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Maternal exercise improves insulin sensitivity in mature rat offspring

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Abstract

Purpose—Recent findings have shown the intrauterine environment can negatively influence long-term insulin sensitivity in the offspring. In an attempt to be pro-active, we set out to explore maternal voluntary exercise as an intervention in order to improve offspring insulin sensitivity and glucose homeostasis.

Methods—Female Sprague Dawley rats were split into sedentary and exercise groups with the exercise cohort having voluntary access to a running wheel in the cage prior to and during mating, pregnancy, and nursing. Female offspring were weaned into sedentary cages. Glucose tolerance tests and hyperinsulinemic–euglycemic clamp were performed in adult offspring to evaluate glucose regulation and insulin sensitivity.

Results—Adult female offspring born to exercised dams had enhanced glucose disposal during glucose tolerance testing ($P < 0.05$) as well as increased glucose infusion rates ($P < 0.01$) and whole body glucose turnover rates ($P < 0.05$) during hyperinsulinemic–euglycemic clamp testing compared to offspring from sedentary dams. Offspring from exercised dams also had decreased insulin levels ($P < 0.01$) and hepatic glucose production ($P < 0.05$) during the clamp procedure compared to offspring born to sedentary dams. Offspring from exercised dams had increased glucose uptake in skeletal muscle ($P < 0.05$) and decreased heart glucose uptake ($P < 0.01$) compared to offspring from sedentary dams in response to insulin infusion during the clamp procedure.

Conclusions—Exercise during pregnancy enhances offspring insulin sensitivity and improves offspring glucose homeostasis. This can decrease offspring susceptibility to insulin resistant related disease such as type 2 diabetes mellitus. Maternal exercise could be an easy, short–term, non–pharmacological method of preventing disease in future generations.

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Author Contributions

L.G.C. and K.J.P. designed research; L.G.C. and N.Q. performed research; L.G.C., N.Q., R.de C., and K.J.P. analyzed data; and L.G.C., N.Q., R.de C., and K.J.P. wrote the paper. K.J.P. is the guarantor and takes responsibility for the contents of the article.

Keywords

Pregnancy; running; programming; hyperinsulinemic-euglycemic clamp; rat

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by peripheral tissue insulin resistance and dysfunctional insulin secretion caused by prolonged hyperglycemia. Genetic factors are also thought to play a role in susceptibility to the disease. There have been many epidemiological studies that have shown a link between low birth weight and higher incidence of T2DM in adulthood (12, 33). David Barker showed an association between prenatal environment and adulthood disease (4). Hales and Barker coined the term ‘thrifty phenotype’ to describe the phenomenon in which low birth weight babies, exposed to low maternal nutrient intake during gestation, have higher incidences of T2DM and cardiovascular disease later in life (21). They hypothesized that development is plastic and can shift depending on environmental cues received during gestation. In the case of poor maternal nutrient intake, the fetus receives cues that the environment outside the womb is low in nutrient availability, thus development shifts to adapt to survive in such an environment. When the postnatal environment, however, is rich in nutrient sources, contrary to what was predicted, the offspring are predisposed to diseases associated with excess nutrient intake such as cardiovascular disease and T2DM.

Many animal models have been created to study the mechanisms that cause late-life outcomes in low birth weight offspring. Maternal nutrient restriction and uterine artery ligation have been used to promote intrauterine growth restriction (IUGR) in the fetus (30, 35). These studies have found impaired glucose regulation and insulin sensitivity in adult offspring exposed to IUGR (35). Other studies have shown that offspring exposed to IUGR have decreased expression and activation of key insulin signaling pathway proteins in skeletal muscle and adipose tissue suggesting a mechanism for the observed insulin insensitivity (18, 28).

Regular exercise is well known to decrease susceptibility to T2DM by enhancing insulin sensitivity and promoting non–insulin stimulated glucose uptake (34, 39). In animal models, exercise by the offspring reversed the negative metabolic impact of IUGR (19). Still, given the numerous benefits of physical activity, seemingly few people have the time or motivation to maintain an active lifestyle. Pregnant women, however, might be more inclined to eat a healthy diet and remain physically active if these things could positively affect the long–term health of their developing child by decreasing susceptibility to T2DM. Physical activity during pregnancy is already known to have many maternal benefits including weight and body composition control and improved cardiovascular health (11). Exercise during pregnancy has also been shown to have beneficial effects for offspring in humans. Maternal aerobic exercise has been found to decrease cord growth hormone levels and infant body mass index (23). Other benefits of exercise during pregnancy include decreased fat mass at birth and in childhood as well as improved cognitive characteristics (9). Changes in adiposity are thought, in part, to be the result of small decreases in fetal nutrient availability due to intermittent reduction in uterine blood flow during times of physical activity (10). In animal studies, offspring of exercised dams show improved neurogenesis and memory (24). Our laboratory has recently shown that perinatal exercise in chow fed mice enhances insulin sensitivity in adult male and female offspring (7). One study also found that exercise during pregnancy in streptozotocin-induced diabetic rats affected 28 day old offspring glucose regulation compared to offspring from sedentary diabetic rats (38).

However, no studies in rats have researched the long–term effects of exercise during healthy pregnancy on offspring glucose homeostasis and insulin sensitivity.

It has been well established in animal models and in humans that the intrauterine environment can negatively impact fetal development and future metabolic health. What needs further exploration is how certain maternal behaviors, such as exercise, can positively influence the perinatal environment and improve offspring long–term metabolic health outcomes. Maternal interventions during pregnancy are a novel and unique way of targeting and preventing T2DM and other diseases in future generations. Therefore the objective of the current study was to investigate the effects of maternal exercise prior to and during pregnancy and nursing on offspring insulin sensitivity using a rat model. The hypothesis for the current study was that adult offspring born to exercising dams would have enhanced insulin sensitivity compared to those offspring born to sedentary control dams.

Materials and Methods

Animals and diets for breeding scheme to follow offspring

Forty 12 week old female CD/IGS Sprague Dawley (SD) rats (Charles River Laboratories International, Wilmington, MA) arrived at the University of Kentucky animal facility and were housed 2 per cage for a one week acclimation period with unlimited access to food and water. The animal facility was on a 12 hour light/dark cycle and maintained at a temperature of 68 – 72°F. After the acclimation period, females were split into sedentary and exercise cohorts, ensuring no initial weight differences between the two groups (n = 20 per group). Females were then single housed and placed on Formulab Diet #5008 (Labdiet®, Cincinnati, OH). Female body weight and food intake were measured twice a week during breeding, pregnancy, and nursing. When males were present in the cage, food intake was divided by 2 to account for food intake of both animals. After 7–10 days in sedentary or exercise cohorts, 10 week old male SD rats were housed with the female rats for 10 days for mating. Females in the exercise group had unlimited access to a running wheel (Nalgene® Running Wheel) mounted in the cage prior to and during mating, throughout pregnancy and up through postnatal day 12. A magnetic counter recorded number of wheel turns onto a computer (VitalView Data Acquisition System, Mini Mitter Company Inc., Bend, OR). Wheels were removed on postnatal day 12 to prevent the pups from running and/or being injured. One exercise dam nested her litter too close to the wheel and this litter was removed from the study. Sedentary females were housed in identical cages without a running wheel. Males bred with exercising females had access to the running wheel during the breeding portion of the study. Although wheel turns due to male versus female running were not measured directly, a study comparing male and female SD rats found that females run significantly more than males (17). On postnatal day 1, litters were culled to 10 pups per litter, ensuring 5 males and 5 females per litter when possible. On postnatal days 14 and 21 one male and one female from each litter were culled for serum and tissue collection. On postnatal day 21, remaining female pups were weaned into sedentary cages and housed 2 rats per cage and fed Teklad Global 18% Protein Rodent Diet #2018 (Harlan, Indianapolis, IN). Offspring did not have access to a running wheel for any portion of the study. Analyses in offspring were conducted such that only one female offspring per dam was included. Rats were shipped to the University of Michigan at 14 months of age where they were housed singly for the remainder of the study. A previous study from our laboratory using mice found that perinatal exercise improves glucose disposal in offspring of both sexes (7). Therefore, we chose to monitor insulin sensitivity in female rat offspring for this study as a way to limit costs.

Oral glucose tolerance test (OGTT)

An OGTT was performed in female offspring at 15 months of age. Rats were fasted overnight for 16 hours and given an oral gavage of glucose at 2 g/kg body weight. Blood glucose readings were taken via tail prick prior to injection (minute 0) and 15, 30, 60, and 120 minutes post glucose administration using an Accu-Chek glucometer (Roche, Germany).

Body composition

Body composition was analyzed in 15 month old female offspring using a nuclear magnetic resonance-based analyzer (Minispec LF90II, Bruker Optics, TX, USA). Body fat, lean mass, and free water were measured.

Hyperinsulinemic–euglycemic clamp

The offspring that remained from the cohort used for glucose tolerance testing at 15 months of age underwent hyperinsulinemic–euglycemic clamping to test whole body insulin sensitivity at 17 months of age. Hyperinsulinemic–euglycemic clamp was performed by the University of Michigan Animal Phenotyping Core on conscious, unrestrained rats using the protocol adapted from the Vanderbilt Mouse Metabolic Phenotyping Center with some modifications (1). The right jugular vein and carotid artery of the rats were surgically catheterized a week prior to the clamp and animals that had healthy appearance, normal activity, and body weight regained to or above 90% of their pre-surgery levels were used for the study. After an overnight fast for 16 hours, rats underwent the clamp procedure consisting of a 90 min equilibration period, followed by a 120 min experimental period (t = 0 to 120 min). Insulin was infused at 4.0 mU/kg/min and euglycemia (120~130 mg/dL) was maintained during the clamp by infusing 50% [3-^{3}H]glucose at variable rates. To estimate insulin-stimulated glucose uptake in individual tissues, a bolus injection of [1-^{14}C]-2-deoxyglucose ([^{14}C]2-DG, PerkinElmer) (30 μCi) was given at t = 78 min while continuously maintaining the hyperinsulinemic-euglycemic steady-state. At the end of the experiment, animals were anesthetized with an intravenous infusion of sodium pentobarbital and tissues were collected and immediately frozen in liquid nitrogen for later analysis of tissue [^{14}C] radioactivity. Tissue [^{14}C]2-DG levels, plasma radioactivity of [^{14}C]2-DG and [3-^{3}H]glucose, and plasma insulin levels were analyzed as previously described (8). The body weight of one rat in each group was a significantly outlier compared to the rest of the group (body weight was higher, $P < 0.01$ by the Grubbs' test) and the data was removed for future analyses.

Timed pregnancy

Timed mating was performed in order to evaluate the effects of maternal exercise on the pregnant dams. The design was similar to the earlier experiment with differences highlighted below. After the acclimation period, female rats were split into sedentary and exercise cohorts, ensuring no initial weight differences between the two groups (n = 25 for sedentary and n = 20 for exercise). After 7–10 days in the sedentary or exercise cohorts, females were mated with 10 week old male SD rats. For timed mating, females were removed from their home cage during the dark cycle and placed with a male in a wire cage to allow for plug detection. The females were returned to their home cage during the light cycle but were mated to the same male over multiple days until a plug was found. If there was no plug after 4 nights of breeding, the female was removed from the study. Only pregnant female rats that had plugs detected were included for further analyses, and the date the plug was found was designated gestation day 0 (18/25 sedentary and 14/20 exercise females became pregnant).

On gestation day 14, rats were fasted overnight for 16 hours and a glucose tolerance test was performed. For the fasting period, exercise females remained in their home cage with running wheel access until 1 hour prior to testing. Exercise females were then placed in standard cages throughout the testing period. Blood glucose readings were taken via tail prick prior to administration (minute 0) and 15, 30, 60, and 120 minutes post glucose administration using an Ascensia Breeze 2 meter (Bayer, Mishawaka, IN).

On gestation day 18, rats were fasted overnight for 16 hours for blood and tissue collection. During the fasting period, exercise dams remained in their home cage with the running wheel until 1 hour prior to euthanasia. Heart, soleus, and parametrial and retroperitoneal fat pads were weighed at take–down. Gestation day 18 serum glucose and insulin were measured using a glucose assay kit (BioVision #K606–100, San Francisco, CA) and an insulin ELISA (Crystal Chem Inc #90080, Downers Grove, IL) respectively.

Animal care and use

Studies conducted at the University of Kentucky were approved by the Institutional Animal Care and Use Committee and adhered to American College of Sports Medicine (ACSM) animal care standards. Studies conducted at the University of Michigan Animal Phenotyping Core were approved by the University Committee on Use and Care of Animals and adhered to ACSM animal care standards.

Statistical analysis

Data were analyzed using a Student's t–test (Sigma Plot 11.0 software, Systat, Point Richmond, CA). Data that failed the Shapiro–Wilk normality test were transformed by calculating the natural log of the values. Area under the curves (AUC) for glucose were calculated using "Area Below Curves" function in Sigma Plot 11.0. Fisher's exact test was used to test for differences in delivery rates using GraphPad Prism software (La Jolla, CA).

Results

Maternal and litter outcomes

Female SD rats were split into sedentary and running wheel cages for ~1 week prior to mating, throughout pregnancy, and the first 12 days of nursing. Running distance increased as the female rats became acclimated to the wheel and was highest when males were present in the cage for mating (Figure 1A). While this study did not distinguish between male and female running, a previous study looking at running behavior of male and female SD rats found that females ran significantly more than males (17). As pregnancy progressed, dams decreased running distance per day; distance was lowest on the day of delivery (set to day 33). Maternal running distance increased slightly during the nursing period.

Body weight and food intake were monitored for both groups during breeding. Maternal body weight trended toward a decrease in the exercise group compared to the sedentary group, but was significantly lower at only one time point (Figure 1B). Exercise, however, had no effect on maternal food intake (Figure 1C). Figures 1B and C are not matched for day of delivery.

There were no significant differences in delivery rates between sedentary (20/20) and exercise (18/20) dams ($P = 0.4872$ by Fisher's Exact Test). At birth, the number of pups per litter was recorded. There was no significant difference in number of pups per litter between sedentary (13.9 ± 0.39) and exercise (14.61 ± 0.59) dams ($P = 0.312$). Pup body weights were calculated by averaging pup body weights per litter then taking each litter mean. There

were no significant differences in pup body weight when they were weighed at postnatal days 1, 7, 14, and 21. (Figure 1D).

Offspring body weight and glucose tolerance

Body weight of offspring from sedentary and exercised dams was measured from weaning (week 3) up through 15 months of age with no recorded differences in body weight. Glucose tolerance testing was performed at several ages, starting at 2 months of age, however no significant differences were observed until offspring reached 10 months of age (data not shown). An oral glucose tolerance test was performed when offspring were 15 months of age. Rats were fasted for 16 hours and blood was collected to assess fasting insulin and glucose levels. Offspring from exercised dams had significantly lower plasma insulin levels compared to offspring from sedentary dams ($P = 0.017$) (Figure 2A), but fasting glucose levels at time 0 were not different (Figure 2B). Rats were then given an oral glucose challenge, and blood glucose levels were significantly lower in offspring born to exercised dams compared to offspring from sedentary dams at the 30 and 120 minute time points compared to those from sedentary dams ($P = 0.018$ and $P = 0.017$, respectively) (Figure 2B). AUC, a measure of overall glucose disposal, was significantly lower in offspring from exercised dams ($P = 0.019$) (Figure 2C). At 15 months of age, female offspring body composition was analyzed and there were no significant differences in body fat, lean mass or free water between the two groups (data not shown). These data suggest that offspring born to exercised dams have enhanced glucose disposal compared to offspring from sedentary dams that is independent of body composition.

Offspring hyperinsulinemic–euglycemic clamp

At 17 months of age, offspring underwent hyperinsulinemic–euglycemic clamp testing to assess whole body insulin sensitivity. At this age point, a body weight difference developed between the two groups; offspring from exercised dams weighed significantly less than those born to sedentary dams (19.8% less, $P = 0.003$). The decrease in body weight in the offspring from the exercised dams was not detected earlier when the glucose disposal differences were observed. Prior to the clamp procedure, rats were fasted for 16 hours. They were then infused with insulin at a constant rate of 4 mU/kg/min for 120 minutes. Rats were simultaneously infused with glucose at varying rates in order to maintain blood glucose levels of approximately 120–130 mg/dL. Blood glucose and the glucose infusion rate (GIR) needed to maintain physiological blood glucose levels were monitored throughout the 120 minute procedure (Figures 3A and B, respectively). There were no differences in blood glucose levels in the offspring from sedentary or exercised dams during the procedure (Figure 3A). The GIR was significantly higher in offspring born to exercised dams compared to offspring from sedentary dams at all time points after 40 min ($P < 0.05$) (Figure 3B). The average steady–state GIR (80–120 minutes) was significantly higher in offspring born to exercised dams ($P < 0.001$) (Figure 3C).

Both basal and clamp plasma insulin levels were significantly lower in offspring from exercised dams compared to offspring from sedentary dams ($P < 0.001$ for both) (Figure 3D). There were no differences in basal whole body glucose turnover rates however during the clamp, offspring from exercised dams had significantly increased glucose turnover rates compared to offspring from sedentary dams ($P = 0.011$) (Figure 3E). Also, there were no differences in basal hepatic glucose production (HGP) however in response to insulin infusion during clamp, offspring from exercised dams had significantly lower HGP compared to those from sedentary dams ($P = 0.037$) (Figure 3F). Suppression of HGP was also significantly increased in offspring from exercised dams ($P = 0.013$) (data not shown). These data suggest that mature offspring born to exercised dams have enhanced insulin sensitivity compared to offspring from sedentary dams.

Offspring tissue specific glucose uptake

At the end of the clamp procedure, the aged offspring were euthanized and insulin sensitive tissues were analyzed for 2–deoxyglucose uptake. Under the hyperinsulinemic–euglycemic steady–state, both the extensor digitorum longus (EDL) ($P = 0.035$) and gastrocnemius (gastroc) ($P < 0.001$) muscles from offspring born to exercised dams had significantly increased glucose uptake (Figures 4A and B, respectively), while soleus muscle uptake was unchanged (Figure 4C). There were no differences in white adipose glucose uptake as represented by visceral and subcutaneous fat pads (Figures 4D and E, respectively). Interestingly, offspring from exercise dams had significantly decreased glucose uptake in the heart compared to offspring from sedentary dams ($P = 0.006$) (Figure 4F). These data suggest that there are tissue specific effects in insulin sensitivity in offspring that result from maternal exercise.

Timed mating maternal outcomes

There were no significant differences in body weight between sedentary and exercising dams prior to or during pregnancy. Further, glucose tolerance was not significantly affected by exercise in the pregnant dams at gestation day 14 (data not shown). Table 1 provides a summary of maternal outcomes after tissue and blood collection at gestation day 18. There were no differences in heart or soleus muscle weight between sedentary and exercise dams. Exercise females, however, had significantly smaller parametrial and retroperitoneal fat pads at gestation day 18 compared to sedentary dams ($P = 0.017$ and $P < 0.001$, respectively). Similar to the findings with glucose tolerance testing on gestation day 14, there were no differences in serum glucose levels at gestation day 18. Serum insulin levels, however, were significantly reduced in exercise dams compared to sedentary dams which could suggest a level of heightened insulin sensitivity.

Discussion

This study has shown that maternal running during pregnancy can improve glucose homeostasis and enhance insulin sensitivity in adult offspring. We found that mature offspring born to exercised dams had improved glucose disposal following a glucose challenge and enhanced whole body insulin sensitivity as determined by hyperinsulinemic–euglycemic clamp. Although glucose tolerance testing was conducted in younger animals, differences were not observed until approximately ten months of age (data not shown). This is not surprising given that numerous developmental programming studies, in animals as well as humans, do not detect differences in offspring until advanced age (3, 18). This could be due to the dysregulation of metabolic processes that occur with age or a number of other factors. Regardless, we have shown that a maternal intervention, exercise during healthy pregnancy, can have a long-lasting positive impact on offspring metabolic health.

In this study we also looked at tissue specific glucose uptake in response to insulin infusion during clamp. Skeletal muscle and white adipose tissues are the main sites of insulin stimulated glucose uptake in the body (13, 14). Compared to offspring from sedentary dams, those born exercised dams had significantly increased glucose uptake in response to insulin in skeletal muscle but not adipose tissue. Differences in skeletal muscle glucose uptake were detected in the EDL and gastroc muscles while no differences were observed in the soleus muscle. This may be due to different fiber types in the various muscles. Type I fibers (slow twitch) are used for slow contractions and have a high oxidative capacity but low glycolytic capacity (40). Type II fibers (fast twitch), used for fast contractions, have a high glycolytic capacity (40). In the rat, the EDL muscle is primarily composed of type IIB fibers, while the soleus is mostly made up of type I fibers (31, 37). The gastroc muscle is comprised of a mixture of both muscle fiber types (37). Taking this into account, it is not surprising that

glucose uptake differences were observed in the EDL and gastroc as opposed to the soleus. It will be necessary in upcoming studies to investigate whether there are changes in expression or activation of insulin signaling pathway proteins in the EDL and gastroc muscles.

In addition to differences in skeletal muscle glucose uptake during insulin infusion, we observed that heart glucose uptake was significantly decreased in offspring born to exercised dams compared to those from sedentary dams. Fatty acids are the main substrate for metabolism in the heart and oxidation of fatty acids prevents glucose metabolism (15). Substrate utilization in the cardiac tissue shifts from primarily fatty acids to glucose when there is increased workload on the heart (27). Glucose also becomes the main substrate of metabolism when there are high levels of circulating glucose and insulin (5, 15). This may suggest that the decreased glucose uptake in the heart observed in offspring from exercised dams is actually indicative of improved cardiovascular and metabolic health. Future studies in the lab will explore in depth the cardiovascular effects of maternal exercise in offspring.

Through the clamp procedure we were also able to evaluate basal and insulin stimulated HGP. Although there were no differences in fasting HGP, insulin stimulated HGP was significantly decreased in offspring from exercised dams compared to those from sedentary dams suggesting the liver in offspring from exercised dams had enhanced insulin sensitivity. Insulin is a major signaling hormone released in the fed state that suppresses endogenous energy (glucose) production while promoting energy storage. After skeletal muscle and white adipose tissue, the liver is a main site of insulin action and glucose uptake (29). With insulin resistance, there is increased gluconeogenesis and decreased glucose uptake which contributes significantly to hyperglycemia seen with T2DM (25). Given the importance of insulin action on the liver, enhanced hepatic insulin sensitivity, as observed in the offspring from exercised dams, is essential to improving overall metabolic health. Future studies will look at hepatic expression of insulin pathway proteins and markers of hepatic function such as glycogen storage and expression and activation of proteins involved in glycogen synthesis and gluconeogenesis.

It is important to note that throughout the majority of the offspring lifespan, there were no body weight differences between the two groups. When the offspring were shipped to the University of Michigan at 14 months of age and during the OGTT at 15 months of age, there were still no differences in body weight. However, at the time of clamp testing, when the offspring were 17 months old, offspring from exercised dams weighed significantly less than those from sedentary dams. There could be several reasons for this observed difference in body weight. Weight loss is a well known response in rats to stress (16). The two groups may have responded differently to the shipping and transition from group to single housing conditions resulting in weight differences between the two groups. Also, an equal number of rats per group (n = 5) had to be removed from the study while at the University of Michigan due to health or experimental problems, and this could have led to unintentional differences in body weight. Regardless, the clamp procedure corrects for differences in body weight because insulin is infused at a rate relative to the rat body weight.

This study provides strong evidence that maternal exercise during pregnancy can improve insulin sensitivity and glucose homeostasis in adult offspring. There are, however, several limitations in the current study that should be considered. The running wheel in the cage of exercise group dams could be a form of environmental enrichment. Maternal environmental enrichment has been found to have developmental programming effects in offspring (36); however, none of these studies have shown maternal environmental enrichment affects offspring insulin sensitivity or glucose regulation. In addition, male rats had access to the running wheel during breeding so paternal running could have an influence on the outcomes

observed in offspring. Recent papers have shown that a paternal low protein diet can cause changes in offspring gene methylation (6) and that paternal high fat diet consumption can induce glucose intolerance in female offspring (26). Finally, only female offspring were used in the current study although developmental programming models have been shown to have sex specific effects in offspring (20). Regardless, our laboratory has found that exercise during pregnancy enhances insulin sensitivity in both male and female offspring in mice suggesting that maternal exercise can positively impact metabolic health in both sexes (7). It is important to note that female offspring estrous cycle was not monitored during testing and this may have impacted results as sex hormones are known to affect insulin sensitivity and glucose tolerance (2). It will be necessary to take all of these confounding factors into consideration in future studies.

Other upcoming studies will focus on the timing (prior to conception, during gestation, and/or during nursing) and intensity of maternal exercise that is key for enhancing insulin sensitivity in offspring. We speculate however, that exercise during gestation, more so than prior to conception or during nursing, is essential for creating the life–long changes in offspring sensitivity. Acute exercise during pregnancy causes intermittent decreases in oxygen and glucose availability to the fetus (10) which most likely impacts fetal metabolic development. Results from the timed mating in this study suggest that maternal insulin levels and fat mass, both affected by exercise prior to and during pregnancy, may also impact fetal metabolic development. Thus, it will also be necessary to investigate epigenetic modifications in offspring, specifically of genes involved in insulin and glucose regulation in an attempt to elucidate the mechanism by which exercise during pregnancy improves adult offspring insulin sensitivity. Many studies, in particular those looking at the effects of IUGR on offspring, have already shown that altering the intrauterine environment can influence epigenetic modifications of genes involved in glucose homeostasis. For example, histone modifications have been found to be responsible for the decrease in glucose transporter type 4 expression in offspring from protein restricted dams (32). IUGR has also been associated with methylation and silencing of the pancreatic and duodenal homeobox 1 gene which is involved in pancreatic development and function in rats (30). A recent human study has found that offspring exposed to nutrient restriction during gestation have changes in methylation patterns of the maternally imprinted insulin –like growth factor 2 gene that is involved in human development (22).

More work needs to be done in this area, but our results are particularly exciting because they confirm that the benefits of maternal exercise on offspring insulin sensitivity are not species specific. The incidence of T2DM is increasing worldwide, and it is necessary to find novel ways to prevent the disease. Exercise during pregnancy could be a short–term, easily accessible and achievable way to target T2DM in future generations.

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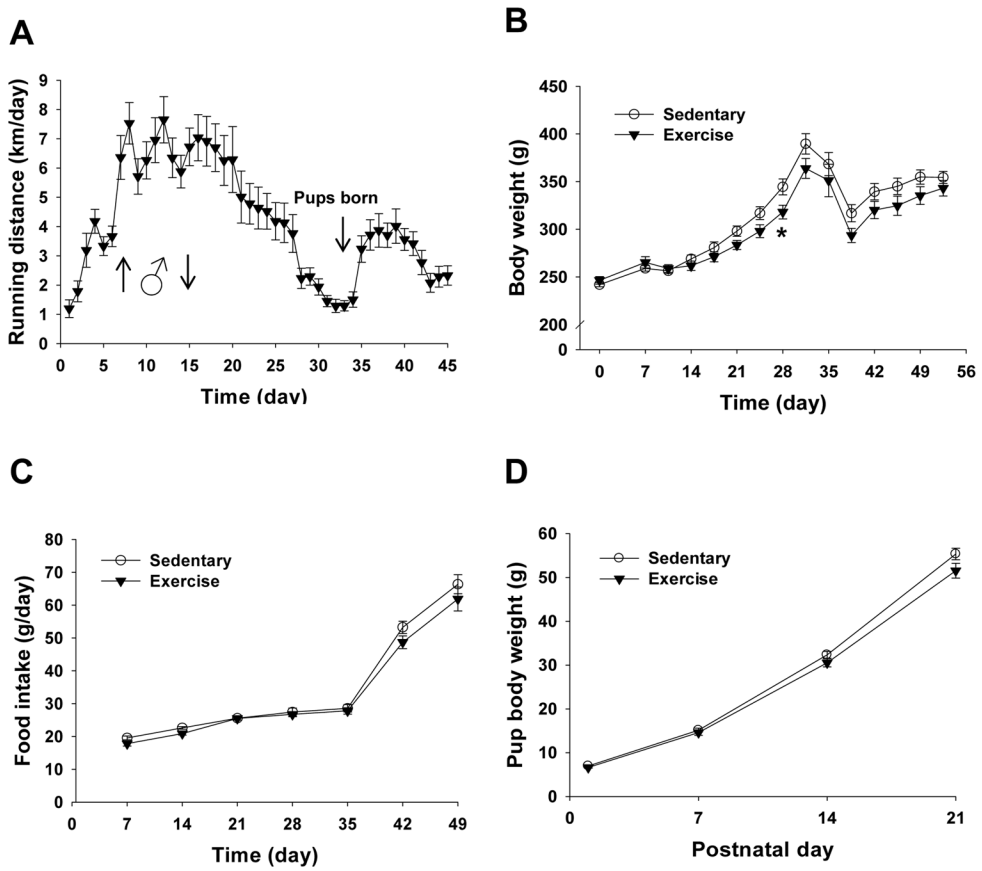

Figure 1. Maternal body weight and food intake

(A) Mean running distance per day for female Sprague Dawley rats set up in a breeding scheme. Arrows indicate when male rats were in the cage for breeding and when pups were born. Day 33 corresponds to day of delivery. (B) Voluntary exercise during pregnancy and nursing caused a trend toward decreased dam body weight compared to sedentary control dams at most time points. (C) There were no differences in food intake between sedentary and exercise dams. (D) There were no differences in pup body weights during nursing. Data were aligned for day of delivery in A but not B and C. * $P < 0.05$ compared to sedentary dams; n = 20 for sedentary and n = 17 for exercise in A – D. Error bars indicate s.e.m.

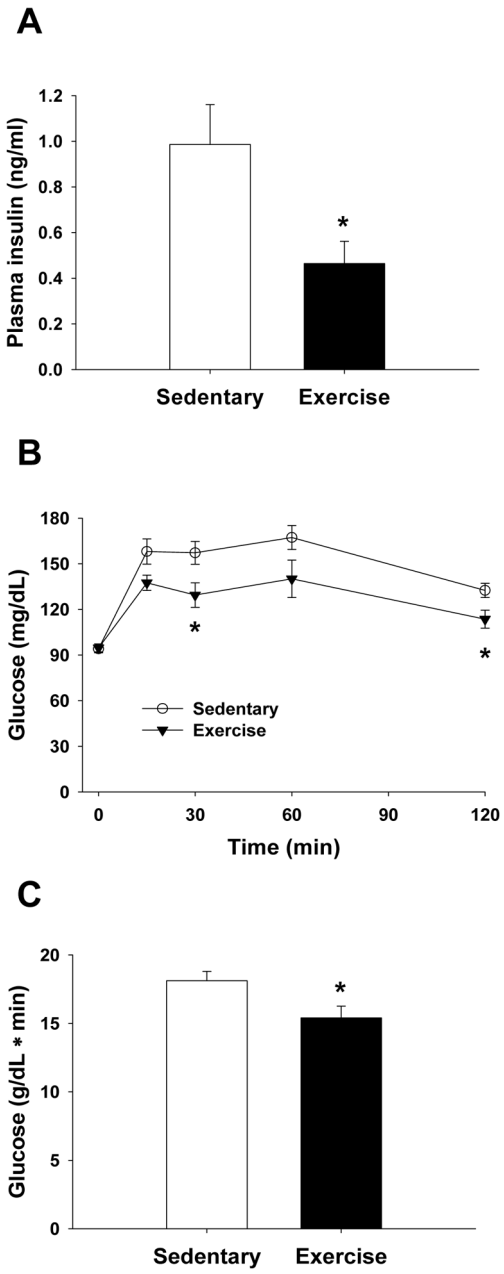

Figure 2. Female offspring born to exercised dams had improved glucose disposal following a glucose challenge

Fifteen month old female offspring were fasted for 16 hours then given an oral gavage of glucose (2 g/kg body weight). (A) Offspring from exercised dams had significantly decreased fasting insulin compared to offspring from sedentary dams. (B) At 30 and 120 minutes post glucose administration, blood glucose was significantly lower in offspring from exercised dams compared to offspring from sedentary dams. (C) Area under the curve (AUC) of blood glucose levels during the glucose tolerance test was also significantly lower in offspring from exercised dams compared to those from sedentary dams. * $P < 0.05$ compared to sedentary control; n = 13 for sedentary and n = 10 for exercise in A; n = 16 for sedentary and n = 14 for exercise in B and C. Error bars indicate s.e.m.

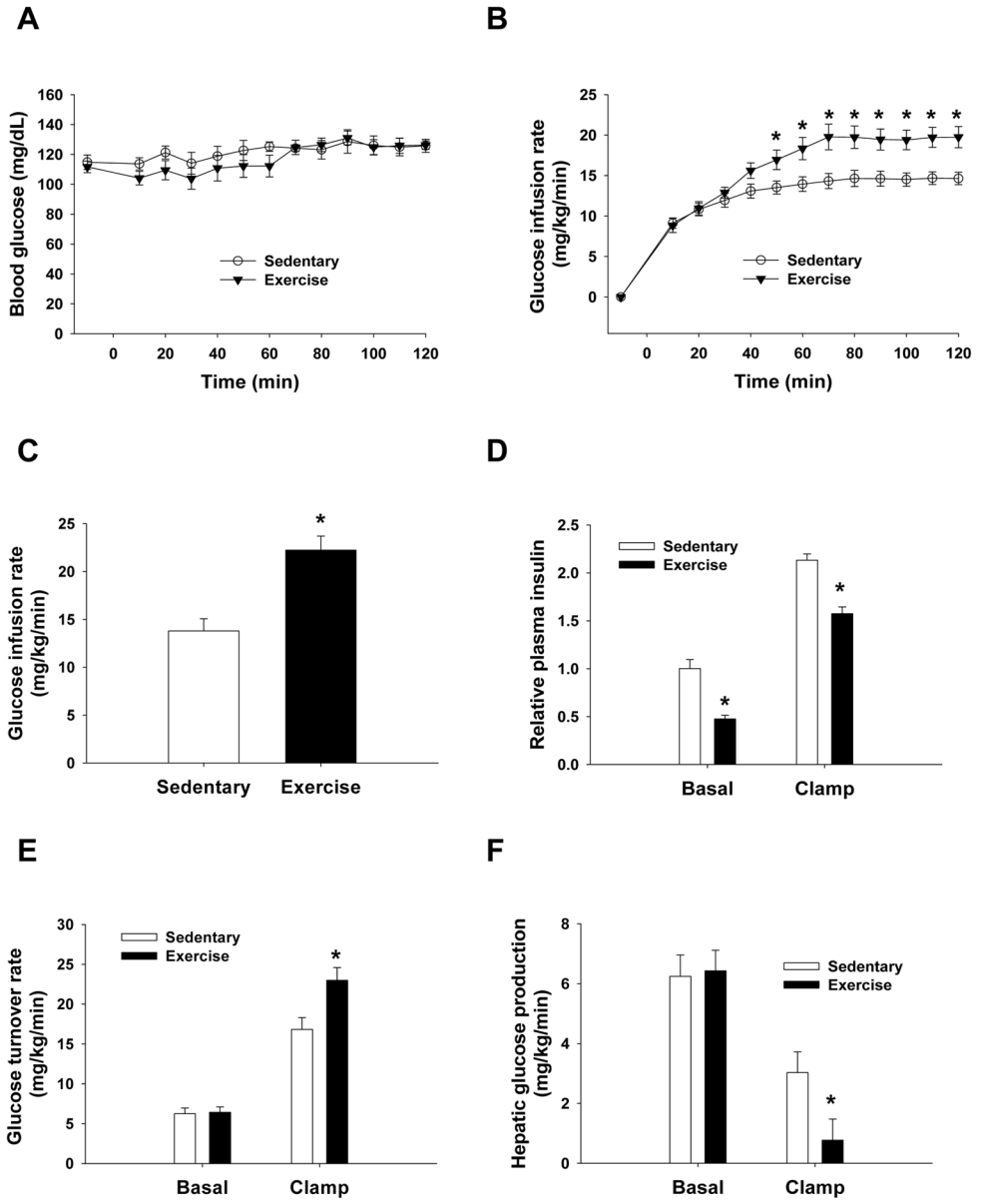

Figure 3. Adult female offspring from exercised dams had increased glucose infusion rate during hyperinsulinemic – euglycemic clamp

Female offspring at 17 months of age underwent the hyperinsulinemic – euglycemic clamp procedure to assess whole body insulin sensitivity. Following a 16 hour fast, insulin was infused at a constant rate of 4.0 mU/kg/min for 120 minutes. Glucose was infused simultaneously at varying rates in order to maintain a 120 – 130 mg/dl blood glucose level in the animal. (A) There were no differences in blood glucose levels during the procedure between the two groups. (B) Glucose infusion rate (GIR) needed to maintain target body blood glucose levels was significantly increased in offspring from exercised dams compared to those from sedentary dams. (C) Steady – state GIR (average of the 80 – 120 min GIR) was significantly increased in offspring from exercised dams compared sedentary dams. (D) Plasma insulin levels were significantly lower under basal and clamp conditions in offspring born to exercised dams compared to offspring from sedentary dams. (E) There were no differences in basal condition whole body glucose turnover rates. Under the clamp

condition, glucose turnover rate was significantly increased in offspring from exercised dams compared to those from sedentary dams. (F) There were no differences in basal condition hepatic glucose production (HGP). In response to insulin stimulation during the clamp HGP was significantly decreased in offspring from exercised dams compared to offspring from sedentary dams.* $P < 0.05$ compared to sedentary control; $n = 12$ for sedentary and $n = 9$ for exercise in A – F. Error bars indicate s.e.m.

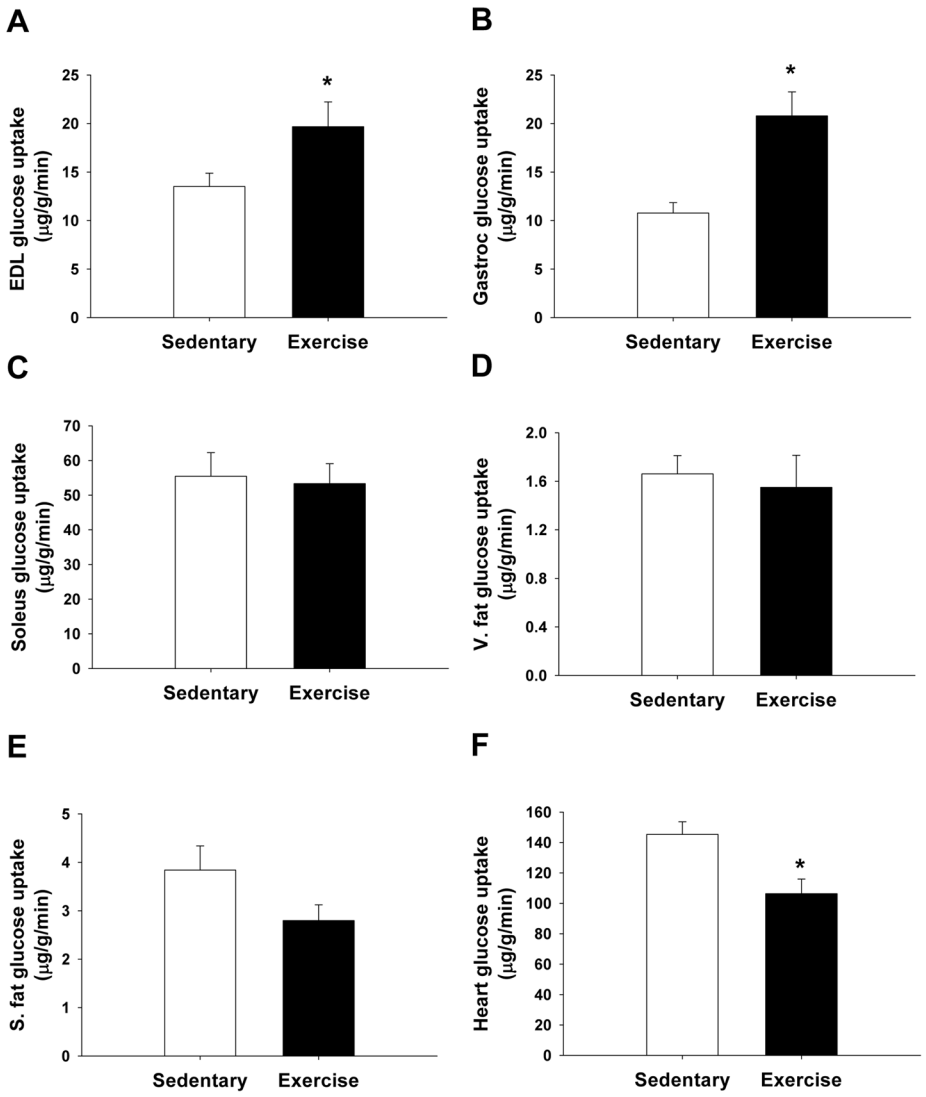

Figure 4. Offspring from exercised dams had increased skeletal muscle glucose uptake during hyperinsulinemic – euglycemic clamp

Following the clamp procedure, tissues were collected to evaluate insulin stimulated 2 – deoxyglucose uptake. Offspring from exercised dams had significantly increased glucose uptake in (A) extensor digitorum longus (EDL) and (B) gastrocnemious (gastroc) muscles compared to offspring from sedentary dams. There were no differences in (C) soleus muscle, (D) visceral (V.) adipose, or (E) subcutaneous (S.) adipose glucose uptake. Offspring from exercised dams had significantly decreased (F) heart glucose uptake compared to those from sedentary dams. * $P < 0.05$ compared to sedentary control; $n = 12$ for sedentary and $n = 9$ for exercise in A – F. Error bars indicate s.e.m.

Table 1

Maternal outcomes on gestation day 18

Parameter	Sedentary (s.e.m.)	Exercise (s.e.m.)	P value
Heart/body weight (x1000)[a]	3.769 (0.092)	3.777 (0.123)	0.952
Soleus muscle/body weight (x1000)[a]	0.407 (0.031)	0.395 (0.063)	0.551
Parametrial fat/body weight (x1000)[a]	8.219 (0.723)	5.288 (0.798)	0.017
Retroperitoneal fat/body weight (x1000)[a]	7.417 (0.645)	4.039 (0.311)	< 0.001
Serum glucose (nmol/μl)[b]	4.347 (0.356)	4.571 (0.359)	0.672
Serum insulin (ng/ml)[b]	0.866 (0.136)	0.385 (0.035)	< 0.001

[a] sedentary n = 17 and exercise n = 9;

[b] sedentary n = 13 and exercise n = 9.