2.

Frequency distribution of fiber area range from small (i.e., 1,000-1,499) to large (5500+) for the lateral gastrocnemius muscle.

Figure 3.

Panel A: The SDS-gel and Western blots of the lateral gastrocnemius muscle for the expression of slow skeletal muscle TnT and TnI isoforms (ssTnT and ssTnI) with normal adult soleus muscle as control. The glycerol-SDS-gel used to resolve the myosin heavy chain isoforms. Panels B-D: Densitometry quantification of TnT and TnI Western blots and MHC bands. (mean $\pm$ SEM; N=4 in offspring from sedentary dams and n=6 in offspring from exercise dams).

Figure 4. Measures of capillarity between the two groups of offspring for the lateral gastrocnemius muscle (mean $\pm$ SEM).

Figure 5. Representative Western Blots and quantification for key regulators of angiogenesis and muscle fiber type for the plantaris muscle (mean $\pm$ SEM; 5-8 per group).

Figure 6. Representative electron microscopy images of mitochondrial morphology for cross-section of the plantaris muscle. Scale bar represents 500 nm. Ultrastructure analysis of mitochondria from the plantaris muscle for the two groups of offspring (mean $\pm$ SEM; n = 5 per group).

Figure 7. Measures of citrate synthase activity for the plantaris and soleus muscles for the two groups of offspring (mean $\pm$ SEM; n = 5 per group).

Figure 8.

Measurement of specific cytochrome *c* oxidase activity (i.e., oxygen uptake) and [ATP] for the quadriceps femoris muscles (mean $\pm$ SEM; $n = 7-8$ per group).

Figure 9. Representative Western Blots and quantification for key mitochondrial regulators (mean $\pm$ SEM; 5–8 per group).

Table 1Anthropometrics of the offspring (mean $\pm$ SEM).

	Groups	
	Offspring from Sedentary Dams (n=9)	Offspring from Exercised Dams (n=13)
Body mass (g)	20.98 $\pm$ 0.45	21.20 $\pm$ 0.18
Soleus mass (mg)	7.22 $\pm$ 0.64	6.23 $\pm$ 0.41
Soleus/body mass (mg/g)	0.35 $\pm$ 0.03	0.29 $\pm$ 0.02
Plantaris mass (mg)	11.33 $\pm$ 0.75	11.46 $\pm$ 0.40
Plantaris/body mass (mg/g)	0.54 $\pm$ 0.04	0.54 $\pm$ 0.02
L. Gastrocnemius mass (mg)	75.78 $\pm$ 2.12	54.85 $\pm$ 1.97 *
L. Gastrocnemius/body mass (mg/g)	3.61 $\pm$ 0.07	2.69 $\pm$ 0.09 *
Quadriceps Femoris mass (mg)	148.44 $\pm$ 5.35	154.62 $\pm$ 3.34
Quadriceps Femoris/body mass (mg/g)	7.09 $\pm$ 0.26	7.29 $\pm$ 0.13

Note:

* Significantly ($p < 0.001$) different between groups.

Table 2Muscle characteristics for lateral gastrocnemius muscle (mean $\pm$ SEM).

	Groups		
	Offspring from Sedentary Dams	Offspring from Exercised Dams	p-value
<u>Lateral Gastrocnemius</u>			
Area (μm²)	2766 ± 59	3341 ± 226	0.07
Perimeter (μm)	231 ± 4	253 ± 12	0.15
<i>R</i> ₉₅	37.1 ± 1.1	38.8 ± 0.4	0.22

Note: R_{95} = estimated diffusion distance.