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ABSTRACT
The perinatal exposome can modify offspring metabolism and health later in life. Within this concept, maternal exercise during gestation has been reported modifying offspring glucose sensing and homeostasis, while the impact of such exercise during lactation is little-known. We thus aimed at evaluating short- and long-term effects of it on offspring pancreatic function, assuming a link with changes in breast milk composition. Fifteen-week-old primiparous female Wistar rats exercised during lactation at a constant submaximal intensity (TR) or remained sedentary (CT). Male offspring were studied at weaning and at 7 months of age for growth, pancreas weight, glycemia and insulin responses. Milk protein content was determined by the bicinchoninic acid assay (BCA colorimetric method), and lipid content and fatty acid composition by gas chromatography. Mature milk from TR rats contained significantly less saturated (-7%) and more monounsaturated (+18%) and polyunsaturated (PUFA +12%) fatty acids compared to CT rats, with no difference in total lipid and protein concentrations. In offspring from TR vs CT mothers, fasting glycemia was lower, pancreas weight was higher with a lower insulin content (-37%) at weaning. Such outcomes were correlated with milk PUFA levels and indices of desaturase or elongase activities. These effects were no longer present at 7 months, whereas a more efficient muscle insulin sensitivity was observed. Maternal training during lactation led to a specific milk phenotype that was associated with a short-term impact on glucose homeostasis and pancreatic function of the male offspring.

Key words: exercise, lactation, breast milk, offspring, glycemia, DOHaD

Abbreviations:
Akt: Akt kinase, ALA: alpha-linolenic acid, AOC: area over the curve, ARA: arachidonic acid, AUC: area under the curve, CT: control, D5D: delta5-desaturase, D6D: delta6-desaturase, DGLA: dihomo gamma linolenic acid, DHA: docosahexaenoic acid, DNL: de novo lipogenesis, DOHaD: developmental origins of health and diseases, DPA: docosapentaenoic acid, DTA: docosatetraenoic acid, EFA: essential fatty acid, ELOVL: elongase, EPA: eicosapentaenoic acid, FAME: fatty acid methyl esters, FBS: fetal bovine serum, GSIS: glucose stimulated insulin secretion test, HBSS: Hank's balanced salt solution, ipITT: intraperitoneal insulin tolerance test, LA: linoleic acid, MCFA: medium-chain fatty acid, MUFA: monounsaturated fatty acid, OGTT: oral glucose tolerance test, PKB: protein kinase B, PUFA: polyunsaturated fatty acid, RIA: radio-immuno assay, SCD-1: stearoyl-CoA-desaturase 1, SFA: saturated fatty acid, TR: trained, VLDL: very low density lipoprotein

1. Introduction

Early life exposome concept deals with the impact of societal and individual environmental factors on the programming of offspring development and its future health. Among individual factors of concern, maternal nutrition is the one the most studied, firstly by Barker and colleagues showing an association between undernutrition, as well as overnutrition, in women during pregnancy and higher risk of cardiovascular and metabolic diseases, such as diabetes type 2, in adult offspring, raising the concept of Developmental Origins of Health and Diseases (DOHaD) [1, 2]. This concept could partly explain the increase of diabetes type 2 prevalence in adult estimated to be about 5.4% worldwide in 2025 comparatively to 4% in 1995 [3]. Another much less studied but equally important factor is the degree of maternal sedentarity. So far, few studies were conducted in rats to better understand the impact of physical exercise practice during gestation or all over the perinatal period on offspring glucose homeostasis on either a short or a long-term basis [4-7]. It has thus been shown that maternal voluntary perinatal exercise enhanced insulin sensitivity and improved glucose homeostasis in rat offspring once adult [4]. In our previous studies, submaximal maternal endurance training before and during gestation showed beneficial effects on muscle insulin sensitivity in rat offspring at weaning, and on limiting fat mass gain in offspring when exposed after weaning to a high-fat/high-sucrose diet [5], but harmful effects on glucose-insulin metabolism at adulthood when exposed to a standard diet and lifelong [6]. In addition, a study reported that low-intensity maternal exercise during gestation and lactation has beneficial effects on glucose-insulin metabolism in the adult rat offspring exposed to early postnatal overnutrition (set up by reducing litter size to 3 pups/dam), possibly via higher insulin levels in the milk of trained dams [7]. The fact that maternal exercise during lactation led to milk composition changes was reported by few other studies such as a decrease in lactose concentration [8] or an increase in total protein and lipid concentrations [9], suggesting plausible involvement of milk components in exercise-related impact.

But, while women are usually encouraged to exercise after childbirth to recover and to lose weight [10,11], the specific role of exercise during lactation on offspring body composition, glucose metabolism and pancreatic function, especially over a long period of life, is not yet characterized as published studies investigated the physical exercise taking place during gestation or over the entire perinatal period.

We hypothesized that endurance exercise performed during lactation would modify breast milk composition which could then alter the metabolism of the offspring fed with this milk on a short-term period but also during aging. More specifically, our purpose was to test whether compulsory exercise training at a submaximal constant intensity during the whole lactation period could enhance growth, glucose sensing and homeostasis, and pancreatic functioning.

2. Materials and methods

2.1. Animals

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 (Cometh 12) and authorized by the French Ministry of Research (#01922.02). Animal facility was on a 12h:12h light/dark cycle and thermoneutral (22°C±2°C).

Nulliparous 15 wk-old female Wistar rats (Charles River Laboratories, Saint Germain-Nuelles, France) were housed 3 per cage with access to CHOW diet (A03) specific for growing-gestation-lactating stages and nursing animals (21.4% protein, 5.1% lipid, 52% carbohydrates; Essential fatty acid (EFA) ratio i.e., linoleic acid/alpha-linolenic acid (LA/ALA): 8.9/1, SAFE Diets, Augy, France) and water *ad libitum*. After a 1-week acclimatization period, female rats were assigned to either sedentary (Control CT) or trained (Trained TR) groups. Females from

each group were housed with male rats during 1 week for mating. The male rats did not exercise during the study. Female body weight and food consumption were monitored once a week during breeding, pregnancy and lactation. On postnatal day 2, litter sizes of the primiparous female rats were equalized to 8 pups/dam. Pups' Sex was determined within the 1 week of age period through visual examination by experienced technicians from our animal facilityPups were cross-fostered with other litters from the same group and the same age to maximize the number of males per litter. Then, considering the issue of sexual dimorphism, we only used male offspring in this study. TR females were exercised using a motorized treadmill (Bioseb, Vitrolles, France) following a protocol used in previous studies [5,6]. They were placed on the treadmill the 3 days before mating to become familiar with it, and then were trained every day from day 2 after delivery until the end of lactation. During the familiarization, animals were encouraged to run by hits on the cover of the treadmill, concurrent with mild electrical shocks (0.1 mA max). After the period, electrical shocks became un-necessary as hitting noise was sufficient. The treadmill speed and the duration of the training session were gradually increased during the first week of training to reach a speed of 25 $m.min^{-1}$ for 60 min. CT females remained into their cage during the entire study. Mothers were euthanized after nursing and offspring were weaned at 3 weeks. Some pups were euthanized at 3, 4 or 6 weeks of age depending on the experiments (see below) but were all referred as weaned rats. The other pups were housed two per cage up to 7 months of age without any controlled physical activity and fed a CHOW diet (A03) diet for growing-nursing rodents (until 2 months of age), then a CHOW diet (A04) designed for adult and maintenance rodents (16.1% protein, 3.1% lipid, 60.4% carbohydrates; LA/ALA (18:2n-6/18:3n-3): 12.5/1, SAFE Diets, Augy, France) and water, *ad libitum*. After euthanasia, selected fat depots, skeletal muscles and organs were dissected and weighed to estimate the changes in body composition and/or collected and stored at -80°C for other measurements.

2.2. Collection and composition analysis of milk from rat mothers

Breast milk was collected on days 2 (colostrum also called "early milk") and 20 (mature milk) after delivery according to a published procedure [12], and was stored at -80°C until proteins and lipid concentrations measurements and fatty acid profile determination. Protein concentration was determined by the bicinchoninic acid assay (Pierce) using bovine serum albumin as a standard. Fatty acid profile, as relative proportion level of each fatty acid expressed as % of total fatty acids, was determined by gas chromatography (Perkin Elmer Clarus 680, flame ionization detector, hydrogen as gas carrier, total chrome software). A direct methylation was performed on 10 µL milk sample at 100°C for 1h using acetyl chloride and methanol/hexane solution as previously published [13]. Then the fatty acid methyl esters (FAME) were separated on a fused silica capillary fast column (BPX70, 10 m x 0.1 mm i.d., 0.2 µm film thickness), and identified by their specific retention time using an external calibrator FAME standard (GLC 674, Nu-Chek Prep, Waterville, NM, USA). Quantification of total fatty acids (lipid, mg/mL) was performed using an internal standard (tridecanoic acid, Sigma) added in each sample in a well-known amount. We further calculated the sum of specific fatty acids as surrogate indices of de novo lipogenesis (ΣDNL) in the liver (sum of 16:0, 16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9) or in the breast (sum of 6:0, 8:0, 10:0, 12:0, 14:0, 14:1). Specific fatty acid ratios (product-to-precursor or product-to-substrate ratios) were calculated as proxies for specific hepatic DNL activity (product-to-precursor ratio: 16:0/LA; 18:0/LA; 16:1n-7/LA; 18:1n-7/LA; 18:1n-9/LA), for Stearoyl-CoA-Desaturase 1 (SCD-1) i.e., Δ9-desaturase enzyme activity involved in monounsaturated fatty acids (MUFA) biosynthesis (product-to-substrate ratio: 16:1n-7/16:0; 18:1n-9/18:0; 18:1n-7/16:0), for the activity of elongases (ELOVL) 2 (22:4n-6 or DTA/20:4n-6 or ARA), 5 (22:5n-3 or DPAn-3/20:5n-3 or EPA) and 6 (18:0/16:0; 18:1n-7/16:1n-7), and for the activity of the Δ5-desaturase (D5D) (ARA/20:3n-6 or DGLA)

and of the Δ6-desaturase (D6D) (DGLA/LA; 22:6n-3 or DHA/EPA; DPAn-6/ARA; DHA/DPAn-3; DPAn-6/DTA) both enzymes involved in the biosynthesis of PUFA from the EFA (LA and ALA) as previously published for milk [13, 14].

2.3. Oral Glucose Tolerance Test in 3-week and 7-month offspring

Oral Glucose Tolerance Tests (OGTT) were performed on 3-week-old and 7-month-old offspring after a 16-hour overnight fast. Glucose was orally given at 2 $g.kg^{-1}$ body weight. Blood glucose was measured in blood from tail vein before (T = 0 min) and 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90, 120 min after glucose administration, using an Accu-Chek glucometer (Roche Diabetes Care ®, Meylan, France at weaning and Sensostar, DiaSys Diagnostic Systems, Holzheim, Germany at 7 months of age). Areas under the curve (AUC) were related to T = 0 min. Blood samples were also collected at T = 0 min to determine plasma insulin concentration using commercial radioimmunoassay (RIA) kits (RI-13K, Merck Millipore Corporation, Germany).

2.4. Intraperitoneal Insulin Tolerance Test in 4-week and 7-month offspring

Intraperitoneal Insulin Tolerance Tests (ipITT) were performed after a 6-hour fast (instead of 16 hours as for OGTT, to prevent strong hypoglycemia following insulin administration) on pups from each group used for OGTT, at 4 weeks and 7 months of age. Insulin was intraperitoneally injected at 1 $mIU.g^{-1}$ body weight. Blood glucose was measured before (T = 0 min) and 10, 20, 30, 40, 50, 60, 90, 120 min after insulin injection as above. Areas over the curve (AOC) were related to T = 0 min. Blood samples were also collected at T = 0 min to determine plasma insulin concentrations as mentioned above.

2.5. Insulin load test and analysis of insulin signaling in skeletal muscle in 3-week and 7-month offspring

After a 6-hour fast (as justified above), 3-week-old and 7-month-old rats were intraperitoneally injected either with NaCl (0.9%) (CT- and TR-) or with insulin (10 $mIU.g^{-1}$ body weight) (CT+ and TR+). Rats were euthanized 15 min after injection and gastrocnemius muscle was rapidly removed and frozen. Protein Kinase B (PKB) phosphorylation level was determined by Western blotting as an indicator of insulin sensitivity [15]. 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_3VO_4, 20 mM NaF, 1 mM DTT and protease inhibitor cocktail (Sigma P2714). Protein concentrations were determined using a commercial kit (BCA Protein assay, Pierce, Rockford, IL) and were subjected to SDS-10% PAGE. Separated proteins were transferred onto polyvinylidene difluoride membrane and overnight incubated with primary antibodies (total PKB, Cell Signaling, #9272 and phospho-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 (ECL Select, GE Healthcare, Little Chalfont, UK).

2.6. Pancreas insulin content in 3-week and 7-month offspring

Pancreas total insulin content was determined in pancreas of rats injected with NaCl during insulin load test. Pancreas were dissected just after euthanasia and stored at -80°C. They were then homogenized at 4°C in 6 mL of 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 s each time and then placed during 2 days on a rotative wheel (20 rpm, 4°C). Homogenates were then centrifuged (150 g, 5 min, 4°C). Supernatants were collected and centrifuged (3200 g, 20

min, 4°C). The resulting supernatants were collected and stored at -80°C until insulin content assay as mentioned above.

2.7. Islets isolation and glucose stimulated insulin secretion test (GSIS) in 6-week offspring

Pancreatic islets isolation was performed at 6 weeks of age by collagenase digestion on the rats used for OGTT and ipITT. Rats were anesthetized by an *i.p.* injection of sodium pentobarbital (50 mg/100 g body weight). The abdomen was opened, and the pancreas was exposed as much as possible. The pancreatic duct was clamped with a hemostat at its duodenal insertion ensuring no injury to the pancreatic tissue. 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) was infused slowly. The pancreas was then carefully removed and placed in 7.5 mL of HBSS at 4 °C and then transferred to a water bath pre-set at 37 °C for 11 min. After the incubation, the tube was vigorously hand-shaked for 15 sec before addition of 25 mL of HBSS, 5% Fetal Bovine Serum (FBS). The tube was centrifuged (250 g, 2 min, 4°C) and the supernatant was discarded. The pellet was filtered through a wire mesh. After addition of 25 mL of HBSS, 5% FBS, the tube was centrifuged (250 g, 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. The suspension was layered on the Histopaque® 1.077 phase followed by 10 mL of HBSS on top. The gradient was centrifuged (1750 g, 20 min, 20°C) with slow acceleration and no braking. The islets were then collected from each of the interfaces and washed 3 times in 25 mL of HBSS, 5% FBS (1 time at 350 g and 4°C for 5 min, and 2 times at 250 g and 4°C for 2 min). Islets were then cultured overnight at 37°C in Roswell Park Memorial Institute medium, 10% FBS, 1% sodium pyruvate, 1% antibiotic/antimycotic solution. The day after isolation, the islets were incubated with glucose to determine their insulin secretory capacity. Islets were preincubated for one hour in 2.8 mM “low” glucose medium at 37°C for equilibration. Then some islets were incubated for 1 supplementary hour in the same conditions and supernatants were collected to give the low glucose-stimulated insulin secretion. Some other islets were incubated with 16.7 mM “high” glucose medium for 1 h at 37°C and supernatants were collected to give the high glucose-stimulated insulin secretion. Residual total insulin was extracted in 0.18 M HCl, 70% ethanol as described above. Samples were stored at -80°C until insulin assay by RIA kit as mentioned above. Islets insulin secretion index was calculated by dividing the insulin concentration in the medium after "high" glucose incubation by the insulin concentration in the medium after "low" glucose incubation for the islets of each animal.

2.8. Statistical analysis

Data are expressed as mean ± S.E.M. Data were analyzed using Student’s t-test. Mann-Whitney Rank Sum tests were applied when values were not normally distributed. Strength of association between pairs of variables was assessed using Pearson product moment correlation analysis. For all analyses, $P<0.05$ was considered significant. The box-whisker-plot graphs have been realized with the Graph Pad software ® version 9. Statistical analysis was performed using the Sigma Plot ® software.

3. Results

3.1. Mothers and litters outcomes

Body weight of female Wistar rats was similar in both groups at the beginning of the study. After nursing, body weight of TR dams was higher than that of CT dams (Table 1). Food intake was similar between groups at the beginning of gestation and at the end of lactation (Table 1). Exercise training during lactation period did not affect the weight of liver, kidney, or selected muscles (gastrocnemius, plantaris, soleus) (Table 1). Relative mass of the sum of retroperitoneal and urogenital fats depots were also similar in both groups (Table 1).

The number of offspring per litter and the sex ratio were similar between TR and CT female groups (Table 2). Offspring body weight was not affected by maternal training during lactation at 7, 14 and 21 days of age (Table 2).

3.2. Milk composition

We observed a high variability in values within groups. There was no significant difference in lipid concentration (total fatty acids), individual fatty acid proportions, fatty acid ratios and indices in colostrum between the two groups (Table 3). No significant difference in protein and total lipid concentrations, or for ratios LA/ALA, ARA/DHA, and total n-6 PUFA/total n-3 PUFA showed up in mature milk from TR mothers compared with that from CT mothers 20 days after delivery (Table 3). It is also noticeable that the relatively high LA/ALA ratio from mature milk was close from mother diet A03 during gestation-lactation period (i.e., 11/1 versus 9/1, respectively). Some fatty acid families and individual fatty acids were significantly different. Indeed, saturated fatty acids (SFA, -7%), mainly 12:0 (-12.4%) and 14:0 (-15.7%) proportions were significantly lower in the mature milk from TR dams compared to CT dams. Total MUFA (+18.5%), i.e., both 16:1 and 18:1 from the n-7 (+73.3% and +27.9%, respectively) and the n-9 (+30.4% and +15.3%, respectively) families, total PUFA (+11.6%) and especially total n-6 PUFA (+12.5%) with mainly LA (+12.8%), and ALA (+11%) proportions were significantly higher in TR dams versus CT dams. While total DNL in the liver was not different, specific hepatic DNL indices were significantly lower (-16% for16:0/LA and -21% for 18:0/LA) or higher (+50% for 16:1n-7/LA) in TR versus CT dams. A trend for a lower total DNL in the breast (P = 0.0594) was found in TR dams. All indices reflecting SCD-1 activity were significantly higher in the TR group compared to control (+85.4% for 16:1n-7/16:0, +25.3% for 18:1n-9/18:0, +36.4% for 18:1n-7/16:0). The indices for D5D or D6D enzymes activity were similar except for one index of D6D that was lower (22:5n-6/22:4n-6; -19.7%) in the TR group compared to the CT group. Index for ELOVL6 activity (18:1n-7/16:1n-7) was significantly lower in TR dams compared to CT dams (-24.5%, P = 0.038), while indices for ELOVL2 and 5 activities were higher (+29.5% P = 0.013, trend +37.8% P = 0.052, respectively).

3.3. Offspring outcomes

3.3.1. Body and organs weights

At 4 weeks of age, body weight and organ relative weight were not different in offspring from CT and TR mothers except for the pancreas (Table 4). Indeed, pancreas relative weight was higher in pups from TR mothers (+15%) compared with that of pups from CT mothers (Table 4). At 7 months of age, organs relative weights were not different between the two groups (Table 4).

3.3.2. Blood parameters

After a 6h- and 16h-fast, weanling TR pups showed a lower glycemia compared with CT pups (Figure 1A and 1B, respectively). At 7 months of age, offspring glycemia was not significantly different between the two groups, whatever the fasting duration (Figure 1C and 1D). Basal insulinemia after a 6h-fast was similar between offspring from CT and TR groups at 4 weeks and at 7 months of age, while pancreas insulin content was significantly lower at 4 weeks of age for offspring from TR dams (Table 4).

3.3.3. Glucose tolerance

At 3 weeks of age, pups from the two groups showed the same glucose tolerance as assessed by the AUC from OGTT, an index of the overall glucose disposal (Figure 2A). However, at time T = 0 min of the test, offspring from TR dams displayed a lower glycemia compared with those from CT dams (Figure 2A).

At 7 months of age, glucose tolerance was similar between offspring from CT and TR dams as assessed by blood glucose levels and AUC during OGTT that were not significantly different between the two groups (Figure 2B).

3.3.4. Insulin sensitivity

At 4 weeks of age, AOC during the first 90 minutes of ipITT was similar between pups from the two groups, suggesting no difference in overall insulin sensitivity (Figure 2C). However, at T = 0 min, TR pups 6h-fast glycemia was lower compared with that of CT pups (P=0.022) (Figure 2C).

At 7 months of age, blood glucose levels and AOC during ipITT were similar between animals from CT and TR mothers, suggesting no difference in overall insulin sensitivity (Figure 2 D).

3.3.5. Muscle insulin signaling pathway

Muscle insulin signaling pathway activation was estimated by the ratio of phosphorylated form of PKB (pPKB) over its total content (PKB) after either a NaCl (-) or an insulin load (+). In non-stimulated conditions (NaCl), at 3 weeks of age, we found that muscle insulin pathway activation in weanling offspring was sensitive to maternal exercise training during lactation since the pPKB/PKB ratio was lower in offspring from TR dams compared with those from CT dams (-39%, P=0.018, TR- vs. CT-) (Figure 3A and 3C). In insulin stimulated conditions, insulin pathway was unaffected by maternal exercise during lactation since muscle pPKB/PKB ratio was similar between the two groups (CT+ and TR+) (Figure 3A and 3C).

At 7 months of age, offspring from TR mothers had a higher muscle pPKB/PKB ratio in basal conditions compared with offspring from CT mothers (+60%, P=0.007, TR- vs. CT-) (Figure 3B and 3D). In insulin-stimulated conditions, pPKB/PKB ratio in muscle was not different between TR pups and CT pups (Figure 3B and 3D).

3.3.6. Pancreas insulin content and islets insulin secretion

One week after weaning (4 weeks of age), pancreas insulin content was lower in pups from TR dams compared with pups from CT dams (-37%, P=0.001, TR vs. CT) (Table 4). At 7 months of age, pancreas insulin content was not different between offspring from the two groups (Table 4).

At 6 weeks of age, islets insulin secretion was not different between offspring from the two groups whether in low glucose (2.8 mM G; P = 0.234) or in high glucose (16.7 mM G; P = 0.138) condition. Mean values in TR group were numerically two times higher than in CT groups but differences were not significant due to the large range of the data (Figure 4A). Insulin secretion index was not significantly different between the two groups also the average value was numerically higher in TR group compared with CT group (P = 0.156) (Figure 4B).

3.4. Relationships between mature milk characteristics and offspring glycemia and pancreatic function related parameters at weaning.

Correlation between mature milk fatty acid composition or indices and, glycemia and pancreatic function parameters in offspring at weaning, which showed significant differences between groups, are indicated in Table 5.

Total PUFA, from the n-6 as well as the n-3 families, and more specifically their precursors (LA and ALA) were highly ($r \geq 0.5$) inversely associated with 6h-fasted glycemia, whereas only n-6 PUFA and especially LA were moderately inversely associated with 16h-fasted glycemia. ALA was the only n-3 PUFA highly inversely associated with pancreas insulin content and positively with pancreas weight. In addition, the 16:1n-7/16:0 ratio, as one surrogate index for stearoyl-CoA-desaturase 1 activity (SCD-1), showed a high to moderate inverse association with 6h-fasted or 16h-fasted glycemia and pancreas insulin content, and was highly positively correlated with pancreas weight. The elongase 2 activity as estimated by the DTA/ARA ratio was found to be negatively correlated to the insulin content and to the 16h-fasted glycemia. Similarly, the elongase 5 activity as estimated by the DPAn-3/EPA ratio was found to be highly negatively correlated to the insulin content and to the 6h-fasted glycemia. The elongase 6 activity as estimated by the 18:1n-7/16:1n-7 ratio was found to be highly positively correlated to the insulin content and inversely to the pancreas weight.

4. Discussion

Knowing that perinatal exposome is important for the future health of offspring, our purpose was to study the effects of chronic submaximal maternal exercise specifically during the lactation period on short- and long-term offspring pancreatic function and glucose homeostasis in a context of regular rodent diets. Indeed, while several studies addressed the question of the health interest to the offspring of maternal exercise during critical periods of development showing for most of them a beneficial impact on glucose homeostasis [16,17], such studies concerned only the entire gestation period until lactation and extreme deleterious diets for the dams and/or the offspring (e.g., high fat, high sucrose). Thus, the impact of maternal exercise needed to be investigated during only the lactation period along and within a context of standard CHOW diet (A03 and A04). For that, female Wistar rats performed a treadmill submaximal endurance training during lactation, and we studied the male offspring at weaning (at 3, 4 and 6 weeks of age) and at adulthood (7 months of age). We found that maternal chronic exercise during lactation substantially modifies fatty acid breast milk composition and is associated with a lower fasting glycemia along with changes in endocrine pancreas function (higher pancreas weight and lower insulin content) in the male offspring during the first weeks of life and with a higher activation of insulin signaling pathway in skeletal muscle in adulthood. At final, the relationship between maternal chronic exercise during lactation, type of milk fatty acid supplied to male offspring during suckling and glucose-insulin axis parameters provides novel knowledges in the DOHaD framework.

As regards to outcomes in mothers' rat at the end of the lactation period, the only significant difference due to training exercise was a higher body weight after nursing in TR compared to CT dams that could be explained by a numerically higher food intake during lactation and numerically higher muscle mass (but both not statistically significant). More interestingly, we report, for the first time to our knowledge, a change in fatty acid proportions in the mature milk in response to maternal exercise. While the fatty acid composition of the milk of CT dams was close to data recently published [18], the lower proportion in SFA concomitantly to a higher proportion in MUFA and PUFA in the mature milk of TR dams are likely to be the result of maternal training per se. Indeed, the fatty acid profile of the colostrum, early milk collected 2 days after delivery, i.e., before the start of the exercise training, was similar between both groups. Milk fatty acids derive from different sources such as dietary intakes (via chylomicrons), de novo lipogenesis (DNL) in mammary gland (mainly medium chain fatty acids (MCFA) produced from glucose) and in the liver (via VLDL production), and maternal stores (free fatty acids released from the adipose tissue) [14]. All these sources compete for incorporation into milk triglycerides within the breast. Since the dietary intakes were similar between our two groups, differences in biosynthesis from the mammary gland and

from the liver, and in the mobilization of fatty acids from the adipose tissues were more likely to intervene in milk fatty acid changes in our study. Indeed, the lower level in SFA, mainly due to a lower proportion in C12:0 (-12%) and in C14:0 (-16%), in TR mature milk could be due to a lower de novo lipogenesis of such medium-chain fatty acids in the breast, in accordance with a trend in a lower total de novo lipogenesis in the breast (ΣDNL breast, $P = 0.059$), and might be due to a lower availability of glucose, more orientated towards endurance exercise than to the mammary gland for producing MCFA [14]. The higher proportion of MUFA in TR milk corroborates well with a higher activity of the rate-limiting enzyme in their biosynthesis i.e., SCD-1, as suggested by the three calculated indices. Our finding is consistent with the report of an upregulation of SCD-1 expression in the liver by single-endurance training in rat [17], and with higher SCD-1 activity found in exercised pigs compared to sedentary ones [19]. The near similar indices of D5 and D6 desaturase enzymes activity explain the similar proportion of long-chain PUFA (n-6 or n-3 fatty acids with > 18 atoms of carbon) in both groups, whereas the higher proportion of both EFA in TR milk could likely to be due to a higher mobilization of fatty acids stored in adipose tissue thanks to daily practice of exercise, and among them LA and ALA that incorporated more into milk lipids [20]. While the global de novo lipogenesis in the liver appeared similar in the two groups (ΣDNL liver index), specific hepatic DNL (hDNL) activity indices (lower 16:0/18:2 and 18:0/18:2 together with a higher 16:1n-7/18:2) suggest a different metabolism in the liver in TR lactating rat dams, driving the liver to deliver more LA together with palmitoleic acid (C16:1n-7) into VLDL triglycerides that will be used by mammary glands [14].

Several milk indices were indicators of a better handling of glucose homeostasis and of a higher insulin sensitivity in exercise trained lactating dams. Indeed, in a case-control study in patients with impaired fasting glycemia, indices calculated from plasma fatty acids concluded in a lower estimated activity of ELOVLs 2 and 5 while higher for ELOVL6, together with a reported lower plasma vaccenic acid level (C18:1n-7) in such patients [21]. Such identical feature was found in our study in the mature milk of sedentary lactating dams, and inversely in our exercise trained ones. In addition, mice KO for *ELOVL6* gene were reported to be resistant to diet-induced insulin resistance [22], which allows us to suggest that a lower index of estimated activity of ELOVL6 in the milk of exercise trained dams was a reflect of a higher insulin sensitivity in these animals. It is known that a physiological state of reduced insulin sensitivity/compensatory hyperinsulinemia takes place at the second half of pregnancy, and restoration of insulin sensitivity occurs progressively between delivery and 3 months postpartum [14]. Relying on our findings and on literature reporting that postpartum exercise in mothers improves insulin sensitivity [23], we can assume that such restoration could be faster with physical activity during the lactation period, and this could influence the fatty acid metabolism in mothers' rat and in turn the fatty acid profile of their mature milk.

Overall, the specific characteristic of mature milk fatty acids from training dams in our study matched well with higher MUFA, 18:1 n-9, 18:1/18:0 ratio, MUFA/SFA ratio, 18:2 n-6, 18:3 n-3 profiles reported in subcutaneous adipose tissue and muscular neutral lipids in exercised pigs compared to sedentary ones [19]. Finally, it is not surprising that fatty acid profiling of a biological fluid as milk could reflect a 'physical activity pattern' in training dams since the fatty acid print of milk was previously found as related to other metabolic conditions such as underweight, normal or overweight in lactating women [13]. Therefore, it is likely that fatty acid synthesis and fluxes in lactating mothers are reflected by the content of specific fatty acids and their ratios in their milk since it is a biological fluid as is the plasma.

Maternal exercise during the lactating period was also associated with changes in the offspring's outcomes. It was associated just after weaning to a higher pancreas growth as well as functional changes illustrated by a lower total insulin content together with a numerically

higher islets insulin secretion capacity in high glucose condition and insulin secretion index. Thus, chronic maternal exercise during lactation period has affected offspring pancreas development and function in the first weeks of life, possibly by decreasing the size of the islets [6] but by slightly increasing their ability to secrete insulin. These changes observed in the juvenile pancreas of TR offspring, even if at the margin of significance because of the great variability of the animals' responses, seemed strong enough for significatively improving regulation of fasting glycemia after a 6h- and 16h-fast, leading to normal systemic glucose concentration while values are closer to a hyperglycemia state in the CT offspring [24]. The underlying mechanisms might rely on the modification of the breast milk fatty acid composition in exercise trained dams. Indeed, as regards to the relationship we have found between some milk fatty acids levels and glucose-insulin axis in offspring, the main fatty acids inversely associated with glycemia at fasting in early life were total PUFA, total n-6 PUFA and total n-3 PUFA, and more specifically LA and ALA (the higher the levels in milk, the lower the fasting glucose concentration). Dietary PUFA have been shown to reduce type 2 diabetes mellitus risk and to improve insulin responsiveness in diabetes type 2 subjects [25]. Among PUFA, LA seems to play a key role in the regulation of glucose homeostasis since serum LA level, related to dietary consumption, was found inversely associated with plasma glucose both in fasting condition and 2 h after glucose tolerance test in subjects at risk for type 2 diabetes [26-29]. LA action is either an insulin-sensitizing effect [30] or an increase in insulin secretion capacity [31,32]. ALA was also reported as involved in glucose homeostasis in hyperglycemia state [28]. Another original finding in our study was the inverse association between the SCD-1 activity estimated from specific fatty acids in milk and systemic glucose regulation, a higher activity leading to a better control and to a lower pancreas insulin content, possibly indicative of a higher insulin secretion, and a higher growth of pancreas. A lower estimated elongase 6 activity is also related to a lower pancreas insulin content and a higher growth of pancreas. A high SCD-1 activity and a low ELOVL6 activity converge to the same common point which is an increase in palmitoleic acid (C16:1n-7), three characteristics found in the milk of exercise trained dams. Since this fatty acid present in milk as esterified in triglyceride did not correlate per itself with glucose-insulin axis, we can suggest that the dynamic flux of free palmitoleic acid i.e., its endogenous production by SCD-1 from desaturation of palmitic acid in parallel of its elongation by ELOVL6 for the synthesis of vaccenic acid, rather than its level by itself in milk triglycerides, is involved in glucose homeostasis. The palmitoleic acid (endogenous or from dietary source) is suggested to act as a 'lipokine' once it has reached blood circulation as non-esterified fatty acid (and not when in the form of triglycerides, esterified cholesterol or phospholipids) which improves metabolic parameters impaired in obesity and diabetes type 2 especially the failure of insulin secretion from pancreatic β-cells to regulate blood glucose which is the main hallmark of diabetes type 2 [33]. Circulating palmitoleic acid affects positively β-cell function and survival, insulin secretion, whole body sensitivity to insulin, skeletal muscle insulin response, glucose tolerance, and negatively liver insulin resistance [33-36]. A dietary dose of palmitoleic at 9 mg/d for 4 weeks decreased hyperglycemia and improved insulin sensitivity in genetic diabetes type 2 mice [36]. For comparison the amount provided by the milk was 2.2 mg/ml in our exercise trained dams versus 1.1 mg/ml in sedentary ones (P = 0.0097). Thus, changes in fatty acid metabolism in TR dam possibly due to a better insulin sensitivity were reflected in specific fatty acids profile of their milk that might imprint in turn improvement in the glucose-insulin axis in their offspring.

To note that while a favorable regulation of systemic glucose was reported in offspring of exercise trained rat mothers in our study, the insulin signaling pathway in skeletal muscle (gastrocnemius) did not support an improvement in peripheral glucose uptake in early life. This latter was significantly improved (P = 0.007) later on at adulthood through a higher pPKB/PKB ratio suggesting a higher insulin secretion from pancreatic islets [37], that we were not able to

measure unfortunately due to the lack of animals. A possible explanation for this inversion in the phosphorylation level of PKB in the offspring between 3 weeks of age and adulthood may lie on a direct effect of certain milk fatty acids provided during breastfeeding in the first case, while in the second case it can be related to a 'nutritional programming' effect either by the fatty acids of the milk ingested earlier in life or by other milk bioactive molecules influencing early epigenetics. Indeed, oleic, linoleic and linolenic acids were reported to decrease insulin-mediated PKB phosphorylation in soleus muscle in vitro [38]. Milk from TR dams providing a higher amount of these 3 fatty acids as triglycerides, we can suggest that a higher level of such fatty acids will be find in the circulation of their suckling rats (after triglyceride digestion, fatty acid absorption, redistribution via chylomicrons by the liver and delivery of free fatty acids to the organs, thanks to the lipoprotein lipase [39]) increasing their contact with organs such as muscles. This could explain a lower pPKB/PKB ratio in gastrocnemius in TR pups just at the end of suckling (3 weeks of age) and thus a lower insulin sensitivity in muscle. At weaning all pups were fed standard diets, i.e., the CHOW A03 diet until 2 months of age then they switched to the CHOW A04 diet. So, at adulthood the significant higher insulin sensitivity indicated by a higher pPKB/PKB in offspring issued from TR mothers is probably more the consequence of a "programming effect" in early life by a different amount of bioactive molecules provided by milk acting on epigenetic processes [40]. Such molecules could be specific fatty acids since, 1/ their composition in milk was modified by maternal chronic exercise, 2/ they have been found associated with parameters of the glucose-insulin axis in early life, and 3/ some of them have been shown to impact epigenetics [41]. However, the associations found in early life do not prove any causal effect, and other milk molecules not quantified herein could have been also involved in glucose-insulin axis programming [40]. In a non-exhaustive way and without making this discussion too speculative, we can cite hormones, such as leptin (400-500 pg/ml rat milk) and insulin (1-3 ng/ml rat milk) [42], whose levels could be different in milk from physically active versus sedentary dams. Indeed, while we were not able measuring hormones level due to milk volume collected herein, other authors reported that insulin level was twice higher in milk from exercised rat mothers compared to sedentary ones (2.4-2.5 ng/ml vs 1.2-1.3 ng/ml) leading to improved insulin sensitivity and glucose homeostasis in offspring [7]. We can also think about specific miRNAs, since some common circulating miRNAs increasing during endurance training [43] are also present in milk [44,45] and possibly in association with specific fatty acid (as 16:1 for miR-26) [45], and are involved in the glucose-insulin pathways [46,47]. All these molecules were not possible to analyze in our study due to the small volume of milk collected, even considering pooled samples. Our results also suggest that maternal exercise benefits decrease in offspring aged of 7 months. This may be related to a progressive decline of the impact of the specific milk composition from trained exercise mothers (different levels of oleic, LA and ALA) due to the contribution of high levels of oleic (as MUFA), linoleic and linolenic (as PUFA) acids provided by CHOW diets A03 and A04 from weaning in similar ways in both offspring from sedentary and trained mothers. Such similar supply in these fatty acids may have progressively overcome the effects attributable to the differences in fatty acids from milk due to the mother's training. Although we did not explore that point, we can hypothesize that the protective effect of maternal exercise would have been still detectable or even amplified in the offspring if differences in such fatty acids supply have been maintained in diets used from weaning to 7 months of age.

In conclusion, our study is the first one to our knowledge that explored the specific effects of chronic maternal exercise during only the lactation period on fatty acid composition of milk as well as short- and long-term offspring glucose homeostasis and pancreatic function. Here we showed that maternal regular submaximal exercise during lactation, starting at the third day after delivery, lead to a specific "phenotype" of milk in terms of fatty acid profile that seems

to be beneficial for the development of the glucose-insulin axis in offspring. Furthermore, the examination within milk of the indices of enzymes involved in fatty acid metabolism suggests a higher insulin sensitivity in physically active lactating mothers as a main explanation. Beyond the fatty acid profile, it might be highly possible that other milk bioactive molecules could exert health programming via early epigenetic modifications. In this way, future investigations on the changes in milk composition due to physical exercise through a holistic approach using omics' technology are necessary for a larger knowledge of milk impact. Another novelty of our study lies in that a regular exercise training versus sedentary state of the lactating rat mothers showed beneficial impact despite a standard CHOW diet during gestation and lactation. Lactation appears clearly as a window of opportunity to improve outcomes in offspring at short- and long-term via chronic maternal exercise. These findings support the DOHaD concept and provides new insights that could help to update the actual guidelines about exercise for lactating women.

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Credit authorship contribution statement

Charline Quiclet: investigation, visualization, writing – original draft, writing – review and editing. **Martine Armand:** investigation, visualization, writing – original draft, writing – review and editing. **Hervé Dubouchaud**: investigation, visualization, writing – review and editing. **Guillaume Vial**: investigation, writing – review and editing. **Eric Fontaine**: conceptualization, resources, funding acquisition, writing – review and editing. **Cecile Batandier**: conceptualization, investigation, visualization, writing – review and editing, supervision. **Karine Couturier**: conceptualization, investigation, visualization, writing – review and editing, supervision, funding acquisition.

All authors have approved the final version of the manuscript and agreed to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Declaration of competing interest

The authors declare that no conflict of interest exists.

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Table 1. Mothers outcomes

	CT	TR
BW before gestation (g)	239±3	247±3
BW after nursing (g)	262±3	276±5*
Food intake during gestation (g/kg BW/day)	93.2±3.2	100.8±6.4
Food intake during lactation (g/kg BW/day)	160.9±17.2	184.6±6.2
Food intake at the end of lactation (g/kg BW/day)	264.8±15.8	232.8±11.8
Liver (g/kg/BW)	26.7±0.51	27.3±0.60
Kidney (g/kg/BW)	3.3±0.07	3.3±0.09
Fat (g/kg/BW)	15.4±1.20	17.8±1.72
Muscles (g/kg/BW)	5±0.58	5.6±0.11

Data are mean ± S.E.M. (n=8, or n=3 to 7 for food intake). Fat mass was calculated as the sum of retroperitoneal and urogenital fats depots. Muscles mass was calculated as the sum of gastrocnemius, plantaris and soleus muscles. *different from CT, $P<0.05$. Data were analyzed using Student's t-test except for data on muscles (Mann-Whitney Rank Sum test used).
Abbreviations: BW, body weight; CT, control; TR, trained.

Table 2. Litter outcomes

	CT	TR
Litter size	12.8±0.5	12.2±0.6
Number of males	7.0±0.8	5.6±0.5
Number of females	5.8±0.5	6.6±0.7
D7 body weight (g)	15.2±0.5	15.3±0.3
D14 body weight (g)	32.3±0.7	30.9±0.7
D21 body weight (g)	50.5±1.1	48.7±1.0

Data are mean ± S.E.M. (n = 4 to 8). Data were analyzed using Student's t-test.
Abbreviations: CT, control; D, days; TR, trained.

Table 3. Protein and lipid concentrations, and fatty acids (FA) proportions (% total FA) in colostrum and mature breast milk

	Colostrum (D2)		Mature milk (D20)	
	CT (n = 8)	**TR (n = 6)**	**CT (n = 6)**	**TR (n = 8)**
Proteins ($mg.mL^{-1}$)	nd	nd	96.4±2.6	104.5±5.1
Lipid (Total FA) ($mg.ml^{-1}$)	99.2±15.1	140.5±19.4	127.6±18.3	145.8±8.5
Total SFA	47.62±3.50	48.58±2.35	62.57±1.14	58.18±0.69*
Total MCFA	20.01±2.59	21.52±2.09	30.94±1.0	29.50±0.85
SFA species				
6:0	0.21±0.02	0.20±0.02	0.21±0.01	0.18±0.02
8:0	3.17±0.33	3.68±0.40	5.28±0.31	5.52±0.33
10:0	8.94±1.18	9.84±1.00	14.18±0.52	13.96±0.52
12:0	7.42±1.11	7.49±0.83	11.17±0.30	9.79±0.21*
14:0	6.19±1.07	6.03±0.77	9.52±0.60	8.03±0.42*
15:0	0.23±0.01	0.19±0.01	0.24±0.06	0.22±0.02
16:0	17.23±1.34	17.14±0.59	17.84±0.52	17.01±0.64
17:0	0.27±0.03	0.23±0.02	0.31±0.06	0.26±0.03
18:0	3.28±0.14	3.05±0.14	3.31±0.28	2.96±0.10
Total MUFA	21.24±2.22	20.99±1.67	15.38±0.83	18.22±0.58*
MUFA species				
16:1 n-9	0.29±0.01	0.29±0.02	0.23±0.02	0.30±0.01*
16:1 n-7	1.25±0.32	1.58±0.26	0.86±0.06	1.49±0.15*
18:1 n-9	16.68±1.88	16.15±1.31	12.28±0.72	14.16±0.48*
18:1 n-7	1.27±0.12	1.43±0.07	0.79±0.03	1.01±0.04*
20:1 n-9	0.86±0.13	0.98±0.19	0.57±0.07	0.57±0.04
Total PUFA	25.15±1.64	26.74±0.92	19.53±0.60	21.80±0.45*
Total n-6 PUFA	22.23±1.54	23.65±0.81	17.22±0.56	19.37±0.42*
n-6 PUFA species				
18:2 n-6 (LA)	16.63±1.45	17.87±0.78	15.60±0.51	17.60±0.39*
18:3 n-6	0.91±0.12	0.98±0.08	0.22±0.03	0.23±0.03
20:2 n-6	0.56±0.05	0.51±0.02	0.28±0.02	0.31±0.02
20:3 n-6 (DGLA)	0.74±0.04	0.70±0.03	0.16±0.02	0.17±0.02
20:4 n-6 (ARA)	2.64±0.14	2.76±0.16	0.65±0.05	0.73±0.06
22:4 n-6 (DTA)	0.49±0.04	0.60±0.03	0.11±0.01	0.17±0.02
22:5 n-6 (DPA)	0.19±0.02	0.15±0.01	0.05±0.01	0.07±0.01
Total n-3 PUFA	2.74±0.14	2.93±0.15	2.18±0.06	2.32±0.05
n-3 PUFA species				
18:3 n-3 (ALA)	1.01±0.09	1.06±0.09	1.38±0.05	1.55±0.04*
18:4 n-3	0.18±0.04	0.15±0.03	0.13±0.01	0.12±0.01
20:4 n-3	0.23±0.07	0.15±0.04	0.17±0.04	0.10±0.02
20:5 n-3 (EPA)	0.45±0.01	0.47±0.02	0.18±0.01	0.16±0.01
22:5 n-3 (DPA)	0.41±0.04	0.48±0.03	0.18±0.01	0.21±0.02
22:6 n-3 (DHA)	0.64±0.08	0.77±0.06	0.28±0.02	0.29±0.02
Ratios				
LA/ALA	16.6±0.7	17.2±0.9	11.3±0.3	11.4±0.3
ARA/DHA	4.6±0.7	3.6±0.1	2.4±0.1	2.5±0.1
Total n-6/n-3 PUFA	8.1±0.2	8.1±0.2	7.9±0.2	8.4±0.1
Indices				
ΣDNL Liver	37.29±2.50	37.28±1.68	32.33±0.96	34.25±0.77
ΣDNL Breast	25.95±3.51	27.27±2.59	40.39±1.30	37.52±0.70
hDNL (16:0/LA)	1.06±0.06	0.97±0.04	1.15±0.06	0.97±0.05*
hDNL (18:0/LA)	0.207±0.020	0.172±0.010	0.214±0.020	0.169±0.010*

hDNL (16:1n-7/LA)	0.072±0.010	0.088±0.010	0.056±0.010	0.085±0.010*
hDNL (18:1n-7/LA)	0.077±0.004	0.081±0.010	0.051±0.003	0.058±0.002
hDNL (18:1n-9/LA)	1.109±0.220	0.923±0.110	0.795±0.060	0.809±0.040
SCD (16:1n-7/16:0)	0.069±0.010	0.091±0.010	0.048±0.003	0.089±0.010*
SCD (18:1n-9/18:0)	5.13±0.48	5.28±0.35	3.84±0.36	4.81±0.18*
SCD (18:1n-7/16:0)	0.074±0.005	0.084±0.010	0.044±0.003	0.060±0.004*
D5D (ARA/DGLA)	3.65±0.28	3.98±0.28	4.57±0.81	4.54±0.47
D6D (DGLA/LA)	0.049±0.010	0.040±0.003	0.010±0.001	0.010±0.001
D6D (DHA/EPA)	1.41±0.16	1.62±0.06	1.59±0.12	1.82±0.10
D6D (22:5n-6/ARA)	0.069±0.010	0.056±0.003	0.088±0.010	0.097±0.010
D6D (DHA/22:5n-3)	1.57±0.12	1.60±0.08	1.58±0.11	1.42±0.11
D6D (DPAn-6/DTA)	0.438±0.110	0.260±0.020	0.519±0.070	0.417±0.040*
ELOVL2 (DTA/ARA)	0.188±0.020	0.218±0.011	0.172±0.015	0.237±0.015*
ELOVL5 (DPAn-3/EPA)	0.903±0.081	1.019±0.036	1.018±0.071	1.318±0.108
ELOVL6 (18:0/16:0)	0.19±0.02	0.18±0.01	0.19±0.02	0.17±0.01
ELOVL6 (18:1n-7/16:1n-7)	1.21±0.13	0.99±0.11	0.94±0.09	0.71±0.05*

Data are mean± S.E.M. of the main fatty acids among all those detected by gas chromatography i.e., present at ≥ 0.05% of total fatty acids in milk (n = 28).

Abbreviations: ARA, arachidonic acid; ALA, alpha-linolenic acid; CT, control; D, days; D5D, delta-5 desaturase; D6D, delta-6 desaturase; DHA, docosahexaenoic acid; DNL, de novo lipogenesis; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; ELOVL, elongase ; EPA, eicosapentaenoic acid; hDNL, hepatic specific DNL; LA, linoleic acid; MCFA, medium-chain fatty acids (C ≤ 12); MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD, Stearoyl-CoA-Desaturase; SFA, saturated fatty acids; TR, trained; nd: not determined.

ΣDNL Liver = sum of 16:0, 16:1 n-7, 18:1 n-7, 18:1 n-9, 20:1 n-9.

ΣDNL Breast = sum of 6:0, 8:0, 10:0, 12:0, 14:0, 14:1.

* different from CT, $P < 0.05$. Data were analyzed using Student's t-test.

Table 4. Offspring outcomes, fasting insulin and pancreas insulin content in offspring at 4 weeks (early life) and 7 months (adulthood) of age

	4 weeks		7 months	
	CT	**TR**	**CT**	**TR**
Body weight (g)	78.8±2.7	77.6±2.9	517.0±14.9	567.1±26.3
Organs weight (g/kg/BW)				
Liver	35.09±0.60	35.68±0.36	26.43±1.25	27.10±0.66
Kidney	5.61±0.08	5.34±0.07	2.53±0.09	2.42±0.07
Adipose tissue	5.11±0.28	5.38±0.58	46.74±5.05	49.83±5.10
Muscles	4.35±0.08	4.19±0.08	4.82±0.13	4.63±0.15
Pancreas	4.79±0.20	5.51±0.21*	2.82±0.17	2.66±0.12
6h-fast Insulinemia (ng/mL)	0.47±0.08	0.40±0.10	2.22±0.54	1.89±0.34
Pancreas insulin content (ng/mg fresh weight)	89.4±2.8	56.0±2.7*	124.4±18.7	133.5±19.4

Data are mean ± S.E.M. (n = 7 to 27). Fat mass indicated as "adipose tissue" was calculated as the sum of retroperitoneal, epididymal and mesenteric fats depots. Muscles mass was calculated as the sum of gastrocnemius, plantaris and soleus muscles. *different from CT, $P < 0.05$. Data were analyzed using Student's t-test except for data on body weight at 4 weeks (Mann-Whitney Rank Sum test used).
Abbreviations: CT, control; TR, trained.

Table 5. Correlation coefficients between mature milk principal components and glycemia-related parameters in offspring at weaning.

Association between two continuous variables were performed using Pearson's correlation coefficient test; *P* values are indicated into brackets; number of data used for such analysis were between 17 and 25.

Milk parameters	6h-fasted glycemia	16h-fasted glycemia	Insulin content	Pancreas weight
Fatty acids				
Total PUFA	-0.51 ($P = 0.026$)			
Total n-6 PUFA	-0.50 ($P = 0.030$)	-0.45 ($P = 0.046$)		
18:2 n-6 (LA)	-0.56 ($P = 0.012$)	-0.47 ($P = 0.037$)		
Total n-3 PUFA	-0.61 ($P = 0.006$)			
18:3 n-3 (ALA)	-0.65 ($P = 0.002$)		-0.60 ($P = 0.024$)	0.55 ($P = 0.040$)
Indices				
SCD-1 (16:1n-7/16:0)	-0.59 ($P = 0.0074$)	-0.45 ($P = 0.046$)	-0.66 ($P = 0.010$)	0.54 ($P = 0.046$)
ELOVL 2 (DTA/ARA)		-0.55 ($P = 0.011$)	-0.76 ($P = 0.002$)	
ELOVL 5 (DPAn-3/EPA)	-0.48 ($P = 0.037$)		-0.60 ($P = 0.024$)	
ELOVL 6 (18:1n-7/16:1 n-7)			0.68 ($P = 0.007$)	-0.58 ($P = 0.029$)

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acids; SCD-1, Stearoyl-CoA-Desaturase 1.

Figure captions

Fig. 1. Maternal exercise during lactation period reduces fasting glycemia in offspring early in life but not at adulthood. Box-and-whisker plot of offspring fasting glycemia measured after a 6-h fast (A;C) and a 16-h fast (B;D) at 4 weeks (A), 3 weeks (B) and 7 months of age (C;D). Data represented as plots are median, first quartile (Q1 or 25th percentile) and third quartile (Q3 or 75th percentile), min and max, mean (as a cross) and all individual points (n = 8 to 12). *TR different from CT, $P < 0.05$. Data were analyzed using a Mann-Whitney Rank Sum tests.

Fig. 2. Offspring glucose tolerance and overall insulin sensitivity are not altered by maternal exercise during lactation period. Rat offspring underwent an OGTT at 3 weeks (A) and 7 months (B) of age to assess whole body glucose tolerance. Following a 16h-fast, glucose was orally given at 2 g.kg^{-1} body weight. Blood glucose levels were monitored before (0) and 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90 and 120 min after glucose administration. Inset graphs show the areas under the curve of OGTT related to the value at T = 0 min. Rat offspring also underwent an ipITT at 4 weeks of age (C) and 7 months of age (D) 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. Inset graphs show the areas over the curve of ipITT related to the value at T = 0 min. Data are mean ± S.E.M.; n=8 to 15. *TR different from CT, $P<0.05$. Data were analyzed using Student's t-test.

Fig. 3. Maternal exercise during lactation period modulates offspring basal muscle insulin pathway activation at 3 weeks and 7 months of age. Rat offspring underwent an insulin load test at 3 weeks (A, C) and 7 months (B, D) of age to assess 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 (CT+, TR+) and the others were injected with NaCl (CT-, TR-). Gastrocnemius muscle samples were collected 15 minutes after injection in order to determine pPKB/PKB ratio (Ser473) by Western blotting. Representative Western Blots showing phosphorylated and total (pPKB and PKB, respectively) PKB content in gastrocnemius muscle (C and D). Quantitation of the signals was expressed in arbitrary units. Data are mean ± S.E.M.; n = 4 to 8. * different from CT-, $P < 0.05$; Data were analyzed using Student's t-test.

Fig. 4. Maternal exercise during lactation period numerically but not significantly increases offspring islets insulin secretion index at 6 weeks of age. Offspring pancreatic islets were isolated at 6 weeks of age in order to assess their insulin secretory capacity. The day after isolation, islets were incubated in a low glucose concentration (2.8 mM G) medium and then in a high glucose concentration (16.7 mM G) medium (A). Supernatants were collected and islets insulin secretion determined by RIA. Insulin secretion index was calculated by dividing insulin concentration in the medium after high glucose incubation by that after low glucose incubation (B). Data are represented as box-and-whisker plot with median, first quartile (Q1 or 25th percentile) and third quartile (Q3 or 75th percentile), min and max, mean (as a cross) and all individual points (n = 5 to 7). Data were analyzed using a Mann-Whitney Rank Sum tests.

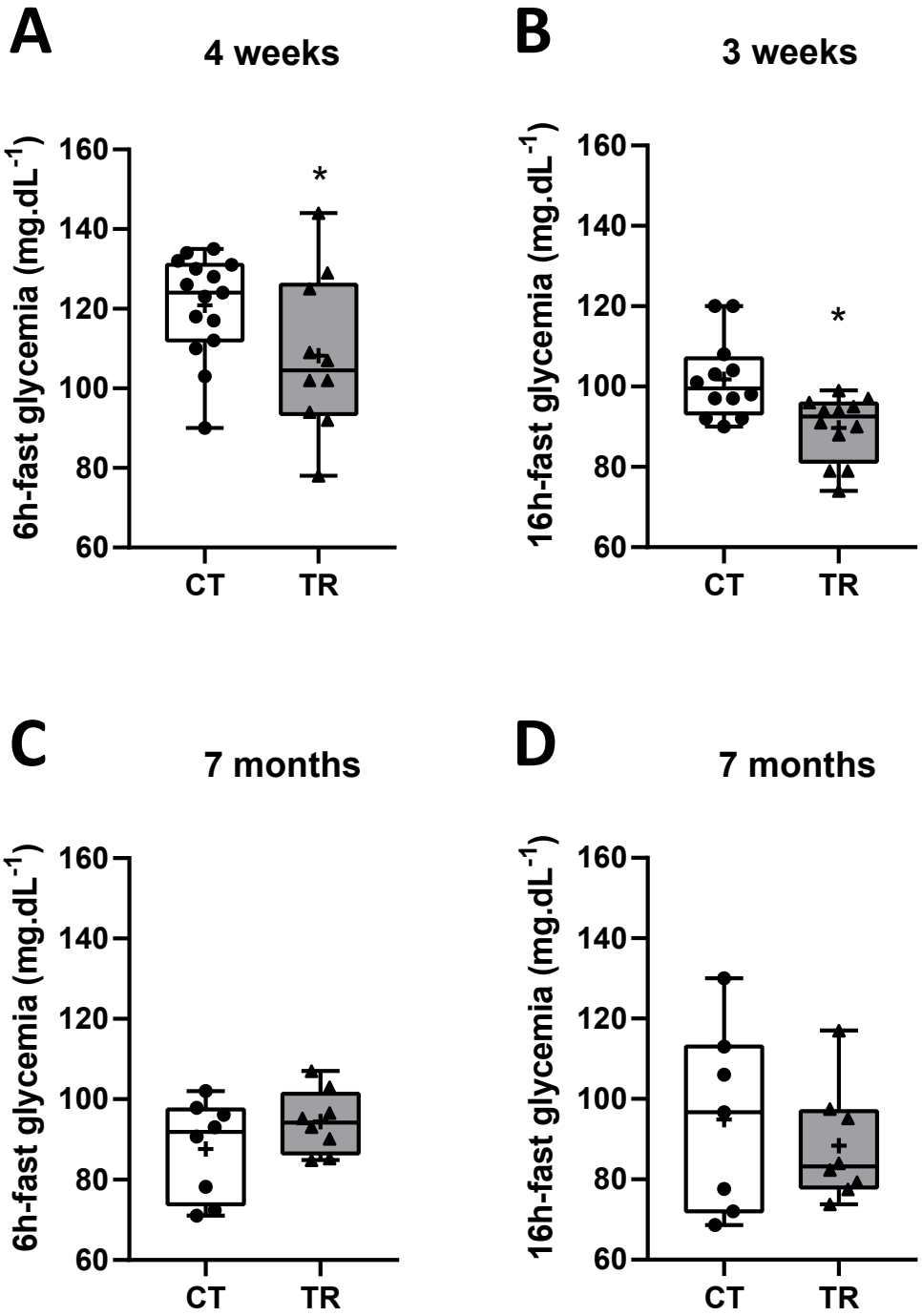
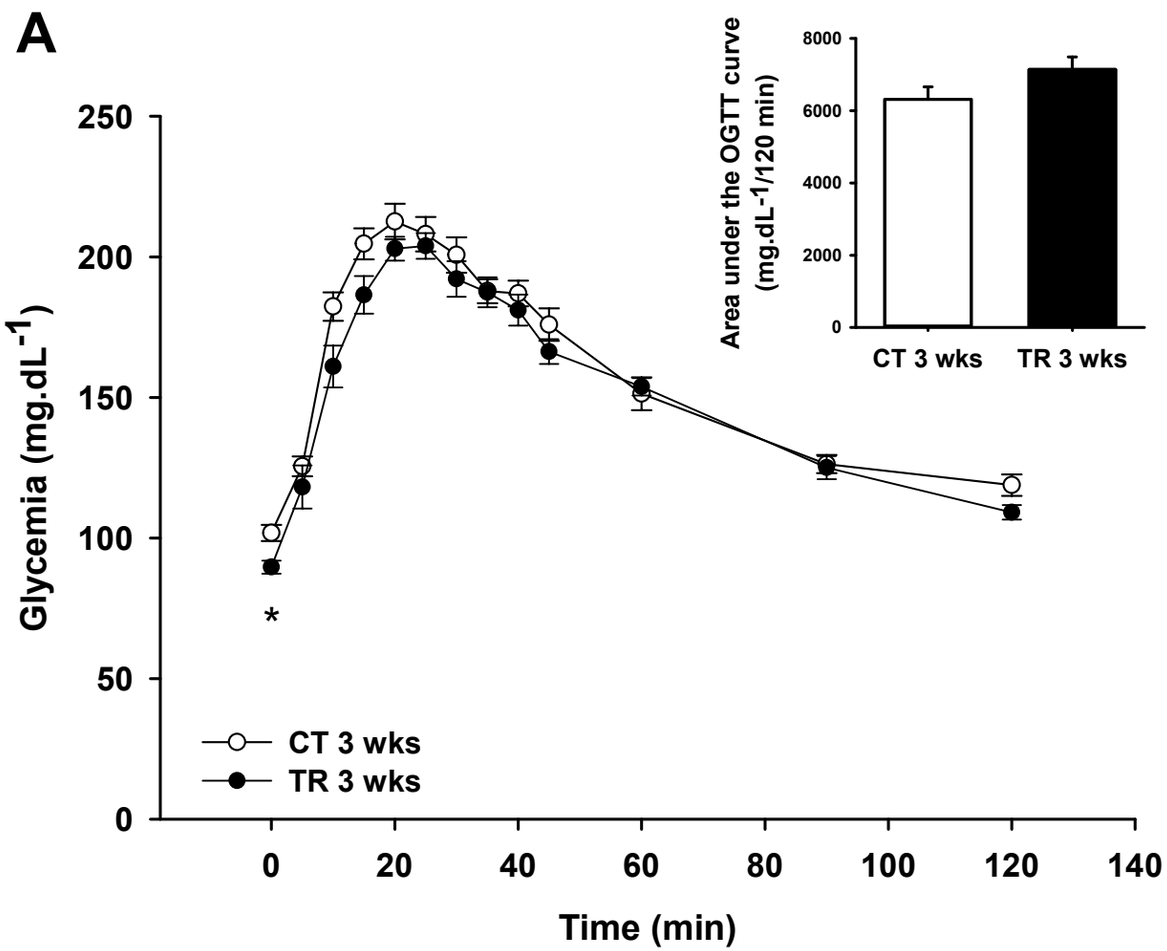
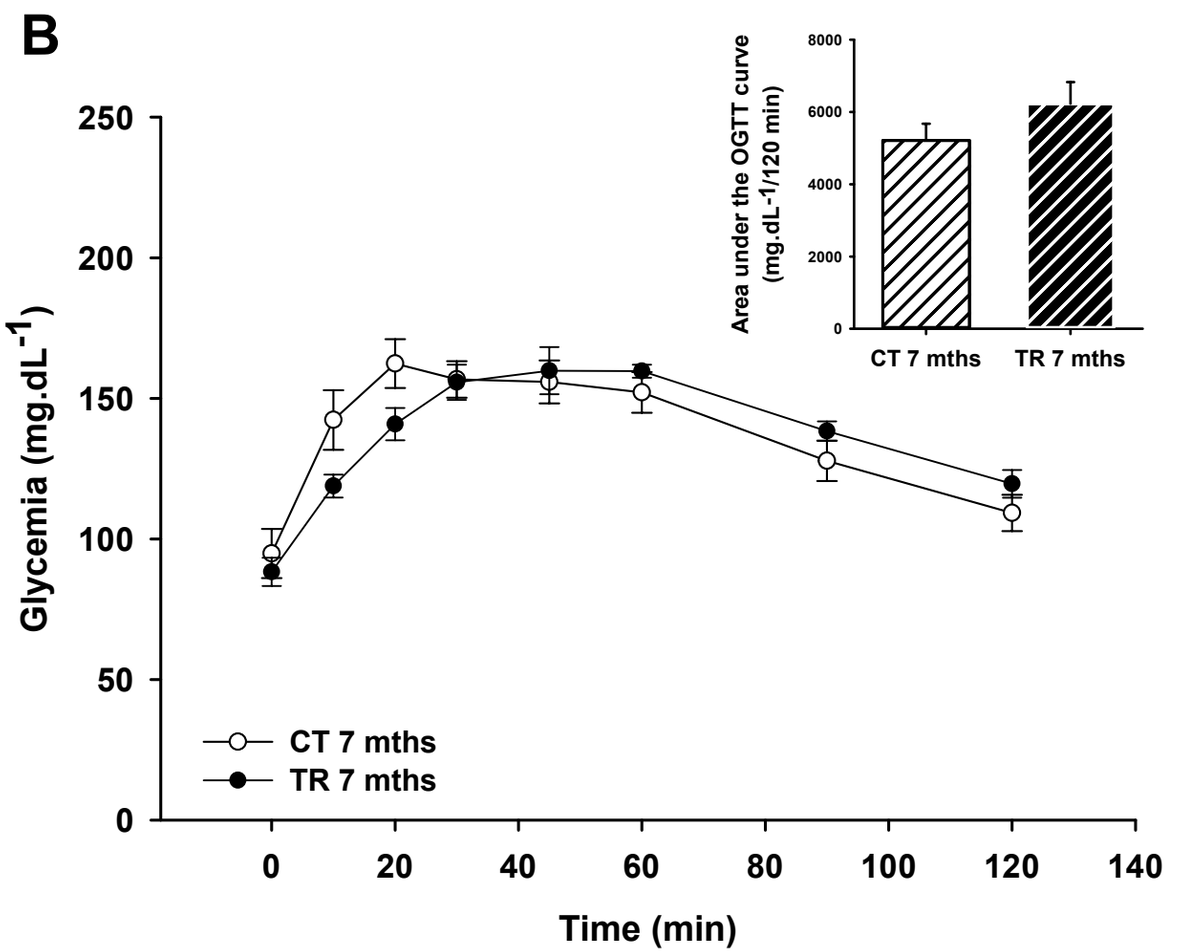
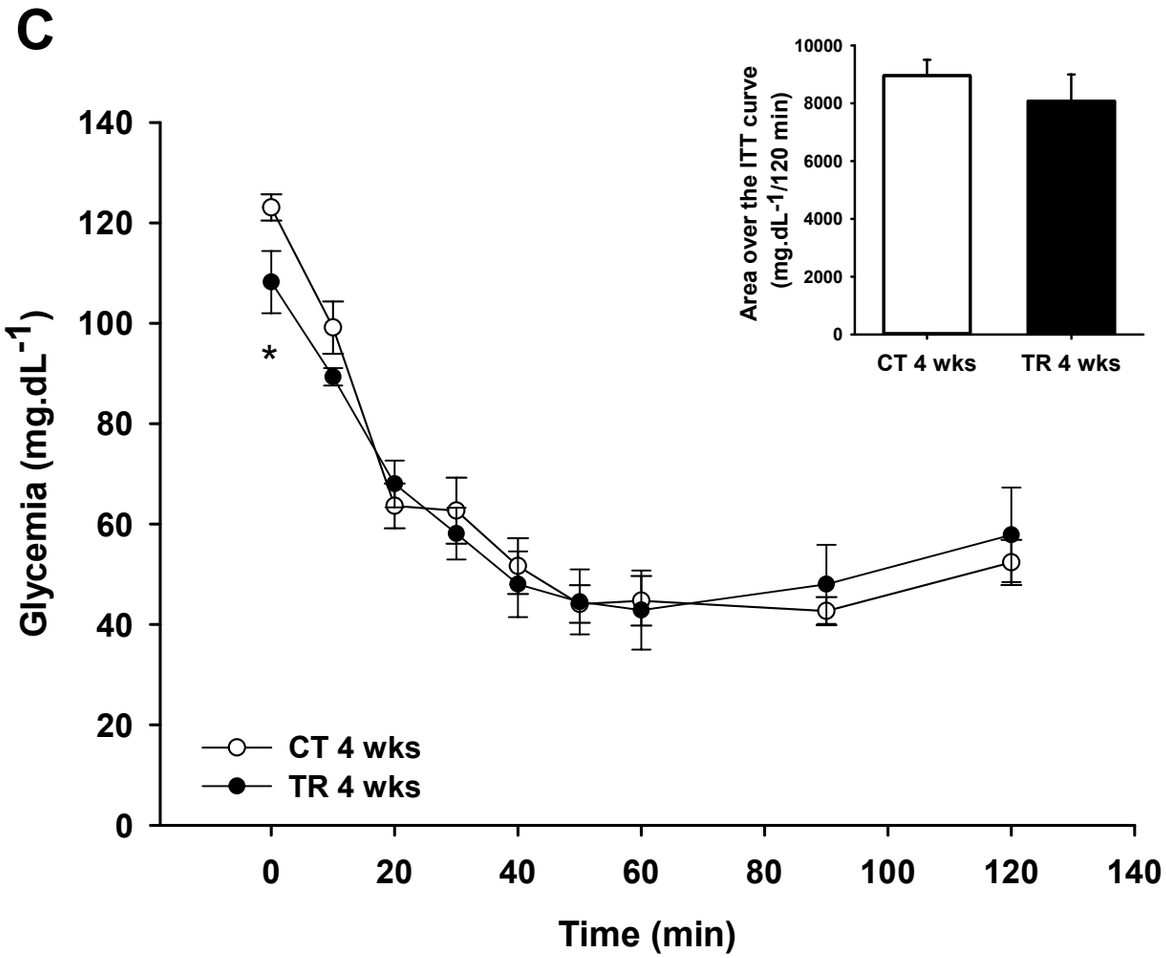

A
4 weeks
6h-fast glycemia (mg.dL-1)
160
140
120
100
80
60
*
CT
TR
B
3 weeks
16h-fast glycemia (mg.dL-1)
160
140
120
100
80
60
*
CT
TR
C
7 months
6h-fast glycemia (mg.dL-1)
160
140
120
100
80
60
CT
TR
D
7 months
16h-fast glycemia (mg.dL-1)
160
140
120
100
80
60
CT
TR

Figure 1


A
Glycemia (mg.dL-1)
Time (min)
CT 3 wks
TR 3 wks
*
Area under the OGTT curve (mg.dL-1/120 min)
CT 3 wks
TR 3 wks


B
Glycemia (mg.dL-1)
Time (min)
CT 7 mths
TR 7 mths
Area under the OGTT curve (mg.dL-1/120 min)
CT 7 mths
TR 7 mths


C
Glycemia (mg.dL-1)
Time (min)
CT 4 wks
TR 4 wks
*
Area over the ITT curve (mg.dL-1/120 min)
CT 4 wks
TR 4 wks

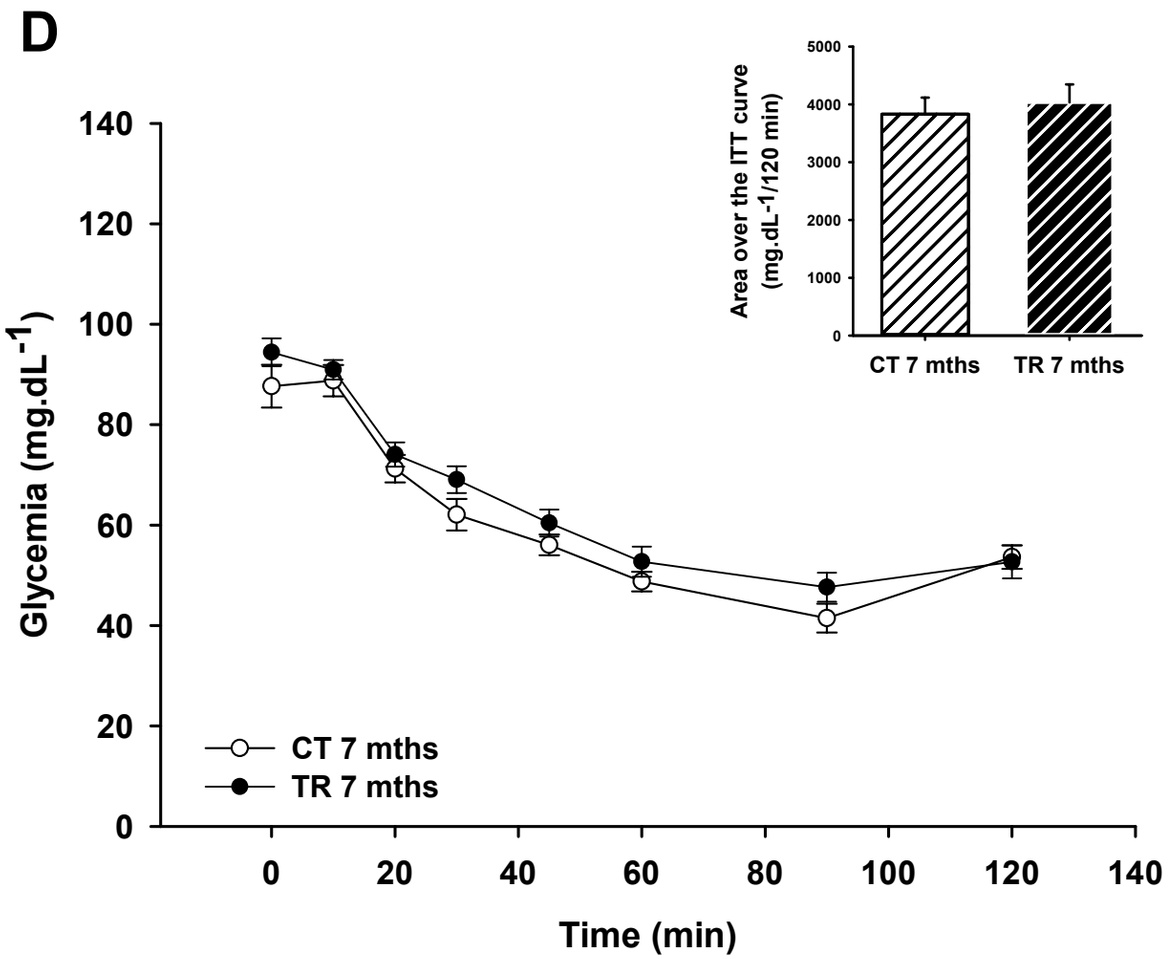

D
Glycemia (mg.dL-1)
Time (min)
CT 7 mths
TR 7 mths
Area over the ITT curve (mg.dL-1/120 min)
CT 7 mths
TR 7 mths

Figure 2

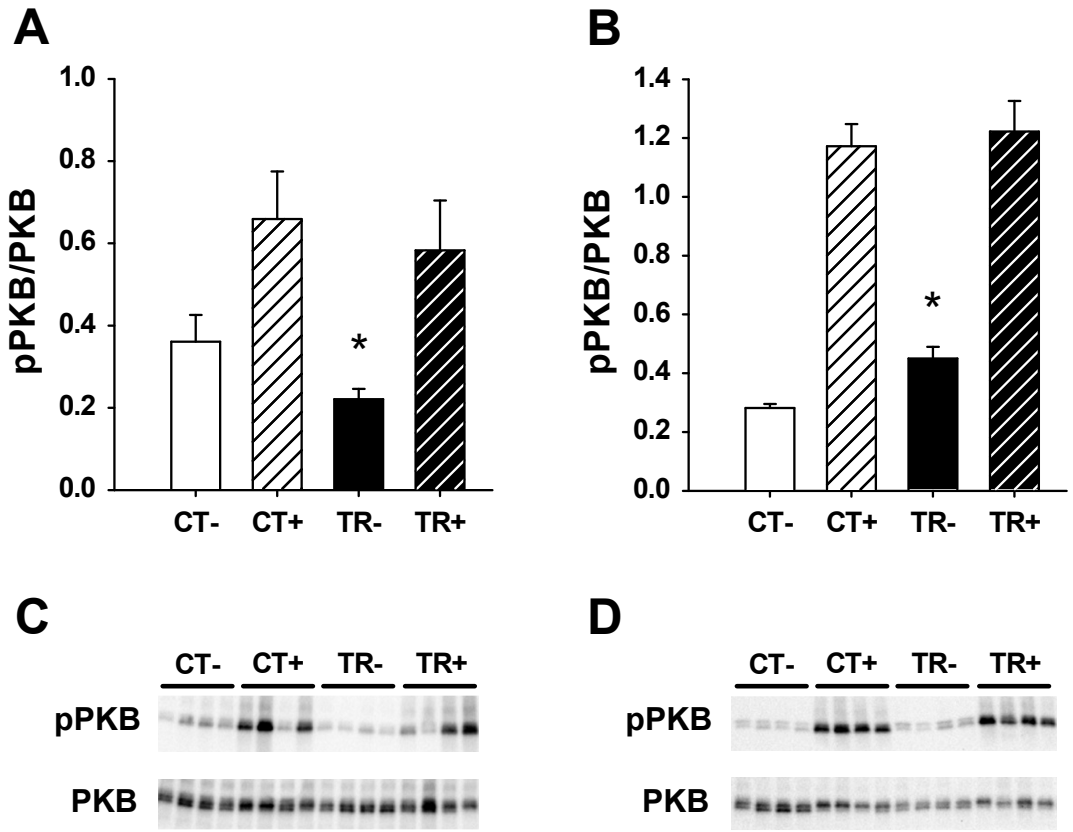

A
B
pPKB/PKB
1.0
0.8
0.6
0.4
0.2
0.0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
CT-
CT+
TR-
TR+
C
D
pPKB
PKB

Figure 3

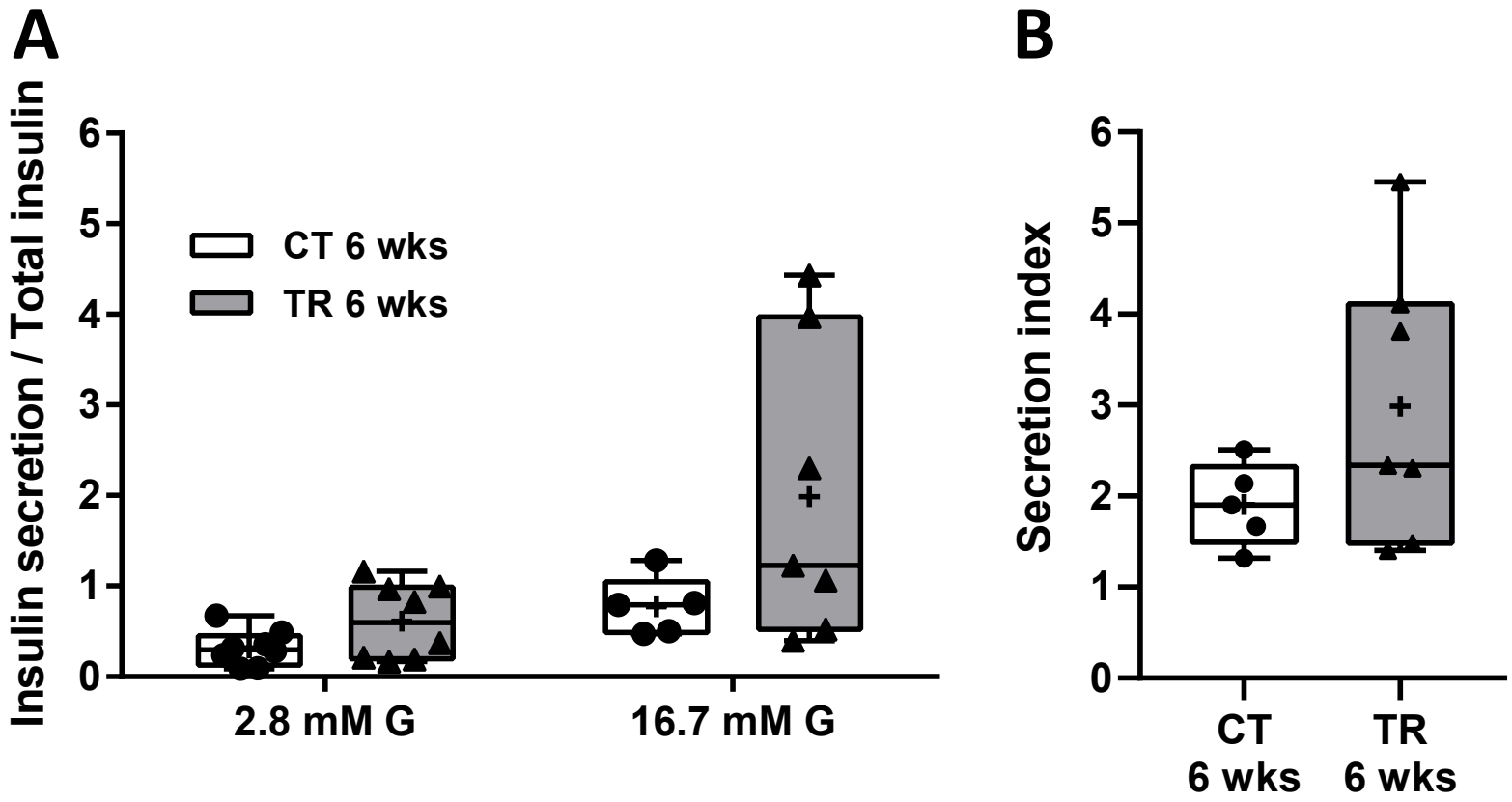

A
Insulin secretion / Total insulin
6
5
4
3
2
1
0
CT 6 wks
TR 6 wks
2.8 mM G
16.7 mM G
B
Secretion index
6
5
4
3
2
1
0
CT
6 wks
TR
6 wks

Figure 4