Research Article

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Gestational exercise protects adult male offspring from high-fat diet-induced hepatic steatosis

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Background & Aims: Mounting evidence indicates that maternal exercise confers protection to adult offspring against various diseases. Here we hypothesized that maternal exercise during gestation would reduce high-fat diet (HFD)-induced hepatic steatosis in adult rat offspring.

Methods: Following conception, pregnant dams were divided into either voluntary wheel running exercise (GE) or wheel-locked sedentary (GS) groups throughout gestation (days 4–21). Post-weaning, offspring received either normal chow diet (CD; 10% fat, 70% carbohydrate, 20% protein) or HFD (45% fat, 35% carbohydrate, and 20% protein) until sacrificed at 4- or 8-months of age.

Results: GE did not affect offspring birth weight or litter size. HFD feeding in offspring increased weight gain, body fat percentage, and glucose tolerance test area under the curve (GTT-AUC). Male offspring from GE dams had reduced body fat percentage across all ages (p <0.05). In addition, 8-month male offspring from GE dams were protected against HFD-induced hepatic steatosis, which was associated with increased markers of hepatic mitochondrial biogenesis (PGC-1α and TFAM), autophagic potential (ATG12:ATG5 conjugation) and hepatic triacylglycerol secretion (MTTP).

Conclusions: The current study provides the first evidence that gestational exercise can reduce susceptibility to HFD-induced hepatic steatosis in adult male offspring.

Keywords: NAFLD; Fetal origins; Rat.
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Introduction

The American College of Sports Medicine (ACSM), the American College of Obstetricians and Gynecologists (ACOG), and the Center for Disease Control (CDC) recommend that healthy pregnant women engage in ⩾30 min of moderate physical activity on most, if not all, days of the week (reviewed in [1]). However, a despairingly small proportion (∼15%) of pregnant women actually achieve these recommendations [2] and a relatively low-proportion of obstetricians (52%) actually convey these recommendations to their pregnant patients [3]. Along these lines, it is necessary to address the current paucity in the literature regarding the long-term effects of maternal exercise on adult offspring health.

Adverse fetal stressors, such as maternal undernutrition or gestational diabetes, are well appreciated to negatively affect adult health outcomes of the offspring [4]. It is believed that the developing fetus responds to the *in utero* environment with epigenetic modifications to increase survival in the postpartum period that, when carried into adulthood, can increase disease susceptibility [5]. In contrast, evidence supporting the notion that healthy maternal behaviors may confer protection to the offspring against adult disease is comparatively understudied. Emerging evidence supports that maternal exercise may protect adult offspring against age and high-fat diet (HFD)-induced insulin resistance and adiposity [6–9]. Interestingly, despite the central importance of the liver to systemic metabolism, little it is currently known regarding the effects maternal exercise may have on the liver.

Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of liver phenotypes from simple steatosis to non-alcoholic steatohepatitis and cirrhosis [10]. It is present in the majority of obese individuals [11,12] and is identified as an independent predictor of liver-related, cardiovascular, and all-cause mortality [13,14]. Given the alarming modern obesity epidemic, the concept that maternal behaviors could affect NAFLD in adult offspring could have significant implications. Indeed, mice born to obese dams have elevated hepatic triacylglycerol (TAG) accumulation and reduced mitochondrial content and function compared to those born to dams of normal weight [15]. Whether maternal exercise can protect adult offspring from NAFLD,

however, has not previously been addressed. Thus, in the current report we tested the hypothesis that gestational exercise during pregnancy would confer protection to adult offspring against HFD-induced NAFLD. Using a rat model of gestational exercise, we demonstrate the novel finding that high-fat fed adult male offspring from sedentary mothers are more susceptible to developing hepatic steatosis than are offspring from exercised mothers.

Methods

Animal protocol

Virgin female Sprague-Dawley rats were singled housed in a temperature controlled environment with a 12:12 h light-cycle and given *ad libitum* access to standard chow (Harlan Rodent Diet 2018; Harlan Laboratories, Indianapolis, Indiana, USA) and water. Prior to pregnancy, rats were acclimatized to running wheels with free access to running wheels for four days, at which point wheels were locked. Male rats were housed with dams for breeding purposes and removed thereafter. Pregnancy was confirmed via copulatory plug formation. Dams were randomly divided into either voluntary wheel running gestational exercise (GE; n = 7) or wheel-locked sedentary (GS; n = 7) groups. Daily running distance was recorded using a magnetic sensor system (Rat Activity Wheel; Lafayette Instrument, Lafayette, Indiana, USA or Schwinn 17 Function Bike Computer; Pacific Cycle, Madison, Wisconsin, USA). Wheels were locked following parturition in order to restrict exercise to the gestational period. Post-weaning (21 days postpartum), four male and four female offspring were randomly selected from each litter and randomly divided into age of either 4- or 8-months. Offspring were single-housed to prevent potential confounding effects of increased spontaneous cage activity of group housing [16] and provided *ad libitum* access to either a normal chow diet (CD; 10% fat, 70% carbohydrate, 20% protein) of standard chow or a high-fat diet (HFD) containing 45% fat, 35% carbohydrate, and 20% protein (D12451; Open Source Diets, New Brunswick, New Jersey, USA). Subsets of offspring (n = 8–10 per age and sex) were euthanized at 4- and 8-months of age following a 6 h fast with an overdose of sodium pentobarbital and livers were rapidly excised and a portion snap frozen in liquid nitrogen or formalin-fixed for later processing and a segment processed for liver explant culture. Animal protocols were approved by the Purdue University Animal Care and Use Committee.

Body composition

Body composition (fat-mass, fat-free-mass, and fluids) of offspring was assessed via an EchoMRI-900 machine (EchoMRI, Houston, TX, USA). The MRI test was administered to all offspring 3 weeks prior to the 4 month and 8 month sacrifices. Conscious rats were placed in a specialized holder that allowed for restricted movement on part of the animal. The holder was then placed in the MRI for up to 3 min. Rats were returned to their home cages immediately after the scan.

Offspring activity

At 2, 4, 6 and 8-months of age, rats were placed in cages with voluntary access to running wheels for three days. Mean daily running distance was monitored daily. To allow for acclimatization to the wheel, mean running distance over days two and three at each age is reported.

Glucose tolerance test (GTT)

A GTT was performed on the conscious offspring at 3 weeks prior to sacrifice. Baseline samples of whole blood were taken from a lateral tail vein and animals were given a single intraperitoneal (IP) bolus of glucose (2 g/kg body weight). Blood glucose was measured at baseline, 10, 20, 30, 60, and 120 min post injection with a handheld glucometer (ACCU-CHEK Advantage, Roche, Indianapolis, IN). Glucose clearance during the GTT was evaluated by calculating the total area under the curve (AUC) using the trapezoidal method [17].

Hepatic TAG, histology, and enzyme activities

Intrahepatic TAG content, citrate synthase activity, and 3-hydroxyacyl-CoA dehydrogenase (β-HAD) activity were measured as described previously [18]. Formalin-fixed livers were embedded in paraffin, serially sliced, and stained with hematoxylin and eosin as previously referenced [18].

Palmitate oxidation

At euthanasia, 500 mg of liver was excised and immediately placed on ice in 25 ml of DMEM containing 1% BSA (Millipore, Billerica, MA). Liver explant cultures were prepared within 10 min of sample collection by slicing liver tissue to a thickness of 0.5 mm in a Thomas Stadie-Riggs Tissue Slicer (Thomas Scientific, Swedesboro, NJ) and then subdividing to smaller pieces (20 to 30 mg) using razor blades. Explants were added to 20 ml flasks containing 2.0 ml DMEM and 1.0 mM [1-^{14}C]palmitate (0.45 μCi; American Radiolabelled Chemicals, St Louis, MO) in complex with fatty acid-free BSA (Millipore, Billerica, MA) in a 4:1 molar ratio. Flasks were gassed for 2 s with carbogen (95% O_2 and 5% CO_2), sealed with a stopper fitted with a Kontes hanging center well (Kimbal Chase, Vineland, NJ) and placed in a 37 °C orbital shaking water bath. After 2 h, incubations were terminated by the addition of 0.2 ml of 40% $HClO_4$ into the incubation medium and $^{14}CO_2$ was collected by the addition of 0.2 ml of phenethylamine to the hanging center well trap [19]. Accumulation of $^{14}CO_2$ was determined by liquid scintillation counting. Tissue was removed, blotted and weighed and oxidization data expressed as nmol of [1-^{14}C] palmitate converted to $^{14}[CO_2] \cdot g\ tissue^{-1} \cdot h^{-1}$.

Western blots

Western blot analysis was completed in whole liver homogenate. Primary antibodies used are as follows: acetyl coenzyme A carboxylase (ACC; #3662, Cell Signaling, Danvers, MA, USA), S79 phosphorylation specific ACC (p-ACC; #3661, Cell Signaling); fatty acid synthase (FAS; #3189, Cell Signaling, Danvers, MA), CD36 (ab133625, Abcam, Cambridge, MA), microsomal triglyceride transfer protein (MTTP; #135994, Santa Cruz Biotechnology, Dallas, TX, USA), apolipoprotein B100 (ApoB100, #11795, Santa Cruz), peroxisome proliferator activator receptor-γ (PPARγ; #7273, Santacruz), AuTophagy related Gene 12 (ATG12, #4180, Cell Signaling), adenosine monophosphate activated protein kinase-α (AMPK; #2603, Cell Signaling) and T172 phosphorylated AMPK (p-AMPK; #2531, Cell Signaling). Blots (n = 7–10/group) were analyzed via densiometric analysis (Image Lab 3.0). Amido-black staining was used to control for differences in protein loading and transfer as previously described [18].

RT-PCR

Quantitative real time PCR was completed with the ABI 7500 Fast Sequence Detection System (Applied Biosystems, Carlsbad, CA) using Fast Sybr Green Master Mix (Applied Biosystems, Carlsbad, CA). Primers pairs were obtained from Sigma (St. Louis, MO, USA) for β-Actin (Forward: 5′-CAG AGC AAG AGA GGC ATC CTC-3′, Reverse: 5′-GTC CAG ACG CAG GAT GGC ATG-3′), PGC-1α (Forward: 5′-TTG ACT GGC GTC ATT CAG GA-3′; Reverse: 5′-GGC AGC ACA CTC TAT GTC ACT-3′), and TFAM (Forward: 5′-GAA TGT GGG GCG TGC TAA GA-3′; Reverse: 5′-CAG ATA AGG CTG ACA GGC GA-3′). PCR product dissociation melt curves were used to assess primer pair-target specificity. β-actin transcript abundance was not different between groups and was used as the reference gene to calculate the expression levels of genes of interest using the $2^{-\Delta\Delta CT}$ method. Data are normalized to expression levels of GS-CD.

Statistical analysis

Statistical analyses were completed in R (v.2.15.1) with $p < 0.05$ used to determine statistical significance of all comparisons. Within sex comparisons across age were assessed via three-way ANOVA (condition × diet × age). Across sex comparisons were not made unless otherwise noted. Variables that were assessed at a single age were analyzed with a two-way ANOVA (condition × diet). Significant interactions were followed post hoc with a Fisher's LSD test with pooled standard deviation to determine individual group differences. Data are presented as means ± SE. n = 7–10 observations are represented per group.

Results

Dam characteristics

Portions of dam and offspring characteristics of the cohort of rats used in the current report have been reported previously [20,21]