Short-term and long-term effects of submaximal maternal exercise on offspring glucose homeostasis and pancreatic function

Running title: Maternal exercise and offspring metabolism

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Author's contributions

C.Q. contributed to experimental design, did the experiments, collected data, contributed to the discussion and wrote the manuscript. F.S. contributed to experimental design and researched data. H.D. contributed to experimental design, discussion and reviewed the manuscript. G.V. contributed to researched data, discussion and reviewed the manuscript. E.F. contributed to the discussion and reviewed the manuscript. P.B. contributed to the discussion. C.B. and K.C. co-supervised the entire study, contributed to experimental design, researched data, contributed to the discussion and reviewed the manuscript. C.Q. is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Abstract

Only few studies explored the effects of maternal exercise during gestation on adult offspring metabolism. We set out to test whether maternal controlled submaximal exercise maintained throughout all gestational periods induces persistent metabolic changes in the offspring. We used a model of 15 week-old nulliparous female Wistar rats who exercised (Trained group) before and during gestation at a submaximal intensity or remained sedentary (Control group). At weaning, male offspring from Trained dams showed reduced basal glycemia (119.7±2.4 vs 130.5±4.1 mg.dl\(^{-1}\), \(P<0.05\)), pancreas relative weight (3.96±0.18 vs 4.54±0.14 g.kg body weight\(^{-1}\), \(P<0.05\)) and islet mean area (22822±4036 vs 44669±6761 µm\(^2\), \(P<0.05\)) compared with pups from Control dams. Additionally, they had better insulin secretory capacity when stimulated by glucose 2.8 mM + arginine 20 mM, compared with offspring from Control dams (+96%, \(P<0.05\)). At 7 months of age, offspring from Trained mothers displayed altered glucose tolerance (AUC = 15285±527 vs 11898±988 mg.dl\(^{-1}\)*120 min, \(P<0.05\)), decreased muscle insulin sensitivity estimated by the phosphorylated-PKB/total PKB ratio (-32%, \(P<0.05\)) and tended to have a reduced islet insulin secretory capacity compared with rats from Control dams. These results suggest that submaximal maternal exercise modifies short-term male offspring pancreatic function and appears to have rather negative long-term consequences on sedentary adult offspring glucose handling.
Based on epidemiological data, Barker et al. initiated the concept of the "Developmental Origins of Health and Diseases" suggesting that the in utero environment and the first stages of life environment may play a role in the occurrence of diseases in adulthood (4, 36, 49). Many epidemiological and experimental studies relate early nutritional environment to metabolic disorders such as diabetes, insulin resistance, obesity as well as hypertension or cardiovascular diseases (3-5, 33, 45, 46, 48, 55, 56, 66, 73, 75, 82). Among them, type 2 diabetes mellitus (T2DM) is growing in modern societies at an alarming rate (54). Environmental (i.e. food intake, obesity, physical activity level among others) and genetic factors are thought to play a role in the susceptibility to this disease (68).

Some studies have also established a link between increased glucose intolerance or T2DM prevalence in adulthood and a maternal nutrient intake during gestation (44, 49, 59, 60, 69, 73) or offspring low (3, 10, 35, 48, 81) or high (3, 8, 48, 81) birth weight. This suggests that an inadequate maternal nutritional environment during fetal life may influence fetal development.

To enhance the chances for a successful parturience and to improve both their health and that of their developing child, women are prone to adopt a healthy lifestyle when pregnant (29, 32). Many of them may be willing to eat a healthier diet and stay physically active during that period (32).

Regular moderate exercise has been shown to decrease susceptibility to T2DM in pregnant women (26, 27, 50, 63), by enhancing insulin sensitivity and fostering non-insulin-stimulated glucose uptake (61, 78). The effects of gestational exercise on the mother are now well known, including improved aerobic capacity and cardiovascular fitness or reduced gestational diabetes incidence (2, 24, 27, 53, 62, 63). However, potential outcomes on offspring are still to be studied. Recent studies show that voluntary exercise during pregnancy and nursing can improve glucose tolerance and insulin sensitivity in adult offspring (13, 14). It has been proven that a controlled mild pace exercise during pregnancy is safe in mouse and human (18, 57, 67, 70, 79). However, effects of a controlled submaximal but yet intense exercise during gestation on offspring health are contradictory (41, 70, 71). Literature reports various effects of maternal exercise on offspring birth weight and body composition (20-23, 37, 38). However, most of them show decreased body and fat mass in offspring from exercised dams (20, 21, 37). Currently,
one hypothesis to explain the differences observed in birth weight would be changes in placental growth and in glucose and oxygen delivery towards the fetus during maternal exercise (17). Even if the reduction in placental blood flow is blunted by gestational exercise training with increased oxygen and substrate delivery during resting conditions, placental oxygen and nutrient delivery remained lower during moderate to high intensity exercise sessions (16). However, the consequences of these changes in uterine blood flow during exercise on fetus wellbeing are conflicting (64, 70).

Taken together, these data suggest that the antenatal period, with many factors including the mother's physical activity level that can influence the intrauterine development and offspring birth weight, could be of major importance in the development of chronic diseases later in life (11, 25, 31, 40, 65, 72).

Considering the known effects of voluntary maternal exercise on glucose tolerance and insulin sensitivity in adult rodent offspring (13, 14), we have developed a model of pregnant rats subjected to compulsory exercise to study - with a functional approach and physiological tools - the impact of this constraint on glucose handling in pups. We postulate that metabolic improvements (13, 14) can result from structural and metabolic changes of the pancreas in the offspring from exercised dams. The literature shows that the effects of exercise during pregnancy on offspring depend on the intensity and the duration of this exercise (19, 47, 79). We wanted to test whether the metabolic adaptations such as improved glucose tolerance and greater insulin sensitivity were also present if the exercise was compulsory, submaximal but yet intense (55% of Maximal Aerobic Speed (MAS)) and maintained until almost the end of pregnancy, rather than voluntary.
Research Design and Methods

Animals

Nulliparous 15 wk-old female Wistar rats (Charles River Laboratories, Saint Germain-Nuelles, France) were housed 3 per cage with access to food (A03, SAFE Diets, Augy, France) and water ad libitum. Animal facility was on a 12-h light/dark cycle and maintained at a temperature of 22°C±2°C. All experimental procedures were carried out in accordance with European Directive 2010/63/UE. They were reviewed by the Institutional Ethics Committee for Animal Care and Use and authorized by the French Ministry of Research (00174.01 accepted in March 2014). After a 1-week acclimatization period, female rats were assigned to either a sedentary (Control, n=9) or trained (Trained, n=12) groups. Body weight and food consumption were monitored once a week during breeding and pregnancy. Trained females were exercised using a motorized treadmill (Bioseb, Vitrolles, France) 5 days per week during the 4 weeks before gestation and during the first 18 days of gestation while female rats from Control group were kept into their cage. The treadmill speed and the duration of the training session were gradually increased during the first three weeks of training to reach a speed of 25 m.min⁻¹ for 60 min. After 4 weeks of controlled exercise, females from each group were housed with male rats during 1 week for mating. The male rats did not exercise during the study. Vaginal smears were performed each day until spermatozoa were found in order to determine the first day of gestation. On postnatal day 2, litter sizes were equalized to 8 pups. Pups were cross-fostered from other litters from the same group and the same age to maximize the number of males per litter. Mothers from the Trained group did not exercise during nursing. Pups were weighted on postnatal days 7, 14, and 21. Only male pups were used in the study. Some pups were tested at weaning (3 to 4 weeks of age) and others were housed two per cage up to 7 months of age without any controlled physical activity and fed with food (A04 rodent diet, SAFE Diets, Augy, France) and water ad libitum. Mothers were euthanized after nursing and offspring at weaning and at 7 months of age. Selected fat depots, skeletal muscles and organs were dissected and weighed in order to estimate the changes in body composition and/or collected and stored at -80°C for other measurements.
Intraperitoneal Glucose Tolerance Test

Intraperitoneal Glucose Tolerance Tests (ipGTT) were performed on pups at 3 weeks and 7 months of age after a 16-hour overnight fast. Only a few pups (n = 8 to 14) from each group were tested at 3 weeks of age. Glucose was intraperitoneally injected at 1 g.kg\(^{-1}\) body weight. Blood glucose readings were taken via tail pick before (T = 0 min) and 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90, 120 min after glucose injection using an Accu-Chek glucometer (Roche Diabetes Care ®, Meylan, France). Areas under the curve were related to T = 0 min.

Intraperitoneal Insulin Tolerance Test

Intraperitoneal Insulin Tolerance Tests (ipITT) were performed on pups from each group used for ipGTT at 4 weeks and 7 months of age after a 6-hour fast. Insulin was intraperitoneally injected at 1 mIU.g\(^{-1}\) body weight. Blood glucose readings were taken via tail pick before (T = 0 min) and 10, 20, 30, 40, 50, 60, 90, 120 min after insulin injection as above. Blood samples were collected before insulin injection to determine fasting plasma insulin by radioimmunoassay (RIA) kit (Merck Millipore Corporation, Germany). Areas over the curve were related to T = 0 min.

Insulin load test and analysis of insulin signaling in skeletal muscle and liver

After a 6-hour fast, 3-wk (others than those used for tolerance tests) and 7-month old rats were intraperitoneally injected with NaCl (0.9%) (Control C- and Trained T-) or with insulin (10 mIU.g\(^{-1}\) body weight) (Control C+ and Trained T+). Rats were killed 15 min after injection, and gastrocnemius muscle and liver were rapidly removed and frozen until their use to determine Protein Kinase B (PKB) phosphorylation level by Western blotting as an indicator of insulin sensitivity (76). Tissues were homogenized in PBS buffer containing 1% NP-40, 0.5 % sodium deoxycholate, 0.1% SDS supplemented with 5 mM EDTA, 1 mM Na\(_3\)VO\(_4\), 20 mM NaF, 1 mM DTT and protease inhibitor cocktail (Sigma P2714). Proteins were subjected to SDS-10% PAGE. Separated proteins were transferred onto polyvinylidene difluoride (PVDF) membrane and overnight incubated with primary antibodies (total
Akt/PKB, Cell Signaling, #9272 and phospho-Akt/PKB Ser 473, Cell Signaling, #9271). Primary antibodies were detected with a horseradish peroxidase-conjugated secondary antibody (Biorad, #172-10-19) and revealed with enhanced chemiluminescence system (Pierce).

**Pancreas insulin content**

Pancreas were dissected just after euthanasia and stored at -80°C. They were then homogenized at 4°C in 6 mL of acidified ethanol (0.18 M HCl in 70% ethanol) and placed overnight on a rotative wheel (20 rpm, 4°C). The day after, homogenates were sonicated 2 times during 10 sec each time and then placed during 2 days on a rotative wheel (20 rpm, 4°C). Homogenates were then centrifuged (150g, 5 min, 4°C). Supernatants were collected and centrifuged (3200g, 20 min, 4°C). The resulting supernatants were collected and stored at -80°C until insulin content assay by RIA kit (Merck Millipore Corporation, Germany).

**Pancreas histology**

In a subset of animals from each group, pancreas were dissected just after euthanasia, fixed in Tissue-Tek® and stored at -80°C. Cross-sections were done using a cryostat on glass slides. Sections were randomly selected and colored using hematoxylin-eosin staining. Islets were outlined and their areas were expressed in $\mu m^2$ using Image J® software.

**Islets isolation and glucose stimulated insulin secretion test (GSIS)**

Pancreatic islets isolation was performed on the rats used for ipGTT and ipITT by collagenase digestion. Rats were anesthetized by an i.p. injection of sodium pentobarbital (5 mg/100 g body weight). The abdomen was opened and the pancreas exposed as much as possible. The pancreatic duct was clamped with an hemostat at its duodenal insertion with care to avoid pancreatic tissue injuries. The bile duct at the proximal end was isolated and cut with fine scissors at one third of the way across. A 26G catheter was inserted and fixed in the bile duct. A collagenase digestion solution (10 ml of Collagenase from *Clostridium histolyticum* type XI at 1 mg/mL, Sigma-Aldrich, Saint-Quentin Fallavier, France) in Hanks'
Balanced Salt Solution (HBSS) were infused slowly. The pancreas was then carefully removed and placed in 7.5 mL of HBSS at 4 °C and was incubated in a water bath pre-set at 37 °C for 11 min. After incubation, the tubes were vigorously hand-shaked for 15 sec before addition of 25 mL of a "wash solution" (HBSS, 5% Fetal Bovine Serum). The tubes were centrifuged (250g, 2 min, 4°C) and supernatants were discarded. The pellets were filtered through a wire mesh. 25 mL of wash solution were then added, the tube was centrifuged (250g, 2 min, 4°C) and the supernatant poured-off. The washing procedure was repeated 2 more times. After the last centrifugation, 5 mL of the supernatant were kept with the pellet. A density gradient was then prepared by pouring 10 mL of Histopaque® 1.119 (Sigma-Aldrich, Saint-Quentin Fallavier, France) in a 50 mL tube that was overlayed with 10 mL of Histopaque® 1.077. Islets were then resuspended with 7.5 mL Histopaque® 1.119 + 3.5 mL Histopaque® 1.077 and the suspension was layered on the Histopaque® 1.077 phase followed by 10 mL of HBSS on top. The gradient was centrifuged (1750g, 20 min, 20°C) with slow acceleration and no braking. The islets were then collected from each of the interfaces. The islets were washed 3 times in 25 mL of wash solution (1 time at 350g, 5 min, 4°C and 2 times at 250 g, 2 min, 4°C). Islets were then cultured in RPMI, 10% FBS, 1% sodium pyruvate, 1% antibiotic/antimycotic solution overnight at 37°C. The day after isolation, the islets were incubated with either glucose or glucose + arginine to determine their insulin secretory capacity. After a 1-hour preincubation in 2.8 mM low glucose medium at 37°C, islets were then incubated for 1 hour in 2.8 mM low glucose medium at 37°C and supernatants were collected. Islet insulin secretion was then stimulated by either incubation with 16.7 mM high glucose medium, for 3 wk-old and 7 month-old rats, or with 2.8 mM low glucose + 20 mM arginine medium, for 3 wk-old rats only (1), for 1 h at 37°C and supernatants were collected. Residual total insulin was extracted in 0.18M HCl, 70% ethanol. Samples were stored at -80°C until insulin assay by ELISA (ALPCO, USA) (3 weeks of age) or RIA (Merck Millipore Corporation, Germany) (7 months of age) kits.

**Statistical analysis**
Data are expressed as mean±SEM. Data were analyzed using one-way or two-way ANOVA with Holm-Sidak post hoc test. Kruskall-Wallis tests were applied when values were not normally distributed (Sigma Plot ®). $P<0.05$ was considered significant and $P<0.2$ was considered as tendency.
Results

Exercise training before and during gestation reduces fat depots in the mothers without any alteration in food intake or other organs

Body weight of female Wistar rats was similar in the two groups at the beginning of the study and after the first 4 weeks of training (Table 1). After nursing, body weight of Trained mothers tended to be lower than that of Control mothers (286±4 vs. 297±7 g for Trained and Control groups, respectively, $P=0.136$) (Table 1). Food intake was similar between both groups from the beginning of the protocol to the end of lactating period (Table 1). Exercise training did not affect the weight of organs such as liver, kidney, pancreas or muscles (Table 1). However, relative mass of the sum of retroperitoneal, urogenital and mesenteric fats depots was significantly lower in Trained mothers compared with Control mothers (39.5±2.7 vs. 54.3±6.0 g.kg$^{-1}$ body weight for Trained and Control groups, respectively, $P<0.05$) (Table 1).

Exercise training before and during gestation in mothers has no effect on the litter outcomes

The number of pups per litter as well as the sex ratio were similar in Trained and Control groups (Table 2). Offspring body weights were not affected by mother exercise intervention at any time (Table 2).

Maternal exercise affects offspring pancreas weight and islets size at 3 weeks of age

At weaning, body weight and organ relative weight were not different in offspring from Control or Trained mothers except for the pancreas (Table 3). Indeed, pancreas relative weight was found to be lower in pups from Trained mothers compared with that of pups from Control mothers (3.96±0.18 vs. 4.53±0.14 g.kg$^{-1}$ body weight for Trained and Control groups, respectively, $P<0.05$) (Table 3). Moreover, mean islet area measured in 3 wk-old pups from Trained dams is significantly smaller compared with that measured in pups from Control dams (Table 3). At 7 months of age, no differences were found in body and organ relative weights, including pancreas (Table 3). However, we can notice that there is a tendency
to a higher relative fat pad mass ($P=0.153$) in rats from Trained mothers compared to those from Control mothers (Table 3).

**Effect of maternal exercise on offspring blood glucose and insulin levels**

After a 16h-fast, 21 day-old pups showed a similar glycemia in both groups (104.6±4.6 vs. 108.0±2.7 mg.dL$^{-1}$ for Trained and Control groups, respectively) (Figure 1A).

After a 6h-fast, 28 day-old pups from Trained mothers showed a lower glycemia compared with pups from Control mothers (119.7±2.4 vs. 130.5±4.1 mg.dL$^{-1}$, respectively, $P<0.05$) (Figure 1B) while insulinemia was similar in both groups even if it tended to be higher in rats from trained mothers (0.31±0.04 vs. 0.21±0.05 ng.mL$^{-1}$ for Trained and Control groups, respectively, $P=0.158$) (Figure 1C).

In 7 month-old rats, neither blood glucose nor insulin levels were different, whatever the duration of the fasting (Figure 1D, 1E and 1F).

**Effect of maternal exercise on offspring glucose tolerance**

At 21 days of age, there was no difference in blood glucose levels between the two groups at any point of the ipGTT (Figure 2A) and area under the curve (AUC), as a measure of the overall glucose disposal, was not significantly different between both groups (Figure 2B).

However, in 7 month-old rats, blood glucose levels were higher in animals from Trained group compared to those from Control group during the first 15 min of the ipGTT (Figure 2E). Moreover, the AUC is significantly larger in the Trained group compared to the Control group (15258±924 vs. 11614±1366 mg.dL$^{-1}$ *120 min) for Trained and Control groups, respectively, $P<0.05$) (Figure 2F).

**Effect of maternal exercise on offspring insulin sensitivity**

At 28 days of age, offspring from Trained dams displayed lower blood glucose levels during the ipITT compared to the pups from Control group (Figure 2C), due to a significantly lower starting glycemia (as already shown in Figure 1B). However, the overall insulin sensitivity estimated by the area over the curve...
(AOC) appeared to be similar in both groups (5318±513 vs. 3507±178 mg.dL\(^{-1}\) *120 min for Trained and Control groups, respectively) (Figure 2D).

At 7 months of age, blood glucose levels were not different at any point of the ipITT between animals from the Trained group compared to those from the Control group (Figure 2G). This is confirmed by the AOC which was similar between both groups (Figure 2H).

**Effect of maternal exercise on offspring insulin signalling in liver and skeletal muscle**

Insulin signalling was studied by measuring the level of expression of PKB and its phosphorylated form (pPKB) both in liver and gastrocnemius muscle after either a NaCl or an insulin load. Figure 3 shows the pPKB/PKB ratios obtained.

At 21 days of age, pups from Trained group showed an increase in the pPKB/PKB ratio compared with pups from Control group in basal condition (respectively T- and C-) both in liver (+77\%, \(P<0.05\)) (Figure 3A) and muscle (+97\%, \(P<0.05\)) (Figure 3C). However, these differences were not seen after an insulin load injection (T+ and C+) neither in liver (Figure 3A) nor in gastrocnemius muscle (Figure 3C).

At 7 months of age, pPKB/PKB ratio was similar in the liver of animals from the two groups whatever the condition (NaCl load or insulin load) (Figure 3E). In muscle, under insulin load, pPKB/PKB ratio was significantly lower in rats from the Trained group (T+) when compared with rats from the Control group (C+) (- 32\%, \(P<0.05\)) (Figure 3G) while it remained similar between groups in basal condition (T- vs C-) (Figure 3G).

**Effect of maternal exercise on offspring pancreas insulin content and islet insulin secretion**

The total insulin content in pancreas was not different between Trained and Control groups whether at 3 weeks of age (Figure 4A) or at 7 months of age (Figure 4C).

At 28 days of age, the islet insulin secretion was not significantly different in pups from Trained dams compared to those from Control dams, both after low glucose (2.8 mM) or high glucose (16.7 mM)
incubation (Figure 4B). However, islet insulin secretion after low glucose (2.8 mM) + arginine (20 mM) incubation was significantly higher in pups from Trained dams compared with those from Control dams (+ 96%, P=0.01) (Figure 4B).

At 7 months of age, islet insulin secretion was higher after high glucose (16.7 mM) incubation compared to the low glucose incubation condition (2.8 mM) (P<0.05) (Figure 4D). However, the islet insulin secretion tended to be lower in rats from Trained group compared with rats from Control group, whether in low glucose (2.8 mM) or in high glucose (16.7 mM) condition (P=0.102 and P=0.255, respectively) (Figure 4D).
Discussion

For the first time, to our knowledge, we show that submaximal maternal exercise alters the offspring pancreatic function. Especially, we observed: i) a decline in insulin secretion with age in animals from the exercised mothers along with ii) a glucose intolerance and iii) a skeletal muscle insulin resistance, that are not present in offspring from sedentary dams.

Our purpose was to test the effect of daily chronic exercise during the gestation on fit females that were already active before gestation. We chose to train them during four weeks before mating as such a duration is usually sufficient to get the first signs of adaptation to chronic endurance exercise (77). Studies using voluntary exercise in rodents with running wheels showed that the running distance dramatically decreased in the last week of gestation (13, 28). So we chose a compulsory treadmill exercise that allowed us to test the effects of exercise throughout all the gestational periods, with no change in training parameters and especially no reduction in exercise intensity and duration until day 18 of the gestation. To ensure that these adaptations were significant, we also chose a sufficiently high intensity. We used intensity parameters that are classically found in the literature (6, 43, 84). Based on data collected in our laboratory, the speed and slope of the treadmill that we used would correspond to an intensity of about 55% of the maximal aerobic speed (MAS). That intensity matches the guidelines for exercise in pregnant women from a lot of countries around the world (30). However, the frequency and the duration are above most of the guidelines except those from Denmark and the United States that recommend at least 30 minutes of moderate intensity exercise most of the days or daily (30).

Moreover, some authors report that voluntary and controlled low-intensity exercise during gestation has no negative consequences on dams and litter (14, 57). Our training protocol appears to be safe for the mother and the litter since no differences were found in sex ratio, number and birth weight of pups. Maternal exercise had no effect on maternal body weight but seemed to change body composition since the weight of the sum of several selected fat pads was lower in Trained mothers. Such reduced relative fat mass after endurance training has been previously described in male and female rats (34, 83). It may be
explained by a higher energy expenditure in Trained mothers while food consumption is the same as for Controls. In the offspring, the maternal training had no consequence on body weight or body composition as suggested by the muscle mass and the fat mass measured. This surprising result could be explained by the duration of exercise sessions (1 hour per day) which is shorter than voluntary exercise using running wheels and the running distance (1.5 km per day in our study vs. 1.5 to 8 km per day at early gestationnal age to about 1 km per day at late gestationnal age on running wheels) (12, 14, 28, 58). Reduced caloric intake in Trained mothers appears to have no negative consequences on offspring growth as judged by a similar body weight in both groups. This could be due to the fact that maternal exercise training is thought to increase resting placental bed blood flow (16) and then nutrient delivery at the maternal-placental interface. During pregnancy, post-prandial maternal blood glucose levels would vary in accordance with the type of carbohydrates ingested. In our study, diet was mainly composed of low-glycemic carbohydrates which are associated with no change in blood glucose levels throughout pregnancy and with normal-size offspring contrary to high-glycemic carbohydrates ingestion (16). However, the training program was associated with a lower relative pancreas weight in weanling rats that was not associated with changes in pancreas total insulin content. Moreover, histologic cuts revealed that islets area is reduced in pups from Trained mothers. We then looked at the insulin secretory capacity of these islets in various conditions. In low glucose condition, basal islet insulin secretion was not affected by maternal exercise at weaning. Under high glucose condition, the stimulated insulin secretion only tended to be higher in weanling rats from the Trained mothers. This may be due to the fact that islet insulin release in response to glucose stimulation is reduced in young rats (7, 9, 42, 51). To alleviate this problem, we then measured the insulin secretion after a glucose + arginine incubation known to be efficient in fetal and young rats (1). In such a condition, we found that maternal exercise enhanced the islet insulin secretion, which is consistent with the litterature. Indeed, a recent study showed that small islets have a higher insulin secretion (39). These changes in islet insulin secretion could be responsible for the trend of higher insulinemia found in pups from trained mothers after a 6h-fast.
This tendency of elevated insulinemia without a hyperglycemia in pups from Trained mothers could be an early marker of insulin resistance. To precise such insulin resistance, we then performed glucose tolerance test (ipGTT) and insulin tolerance test (ipITT) on our animals. Our results show that glucose disposal, as judged by the AUC during ipGTT, and overall insulin sensitivity reflected by the AOC during ipITT were not affected by maternal training. Taken together, these results suggest that, despite a trend of elevated insulinemia, pups from Trained mothers were not insulin resistant. This tendency of higher blood insulin could be also a sign for alterations in the insulin signalling. The main insulin sensitive tissues include skeletal muscle, liver and adipose tissue (52). Serine/threonine protein kinase Akt/PKB (15) and the ratio between phosphorylated-PKB and total PKB content (pPKB/PKB) is classically considered as a good marker of insulin pathway activation (76, 80). Thus, we examined pPKB/PKB ratio in gastrocnemius muscle and in liver, both in basal conditions and after an insulin load. In basal conditions, the higher pPKB/PKB ratio measured in both tissues in 3-wk old rats from Trained mothers might be merely due to the trend in a higher plasma insulin level mentioned above. Results after the insulin load confirm that hypothesis since there is no difference in pPKB/PKB ratio between the two groups in that insulin stimulated condition. So, we can conclude that maternal exercise before and during gestation did not modify liver and muscle insulin pathway activation in offspring.

These effects of maternal exercise on the offspring are age-dependent since results on 7-month old rats are different from those obtained on 3-wk old rats. Indeed, at 7 months of age, organ weights were not different between both groups, suggesting that maternal exercise effects would have no significant impact on body composition including the pancreas. However, relative fat mass tend to be higher ($P=0.153$) and relative muscle mass to be lower ($P=0.183$) in offspring from Trained dams. Together these two results suggest that offspring of Trained rats tend to have more fat and may have a significantly higher percentage of body fat. In another set of similar experiments, food consumption was monitored during the first 10 weeks after weaning (i.e. 3 month-old) and we did not find any difference between rats from both groups (unpublished data). Unfortunately, we don't have any data for the 3-to-7 month period but we can not exclude that food intake becomes different meanwhile that could explain the tendency of increased fat...
mass in rats from Trained group at 7 months of age. It can also be a consequence of the impossibility to
perform their spontaneous activity, resulting in a positive energy balance. Indeed, a study showed that
offspring from females that had access to running wheels during gestation were more active in adulthood
(28). This would suggest that offspring lifelong propensity to exercise depends on maternal physical
activity level during gestation (28). In our study, offspring from active mothers could not exert their
potential higher spontaneous physical activity while fed ad libitum. Taken together, these conditions
would ultimately lead to an increase in adiposity. Moreover, there were no differences in 6-h and 16-h
fasting glycemia but insulinemia tended to be increased in rats from Trained dams after a 6h fast.
However, AUC during ipGTT was significally larger in 7-month old rats born from Trained mothers. This
suggests that submaximal maternal exercise would be associated with a worse glucose tolerance in adult
offspring. Here we show that maternal exercise may have various effects on prospective offspring glucose
tolerance, depending on the training session intensity and offspring sex and age. Indeed, our submaximal
compulsory exercise program led to a decrease in glucose tolerance in adult offspring whereas some
studies showed that low-intensity voluntary maternal exercise improved it (13, 14). These discrepancies
could be expained by several factors. Firstly, these studies were conducted on mice (13) or female
Sprague Dawley rats (14) aged up to 16 months. Moreover, the females had access to running wheels
before and throughout gestation and during lactation. In our study, we used 7 month-old Wistar rats.
Moreover, only male offspring were included and maternal treadmill exercise was compulsory and
conducted only before and during gestation with no change in exercise parameters. In animals from
Trained mothers, the lower glucose tolerance observed may be due to either exhaustion in islet insulin
secretion or to a higher insulin resistance (74). Even if pancreas total insulin content was similar between
both groups, the tendency to have a lower islet insulin secretion both in basal and in glucose stimulated
conditions may play a role in the glucose intolerance observed in 7-month old rats from Trained dams. It
is unlikely that liver could contribute to that glucose intolerance since the phosphorylation level of PKB
in liver are similar in both groups in basal and insulin stimulated conditions. In skeletal muscle, the lower
pKB/PKB ratio under insulin stimulation in the rats from Trained mothers suggests that long term insulin
signalling in muscle could be reduced by maternal exercise done before and during gestation. These results suggest that even if the muscle insulin pathway activation observed with maternal exercise is altered at 7 months of age, this has no consequences on the overall insulin response at the whole body level as shown by the lack of difference in AOC during ipITT.

The smaller relative pancreas weight and the smaller islets size could be related to a suboptimal nutritional state of both the mother and the fetus. Indeed, despite the increase of energy expenditure due to training sessions, Trained dams had the same food intake than the Control dams. This supports the idea that offspring from Trained dams may have encountered reduced substrate availability during maternal exercise. It has already been shown that during exercise sessions, there could be a transient fetal hypoglycemia (17). These two factors (reduced energy balance and hypoglycemia) could impact the islets insulin secretory capacity under stimulation that could contribute to the trend of increased insulinemia that we observed in pups from Trained dams.

Our study shows that submaximal maternal exercise leads to an inappropriate insulin secretion relative to ambient glucose in rodent offspring. Associated with the reduced muscle insulin pathway activation and glucose intolerance that we observed in 7-month old offspring, such maternal exercise could increase the susceptibility to T2DM that could be majored when exposed to bad nutrition such as high-fat diet or physical inactivity during adulthood. This suggests that daily exercise during the late period of the gestation would be detrimental to offspring but further studies are needed to determine if the intensity of such exercise program may play a role as well.
Acknowledgments

The authors thank Cindy Tellier (LBFA, INSERM U1055) for animal care and Anne-Sophie Gauchez (UMR-S INSERM U1039) for insulin assays.

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Duality of interest: None to be declared.
Table 1

Maternal body weight (BW), food intake (FI) and organ relative weight after nursing

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW before the study (g)</td>
<td>260±5</td>
<td>260±4</td>
</tr>
<tr>
<td>BW before gestation (g)</td>
<td>277±22</td>
<td>276±19</td>
</tr>
<tr>
<td>BW after nursing (g)</td>
<td>297±7</td>
<td>286±4</td>
</tr>
<tr>
<td>FI before gestation (g/day)</td>
<td>18.4±0.5</td>
<td>18.4±0.4</td>
</tr>
<tr>
<td>FI after gestation (g/day)</td>
<td>23.0±1.3</td>
<td>20.7±0.7</td>
</tr>
<tr>
<td>FI after nursing (g/day)</td>
<td>70.3±1.5</td>
<td>73.0±1.4</td>
</tr>
<tr>
<td>Pancreas (g.kg BW(^{-1}))</td>
<td>4.34±0.32</td>
<td>4.26±0.18</td>
</tr>
<tr>
<td>Liver (g.kg BW(^{-1}))</td>
<td>26.01±0.98</td>
<td>30.34±1.94</td>
</tr>
<tr>
<td>Kidney (g.kg BW(^{-1}))</td>
<td>2.89±0.13</td>
<td>3.17±0.08</td>
</tr>
<tr>
<td>Fat (g.kg BW(^{-1}))</td>
<td>54.30±5.97</td>
<td>39.45±2.65*</td>
</tr>
<tr>
<td>Muscles (g.kg BW(^{-1}))</td>
<td>5.90±0.24</td>
<td>6.02±0.11</td>
</tr>
</tbody>
</table>

Data are mean±SEM (n=9 for Control and n=12 for Trained). Fat mass was calculated as the sum of retroperitoneal, urogenital and mesenteric fats depots. Muscles mass was calculated as the sum of gastrocnemius, plantaris and soleus muscles.

* \(P<0.05\) vs. Control.
Table 2  

Litter outcomes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter size</td>
<td>12.1±2.0</td>
<td>10.8±3.8</td>
</tr>
<tr>
<td>Number of males</td>
<td>5.2±1.8</td>
<td>5.2±2.5</td>
</tr>
<tr>
<td>Number of females</td>
<td>6.9±2.0</td>
<td>5.7±2.6</td>
</tr>
<tr>
<td>D7 body weight (g)</td>
<td>16.1±1.0</td>
<td>16.9±0.6</td>
</tr>
<tr>
<td>D14 body weight (g)</td>
<td>32.5±1.3</td>
<td>32.9±0.9</td>
</tr>
<tr>
<td>D21 body weight (g)</td>
<td>50.9±1.7</td>
<td>50.1±1.0</td>
</tr>
</tbody>
</table>

Data are mean±SEM (n=9 for Control and n=12 for Trained).
### Table 3

**Offspring body outcomes**

<table>
<thead>
<tr>
<th></th>
<th>Weaning</th>
<th>7 months of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Trained</td>
</tr>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>51.61±3.43</td>
<td>47.49±1.16</td>
</tr>
<tr>
<td><strong>Organs weight (g.kg BW$^{-1}$)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>31.81±0.75</td>
<td>30.97±0.19</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.02±0.11</td>
<td>5.99±0.11</td>
</tr>
<tr>
<td>Fat</td>
<td>8.68±0.57</td>
<td>9.00±0.35</td>
</tr>
<tr>
<td>Muscles</td>
<td>4.58±0.13</td>
<td>4.54±0.07</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4.54±0.14</td>
<td>3.96±0.18*</td>
</tr>
<tr>
<td>Islet area (µm$^2$)</td>
<td>44669±6761</td>
<td>22822±4036*</td>
</tr>
</tbody>
</table>

Data are mean±SEM (n=16 for Control and n=17 for Trained at weaning; n=8 for Control and n=6 for Trained at 7 months of age; n=11 to 13 for islet area). Fat mass was calculated as the sum of retroperitoneal, epididymal and mesenteric fats depots. Muscles mass was the sum of gastrocnemius, plantaris and soleus muscles.

* $P<0.05$ vs. Control.
References


Figure 1. Maternal exercise reduces 6h-fasting glycemia and tends to increase 6h-fasting insulinemia in 28 day-old offspring

Rat offspring fasting glycemia was measured after a 16-h fast (A; D) and a 6-h fast (B; E) at 21 days of age (A), 28 days of age (B) and 7 months of age (D; E). Fasting insulinemia was determined after a 6-h fast (C; F) at 28 days of age (C) and 7 months of age (F). Data are mean±SEM; n=4 for Control and n=6 for Trained in C; n=8 to 14 in A, B, D, E and F. *P<0.05 vs. Control.

Figure 2. Maternal exercise alters 7 month-old offspring glucose tolerance

Rat offspring underwent an ipGTT at 3 weeks of age (A; B) and at 7 months of age (E; F) to assess whole body glucose tolerance. Following a 16h-fast, glucose was intraperitoneally injected at 1 g.kg⁻¹ body weight. Blood glucose levels were monitored before injection (0) and 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90 and 120 min after glucose injection (A; E). Areas under the curve of ipGTT (C; G) were related to the value at T=0 min. Rat offspring also underwent an ipITT at 4 weeks of age (C; D) and at 7 months of age (G; H) to assess whole body insulin sensitivity. Following a 6h-fast, insulin was intraperitoneally injected at 1 mIU.g⁻¹ body weight. Blood glucose levels were monitored before injection (0) and 10, 20, 30, 40, 50, 60, 90 and 120 min post insulin injection (C; G). Areas over the curve of ipITT (D; H) were related to the value at T=0 min. Data are mean±SEM; n =8 to 14. *P<0.05 vs. Control.

Figure 3. Muscle insulin sensitivity is reduced in 7 months-old trained group rats

Rat offspring underwent an insulin load test at 3 weeks (A; C) and 7 months (E; G) of age to assess liver and muscle insulin pathway activation as an indicator of insulin sensitivity.
Insulin bolus was intraperitoneally injected at 10 mIU.g\(^{-1}\) body weight to half of the rats in each group (C+ and T+) and the others were injected with NaCl (C- and T-). Liver (A; E) and gastrocnemius muscle (C; G) samples were collected 15 minutes after injection in order to determine pPKB/PKB ratio (Ser473) by Western blotting. Representative Western Blots show phosphorylated and total (pPKB and PKB, respectively) PKB content in liver and gastrocnemius muscle of 3-week old (B and D, respectively) and of 7 month-old (F and H, respectively) offspring from Control (C) and Trained mothers (T) after NaCl (C- and T-) or insulin (C+ and T+) injection. Quantitation of the signals was expressed in arbitrary units. Data are mean±SEM; n=8 for C-, C+, T- and T+ in (A; B); n=4 for C- and C+ and n=3 for T- and T+ in (C; D). * \(P<0.05\) vs. C-; # \(P<0.05\) vs. C+.

**Figure 4. Maternal exercise does not modify offspring pancreas insulin content but enhances short-term islets insulin secretory capacity and tends to reduce it with aging**

Offspring pancreas was collected at 3 weeks of age (A) and at 7 months of age (C) to determine total insulin content. There were no differences in pancreas insulin content between both groups at (A) 3 weeks of age and at (C) 7 months of age. Offspring pancreatic islets were isolated at 4 weeks of age (B) and at 7 months of age (D) in order to assess their insulin secretory capacity. The day after isolation, islets were incubated with low glucose (2.8 mM G) and then with high glucose (16.7 mM G) (B; D) or low glucose + arginine (2.8 mM G + 20 mM A) (B). Supernatants were collected and islet insulin secretion determined by ELISA (B) or RIA (D). Data are mean±SEM; n=5 to 9. * \(P<0.05\) vs. Control.
Figure 1

A. Weaning

B. Glycemia (mg.dL⁻¹)

C. Insulinemia (mg.mL⁻¹)

D. 7 months

E. Glycemia (mg.dL⁻¹)

F. Insulinemia (mg.mL⁻¹)
Figure 2

A. Weaning

E. 7 months

B.

F.

C.

G.

D.

H.
Figure 3

Panel A: Bar graph showing pPKB/PKB ratio at weaning for different treatment groups. C-, C+, T-, T+.

Panel B: Western blot images showing pPKB and PKB for different treatment groups at weaning.

Panel C: Bar graph showing pPKB/PKB ratio at 7 months for different treatment groups. C-, C+, T-, T+.

Panel D: Western blot images showing pPKB and PKB for different treatment groups at 7 months.

Panel E: Bar graph showing pPKB/PKB ratio at weaning for different treatment groups. C-, C+, T-, T+.

Panel F: Western blot images showing pPKB and PKB for different treatment groups at 7 months.

Panel G: Bar graph showing pPKB/PKB ratio at 7 months for different treatment groups. C-, C+, T-, T+.

Panel H: Western blot images showing pPKB and PKB for different treatment groups at 7 months.
Figure 4

A

Weaning

Insulin content (ng/mg fresh pancreas weight)

Control  
Trained

C

7 months

Insulin content (ng/mg fresh pancreas weight)

Control  
Trained

B

Control  
Trained

Insulin secretion/total insulin

2.8 mM G  
16.7 mM G  
2.8 mM G + 20 mM A

D

Control  
Trained

Insulin secretion/total insulin *100

2.8 mM G  
16.7 mM G