Exercise in obese female rats has beneficial effects on maternal and male and female offspring metabolism

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Abstract

BACKGROUND—Maternal obesity (MO) impairs maternal and offspring health. Mechanisms and interventions to prevent adverse maternal and offspring outcomes need to be determined. Human studies are confounded by socio-economic status providing the rationale for controlled animal data on effects of maternal exercise (MEx) intervention on maternal (F0) and offspring (F1) outcomes in MO.

HYPOTHESIS—MO produces metabolic and endocrine dysfunction, increases maternal and offspring glucocorticoid exposure, oxidative stress and adverse offspring outcomes by postnatal day (PND) 36. MEx prevents these outcomes.

METHODS—F0 female rats ate either control or obesogenic diet from weaning through lactation. Half of each group wheel ran (from day ninety of life through pregnancy beginning day 120) providing four groups (n=8/group) – i) controls, ii) obese, iii) exercised controls and iv) exercised obese. After weaning, PND 21, F1 offspring ate a control diet. Metabolic parameters of F0 prepregnancy and end of lactation and F1 offspring at PND 36 were analyzed.

RESULTS—Exercise did not change maternal weight. Before breeding, MO elevated F0 glucose, insulin, triglycerides, cholesterol, leptin, fat and oxidative stress. Exercise completely prevented the triglyceride rise and partially glucose, insulin, cholesterol and oxidative stress increases. MO decreased fertility, recovered by exercise. At the end of lactation, exercise returned all metabolic variables except leptin to control levels. Exercise partially prevented MO elevated corticosterone.

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F1 Offspring weights were similar at birth. At PND 36 MO increased F1 male but not female offspring leptin, triglycerides and fat mass. In controls exercise reduced male and female offspring glucose, prevented the offspring leptin increase and partially the triglyceride rise.

CONCLUSIONS—MEx before and during pregnancy has beneficial effects on maternal and offspring metabolism and endocrine function occurring with no weight change in mothers and offspring indicating the importance of body composition rather than weight in evaluations of metabolic status.

Keywords

Maternal obesity; exercise; programming

Institute of Medicine (IOM) guidelines on maternal obesity (MO) and pregnancy weight gain (http://www.nap.edu/catalog/12584.html)(1) reflect the need for information on mechanisms of adverse maternal and offspring metabolic outcomes resulting from MO before and during pregnancy to design effective interventions. The importance of the worldwide obesity epidemic is shown by the global numbers of obese individuals in developed and emerging countries. Nearly 1.5 billion people worldwide are overweight (BMI> 25 kg/m^2) or obese (BMI > 30 kg/m^2)(2). Obesity is an epidemic throughout the world including women of reproductive age(3, 4). WHO (www.who.int/nut/obs.htm) has declared obesity and associated diabetes in the top ten adverse worldwide health risks and the top five in developed nations.

MO increases maternal predisposition to gestational diabetes and obstetric complications (preeclampsia) and poor fetal (macrosomia, and stillbirth) and offspring outcomes (metabolic syndrome)(5). Extensive data on MO effects on pregnancy exist in rodents including adverse offspring metabolic phenotype(6, 7). The neonatal leptin surge in MO rat offspring is prolonged and amplified leading to leptin resistance, hyperphagia and obesity(6). Cafeteria diets program feeding behavior and junk food preference(8, 9). Importantly there are no published controlled rat studies on effects of maternal exercise on obese mothers or offspring.

Clinical investigations to determine interventions that improve maternal pregnancy health are in progress(10). The IOM report indicates a need for evidence-based interventions that inform and motivate pregnant women to adopt a healthy lifestyle before and during pregnancy. There is much interest in maternal exercise as an intervention(11–13). The extent to which the maternal metabolic phenotype resulting from MO and high calorie diets is preventable by maternal exercise interventions introduced before conception remains an unanswered question of physiological and clinical importance. A PubMed search with terms *obesity*, *rat*, *pregnancy* and *exercise* yielded only six publications: two from 1977, one a review(14) and the other pregnancy related(15); three reported pregnancy models of nutrient restriction, describing offspring not maternal exercise(16–18). The sixth addressed offspring junk food preference following maternal junk food consumption in pregnancy and lactation(8). This lack of clear, well-controlled experimental data on maternal exercise effects in rodents, the most commonly studied model, constitutes a barrier to design of

evidence-based protocols for management of obese women before and during pregnancy and identification of markers of benefit.

We hypothesized that MO and high fat diet (called MO since in human and animal studies it is difficult to produce obesity without altering diet) induce: 1) altered maternal metabolism; 2) increased maternal oxidative stress and production of reactive oxygen (ROS) and nitrogen (RNS) species; 3) increased maternal glucocorticoid production; 4) offspring pre-puberty metabolic dysfunction and 5) maternal exercise prior to and in pregnancy, at least in part, prevents MO induced maternal and offspring changes in an offspring sex dependent manner.

METHODS

Standardization of females used for the pregnancy study (F0)

To ensure homogeneity of mothers in all groups, female albino Wistar rats were only obtained from Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INNSZ), Mexico. All procedures were approved by the INNSZ, Animal Experimentation Ethics Committee. Animals were held in AAALAC accredited light controlled facilities and fed normal laboratory chow (Zeigler Rodent RQ22-5, USA) containing 22% protein, 5% fat, 31% polysaccharide, 31% simple sugars, 4% fiber, 6% minerals and 1% vitamins (w/w) energy 4.0 Kcal/g. At 15 to 17 weeks, weight 220 ± 20 g, females were bred to randomly assigned, non-litter mate, proven male breeders. At delivery on day 0 litters that provided F0 mothers were culled to 10 pups, each containing at least four females. At weaning (day 21) one female F0 pup from each litter was randomly assigned to one of two control groups that ate laboratory chow or one of two MO groups fed a high energy, obesogenic diet (23.5% protein, 20% animal lard, 5% fat, 21% polysaccharide, 21% simple sugars, 5% fiber, 3.5% mineral mix, 1% vitamin mix (w/w), energy 4.9 Kcal/g). Thus each F0 group had only one female from any litter and F0 females in different groups, but not within groups, were sisters, providing homogeneity in F0 mothers' own developmental programming and genetics.

At day 90, one month before breeding, one of the two C and one of the two MO groups were randomly selected to continue their diet and begin wheel-running exercise (C exercised – CEx; MO exercised - MOEx). The opportunity to wheel run was available through pregnancy. Remaining females continued on their respective diets during pregnancy and lactation (Figure 1A).

Voluntary Exercise

F0 CEx and MOEx rats were trained to wheel run (15 min sessions) on two days the week before day 90. A pilot study established an optimum running schedule of 15 min which they always completed, followed by 15-minute rest and a second 15-minute run between 9 AM to 12 PM. Throughout the study rats were allowed two non consecutive rest days weekly. Prior to pregnancy all rats completed 30 minutes running. During pregnancy rats ran for only one 15-minute session daily. Voluntary exercise varied in late gestation, some animals did not run the day before parturition, others completed the schedule until parturition. Distances run were quantified using a bicycle odometer. Mothers did not exercise while nursing.

Measurement of pre-breeding F0 food intake (day 90 to 120)

Rats from the same experimental group were housed three per cage. Food provided and remaining after 24h was weighed daily and consumption averaged per rat.

Maternal obesity phenotype, blood parameters and breeding

In calculating numbers of F0 to breed we used past fertility rates of 75% (controls) and 40% or less (obese mothers) (C=12, CEx=10, MO=32 and MOEx=22).To study the F0 phenotype pre-breeding, at day 120, six rats per group and end of lactation, 8 rats per groups, were fasted for 4 hours and euthanized by decapitation (Rodent guillotine. Thomas Scientific, USA), trunk serum obtained and stored at −20°C, retroperitoneal fat excised and weighed. Female rats were placed with proven males on day 120 and conceived the next cycle. Lactating mothers were maintained on their pregnancy diet and offered the wheel but they showed no desire to exercise. Litter size and pup weight were recorded at birth. Ano-genital distance was measured to identify males and females(19). Litters over 14 were excluded. To ensure F1 offspring homogeneity, on postnatal day (PND) 2 all litters studied were adjusted to 10 pups with equal numbers of males and females wherever possible. At PND 2 pups die if removed for fasting blood thus pups were taken directly from mothers, blood was pooled from offspring killed by decapitation to equalize litters to 10. Offspring were weaned at PND 21, housed 5/cage and fed Chow diet (Figure 1).

Offspring phenotype

To avoid maternal milk influences F1 offspring were studied pre-puberty at PND 36. At offspring PND 36, one male and one female offspring per litter (n= 8) were randomly chosen, fasted 4 h and euthanized by decapitation, trunk blood obtained and fat depots excised and weighed.

Blood measurements

Glucose, triglycerides, cholesterol enzymatically by Synchron CX auto analyser (Beckman Coulter, Co.), insulin, leptin, corticosterone, testosterone and estradiol were determined by RIA(19–21). Homeostasis model assessment (HOMA) was calculated from HOMA=glucose (mmol/L) × insulin (μU/mL).22.5^{-1}(19).

F0 serum and liver ROS measurement at breeding at day 120

F0 Serum and tissue were frozen at −70°C until analyzed. 100 mg tissue was homogenized with 1 mL of cold 0.9% saline solution and protein quantified by Bradford(22). Serum and tissue malondialdehyde (MDA) were measured by thiobarbituric acid spectrometry assay (TBA)(23). Liver data were normalized to protein concentration. Intra- and inter-assay CVs were < 3 and < 5%, respectively.

ROS Assay

ROS formation was estimated according to a previous report(24) adapted for tissue homogenates and expressed as nmoles DCF formed per mg protein.minute^{-1}(24).

Super Oxide Dismutase (SOD) Activity—SOD activity was measured by commercial kit (RANSOD by RANDOX; Cat. N*o*. SD125 and SD126) and normalized to protein concentration. Intra- and inter-assay CVs were < 7 and < 8%, respectively.

Glutathione Peroxidase (GPx) activity—was measured by commercial kit (RANSEL by RANDOX; Cat. Mo. RSD504). Data were normalized to protein concentration. Intra- and inter-assay CVs were < 3 and < 5%, respectively.

Nitrotyrosine (NT) Histology

5 μm liver paraffin sections were immunostained using methods that have been described previously in detail(25) with affinity-purified mouse monoclonal antibody anti-NT (sc-101358) 1:1000 dilution, ABC elite kit, Vector Laboratories and visualized using 2.5% nickel sulfate sith 0.02% DAB chromogen in 0.175 M sodium acetate.

Statistical analyses

To avoid adverse influences for a single mother in all studies females for each group came from different litters. Data mean ± SEM. Prior to pregnancy (F0 day 90) there were only two groups compared by Student's unpaired t-test. Analysis of maternal diet and exercise intervention effects was by two-way ANOVA.

RESULTS

F0 Morphometrics and changes in MO

When weaned body weights were similar in females randomized to the four groups (C=50±3, MO 49±2 g). At day 77, 56 days after high fat diet initiation was the first day body weight differed between C (232±4 g) and MO (248±3 g) groups. When exercise began at day 90 non-pregnant MO females were 11 % heavier than C (C=253±4, MO=280±3.3 g), at breeding (day 120) MO and MOEx were 17% and 19% heavier than respective C groups and at delivery MO 5% and MOEx 10% heavier than their respective C group. Maternal weight gain in pregnancy was reduced (p=0.05) in MO (124±9 g) compared with C (146± 6 g). Maternal weight after delivery was greater in MO than C mothers but similar at the end of lactation. Exercise had no effect on maternal weight at any stage except for CEx which decreased body weight compared with C at parturition and at the beginning of lactation (Fig 1B).

F0 Food and calorie intake—Using averaged data, calorie intake per day was similar in all four groups - C, 76.5±0.4, CEx = 75.1±0.02, MO = 70.2±0.1 and MOEx = 70.8±0.3 Kcal.day[-1]. Pre-pregnant exercise between day 90 and 120 did not affect F0 calorie or food intake.

F0 Exercise before and during pregnancy

Before pregnancy, average distance run per 30 minute session and total distance run between day 90 and 120 were similar between groups as was average distance run per 15minute session between 0 to 8 d and 9 to 15 d of pregnancy. However, between 16 d and delivery MOEx ran further than CEx (CEx 26±7, MOEx 61±8*m). The same differences

were observed in total distance run (CEx 137±34, MOEx 363±53* m; n= 8.* p< 0.05 vs CEx group). Both groups ran less in the third than in the first and second part of pregnancy (supplementary Fig 1).

F0 Metabolic parameters before breeding

MO increased maternal insulin, glucose, HOMA, leptin, triglycerides, cholesterol and retroperitoneal fat before breeding (Fig 2 A–G). Exercise completely prevented the MO induced triglyceride increase and partially prevented the cholesterol, glucose, insulin and HOMA increases without affecting leptin or retroperitoneal fat (Fig 2D,G).

Maternal obesity and fertility—MO decreased fertility rate from 75% to 25%. Exercise completely prevented this decrease (Fig 2H).

Maternal metabolism at the end of lactation

At the end of lactation maternal insulin, glucose, HOMA, leptin, triglycerides and cholesterol were elevated in MO (Fig 3A–F). Exercise did not affect these variables in C mothers but returned all MO variables except leptin to the normal range. Leptin levels were partially reduced in MO by exercise (Fig 3D).

F0 oxidative stress before breeding

Before breeding, F0 serum and liver MDA and ROS, SOD and GPx activities were increased in MO compared to C (Fig 4). Exercise had no effect on these variables in C except GPx activity, which was reduced in CEx. MO exercise partially prevented MDA serum and MDA, ROS and SOD liver changes (Fig 4A, B, C and D) without affecting GPx activity (Fig 4E).

Before breeding MO increased liver NT. Exercise decreased NT to similar low levels in MOEx and CEx (Fig 4F).

F0 steroid hormones before breeding and the end of lactation

MO increased serum corticosterone before breeding at day 120 and exercise decreased C and MO corticosterone but did not normalize MO values (Fig 5A). Corticosterone was still elevated in MO at the end of lactation and exercise reduced corticosterone in C and MO mothers (Fig 5B). Before pregnancy, MO decreased serum testosterone. Exercise had no effect on MO or C. By the end of lactation exercise increased testosterone serum in CEx compared to C. Serum testosterone was similar in C and MO (Fig 5D). MO decreased serum estradiol before breeding; MOEx partially prevented this decrease (Fig 5E). By the end of lactation serum estradiol was lower in MO and MOEx than C (Fig 5F).

F1 Offspring birth parameters

Litter sizes (C 11.6±0.9, CEx 10.4±0.7, MO 11.4±0.5 and MOEx 11.8±0.9 pups/litter), litter weight (C 65±5, CEx 58±4, MO 62±3 and MOEx 63±5 g), body weight (C 5.6±0.04, CEx 5.4±0.08, MO 5.4±0.06 and MOEx 5.5±0.1 g) and sex ratio were similar in C and MO at birth.

MO increased F1 neonatal (PND 2) serum corticosterone and exercise decreased both C and MO F1 corticosterone but MOEx did not reach C values: (C 689±12a, CEx 559±42b, MO 1087±44c and MOEx 881±24d ng/ml; p<0.05 for data not sharing a letter).

Effect of maternal obesity and exercise on PND 36 male and female F1 offspring metabolism

In both male and female F1 offspring PND 36 body weight, cholesterol and insulin were similar between the groups. MO increased male offspring leptin, triglycerides and fat mass (Fig 6D, E, F) but not females. Exercise in MO prevented the male offspring MO leptin increase and partially prevented the increased triglycerides. Maternal exercise in C reduced male and female F1 offspring glucose and male HOMA (Fig 6B, C, H, I).

DISCUSSION

Extensive literature exists on exercise effects in human pregnancy(26, 27). A recent Cochrane Review's concluded that although regular aerobic exercise in pregnancy appears to confer benefit, there is insufficient evidence to determine risks and benefits(28). Exercising before and during pregnancy reduces the incidence of gestational diabetes(29). In one study on healthy pregnant women of BMI 25.5±4 Kg/m^2, regular exercise in pregnancy reduced birth weight unaccompanied by altered maternal insulin sensitivity(30). This finding would indicate that factors other than maternal metabolic variables may regulate fetal macrosomia in obese pregnancies, at least in women borderline between normal and overweight. Voluntary exercise in lean pregnant rats from 42 days before pregnancy to day 19 of gestation and daily distance run of 3 kilometers(31) reduced placental weight and increased placental efficiency, increased circulating antioxidant factors, VEGF and augmented endothelial mediated vascular relaxation. Exercise training before and during pregnancy attenuates placental ischemia and oxidative stress in the reduced uteroplacental perfusion in the rat(32).

Effects of a carefully controlled maternal exercise regimen on the three important physiological mechanisms studied – metabolism, ROS/RNS and glucocorticoid function, have not been reported in MO in any species. To address our hypotheses on mechanisms that produce adverse effects on MO pregnancy and potential value of exercise before and during pregnancy, we used our established rat MO model before and during pregnancy(7, 21). Exposure of non-pregnant females to the MO diet from weaning to breeding established a dysfunctional metabolic phenotype (elevated glucose, insulin and HOMA), increased ROS and RNS production and elevated corticosterone. An obese maternal phenotype results from multiple and complex interactions between the mother's genetic predisposition, programming by gene-environment interactions during her own development pre- and post-natally, her diet, life-style and socioeconomic factors present prior to pregnancy. This mechanistic complexity results in many confounding factors in interpretation of human epidemiologic data and demonstrates the need for precisely controlled animal investigations to determine mechanisms and efficacy of interventions to direct both further human epidemiological studies and translation to clinical management. We rigorously controlled

both genetic stock and maternal phenotype from her own conception with homogeneity of all F0 females to minimize transgenerational programming(33).

Though distance run in exercised CEx and MOEx F0 females pre-pregnancy was similar, surprisingly in the end of pregnancy MOEx ran further than CEx. One potential explanation of this interesting difference is that the estrogen levels were lower in the MO mothers. One study in obese Latino adolescents showed negative correlation with estrogen levels and physical activity(34). In addition the importance of controlling C phenotype is shown by the observation that maternal exercise in C group decreased weight, cholesterol and GPx, NT and corticosterone in F1 females indicating that even controls are affected by experimental protocols. These interesting findings have implications for optimal interactions of maternal exercise regimens, body weight and composition.

Maternal metabolic effects

Excessive gestational weight gain has been associated with adverse maternal pregnancy outcomes with maternal pre-pregnancy BMI a major maternal and offspring outcome determinant(35). While the definition of 'excessive' gain differs according to pre-pregnancy BMI(36), mothers who gain more than recommendations experience more gestational diabetes, pre-eclampsia, macrosomia and stillbirth(37). In our study MO mothers gained less weight during pregnancy than C without changes in offspring birth weight. In our study maternal exercise improved maternal carbohydrate metabolism but signs of dysfunctional carbohydrate metabolism remained, suggesting the level of exercise, commencing even before pregnancy could not completely suppress the effects of MO. Further studies are required to determine interactive effects of longer activity periods, different energy expenditures and combination with dietary intervention.

Although exercise improved triglycerides and cholesterol in F0 MO females, retroperitoneal fat mass and leptin were unchanged. Care is necessary drawing conclusions from this interesting finding as exercise may have different effects on different fat depots. We would pose the testable hypothesis that exercise induced mechanisms change some adipocyte metabolic pathways leaving others, e.g. leptin production, unchanged. A different exercise regimen may be required to modify them. By the end of lactation all metabolic variables had returned to control levels except leptin, supporting this hypothesis. Importantly short periods (30 minutes a day) of exercise for one month clearly provides benefits to both mother and offspring.

Maternal oxidative stress

In the present study, pre-pregnant MO animals increased serum MDA (a lipid-peroxidation product) and liver and hepatic ROS, superoxide dismutase, and GPx. Increased anti-oxidant enzymes may represent compensation to decrease ROS. Exercise in MO, partially prevents this increase, indicating that exercise in MO benefits maternal metabolism. Pre-pregnancy MO increased liver NT prevented by exercise which also reduced NT in C. ROS/RNS are highly reactive free radicals containing an unpaired electron(38) that reacts with another free radical producing other molecules with unpaired electrons, e.g. peroxynitrite. While ROS/RNS have documented pathological effects they may also have physiological

functions. For example, they participate in responses to small *"physiological"* levels of hypoxia, are involved in apoptosis and mitosis(39) and maintain lung ventilation-perfusion matching(40). ROS/RNS do not readily cross cell membranes and local effects depend on vulnerability of cell components and balance of many physiological and pathological systems including glucocorticoids. The importance of balance is shown by a study in which overproduction of the antioxidant enzyme, GPx initially produces beneficial changes in key pancreatic islet genes PDX1 and UCP2, followed by chronic hyperinsulinemia(41). Under normal circumstances ROS/RNS are continually produced by pro-oxidant mechanisms(39, 42) balanced by removal by antioxidant defenses - superoxide dismutase, catalase and GPx, and scavenger molecules - vitamins E and C and melatonin(39). In addition to potential physiological roles for ROS, compelling evidence exists that ROS play a central role in MO induced damage(43). Physical activity reduces NADPH oxidase activity and superoxide anion production, decreasing ROS generation(44). MO results in ROS induced changes in mouse oocytes(45). This observation and others on increased ROS in obesity indicates the need to study ROS as a potential mechanism underlying adverse maternal and offspring MO induced outcomes.

Maternal steroid hormones

Glucocorticoids stimulate human adipocyte differentiation and proliferation(46). In our study, MO increased maternal corticosterone before pregnancy and at the end of lactation. Maternal exercise decreased corticosterone at breeding and end of lactation but not to control levels. While the most likely source of maternal corticosterone is the adrenal, adipose tissue 11β-hydroxysteroid dehydrogenase-1 reductase can generate active corticosterone from inactive dehydrocorticosterone(47). Our findings lead us to hypothesize a feed-forward cycle in MO in which local adipose tissue corticosterone production feeds back to further increase obesity and corticosterone production. Exercise helps to break this unwanted cycle. The observation of increased maternal and offspring glucocorticoids may be considered as a common factor in the light of the proposal by some workers in the field that increased glucocorticoid are a common factor in many situations of developmental programming. This has been called the "Gatekeeper Hypothesis"(48).

Obesity reduced fertility in women(49) and rodents(50). Women who only exercise experience small weight loss and only when combined with a low-calorie diet(49). Diet, exercise and behavioral life-style modifications improve fertility in obese patients(49), but exercise benefits on reproductive function are greater than the benefits of diet(51). In the present study exercise in MO rats exerts beneficial effects on fertility without affecting weight but improves metabolism, showing the importance of body composition rather than weight in determining metabolic phenotype. Decreased maternal estrogen pre-pregnancy may account for the observed infertility. Estrogen levels are still low in obese mothers after lactation, and probably also during pregnancy with unwanted consequences for key maternal and placental functions e.g. uterine blood flow. The exercise induced return towards normal estradiol levels at conception may thus be beneficial. The observation of lowered maternal testosterone merits further study since maternal testosterone is associated with behavioral differences between male and female offspring(52).

We recently published a dietary intervention study in this model(7) in which a group of obese females was transferred to normal chow 30 days before mating, the same stage at which we introduced exercise here. In our previous dietary intervention study maternal body weight at breeding was partially recuperated whilst in the present study exercise in MO did not alter maternal body weight. For both models, mothers undertaking the intervention presented a better fertility, metabolic and hormonal maternal environment than MO, and both maternal interventions improved the adverse offspring metabolism outcomes produced by MO in diverse ways suggesting dissimilar maternal mechanisms.

Effects on offspring metabolism

The goal of interventions in MO pregnancy is to improve both maternal and offspring outcomes. In the present study, MO affects F1 male offspring but not female metabolism indicating sex dependent programming by MO. F0 Exercise in C and in MO benefits offspring metabolism. Carter et al showed improved glucose disposal in female and male adult offspring of exercised lean mice indicating that maternal exercise can affect the offspring. Male, but not female, offspring of exercised mothers had increased percent lean mass and decreased fat mass percent compared to male control offspring(53). These offspring metabolic phenotype effects show similarities to our observed outcomes.

In conclusion, the results reported in the current study of an exercise intervention help to address issues raised in the IOM report and are essential in determining mechanistic maternal targets with potential to develop predictive and clinical tools in human pregnancy(54). Different interventions to improve outcomes may act through different mechanisms and combined approaches may lead to better results for both mother and offspring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Abbreviations

BMI	Body Mass Index
C	Control
CEx	C exercised
DCF	Dichlorofluorescein
DINT	Dietary intervention

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GPx	Glutathione Peroxidase
HFD	High Fat Diet
HOMA	Homeostasis Model Assessment
IOM	Institute of Medicine
INNSZ	Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
MDA	Malondialdehyde
MO	Maternal Obesity
MOEx	MO exercised
NT	Nitrotyrosine
PND	Postnatal day
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid spectrometry assay
TG	Triglycerides
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

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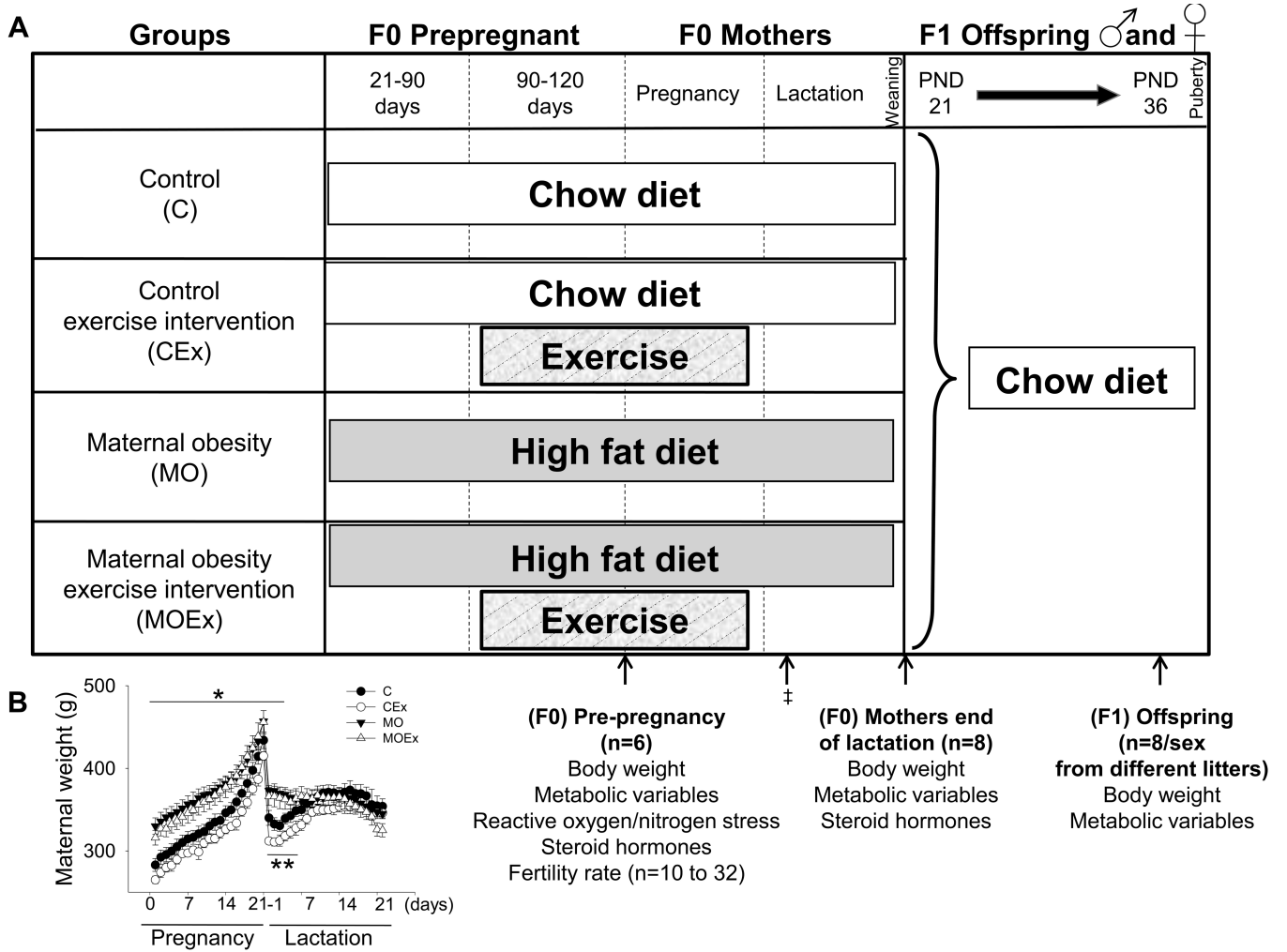
Fig. 1.
A) Time line for study on exercise intervention in maternal obesity; ‡ corticosterone at postnatal day 2 (F1) offspring. **B)** Maternal body weight (g) during pregnancy and lactation. C = control, CEx = Control + exercise, MO = Maternal obesity, MOEx = Maternal obesity + exercise. Data are mean ± SEM, n= 8; * p< 0.05 C and CEx vs MO and MOEx; ** CEx vs C, MO and MOEx.
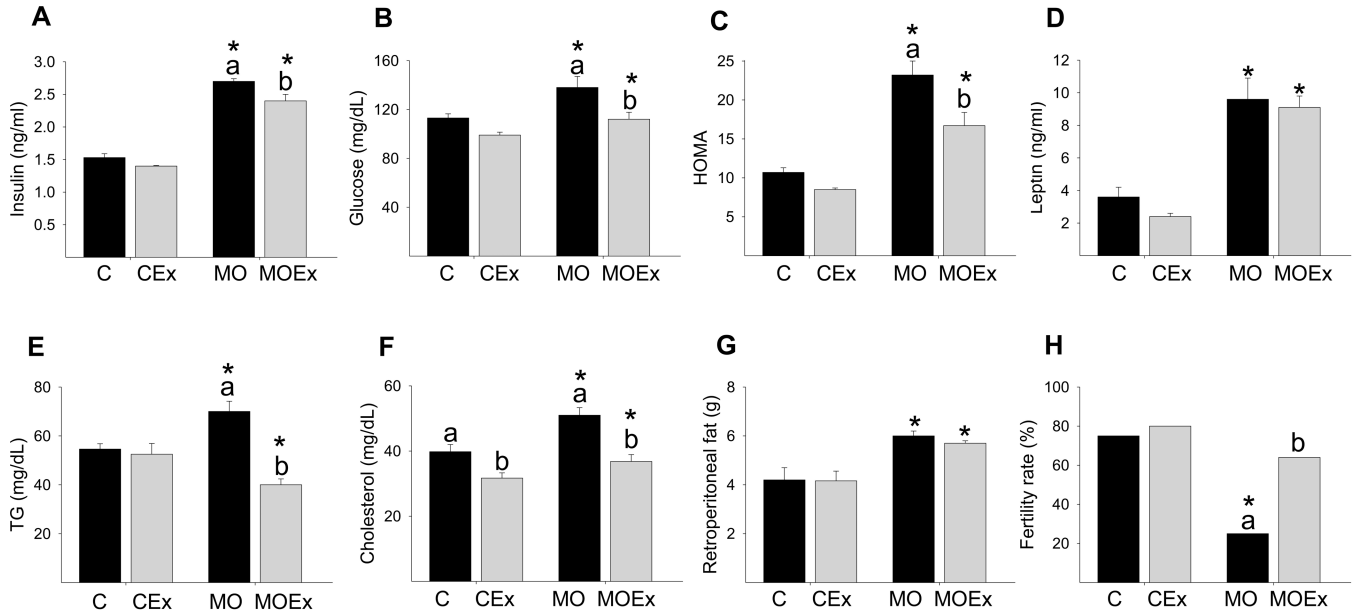
Fig. 2. F0 Metabolism at breeding and fertility
A) Insulin, **B)** glucose, **C)** HOMA, **D)** leptin, **E)** triglycerides, **F)** cholesterol, **G)** retroperitoneal fat and **H)** fertility rate in four groups – breeding at 120 d. C = control, CEx= Control + exercise, MO = Maternal obesity, MOEx = Maternal obesity + exercise. Data are mean ± SEM; pre-pregnancy n=6; the fertility rate is expressed as number of pregnancies/ numbers of animals in the groups C=9/12, CEx=8/10, MO=8/32, MOEx=14/22; * p< 0.05 vs effect of diet between C and MO or CEx and MOEx groups; p<0.05 for different letters between C and CEx or MO and MOEx.
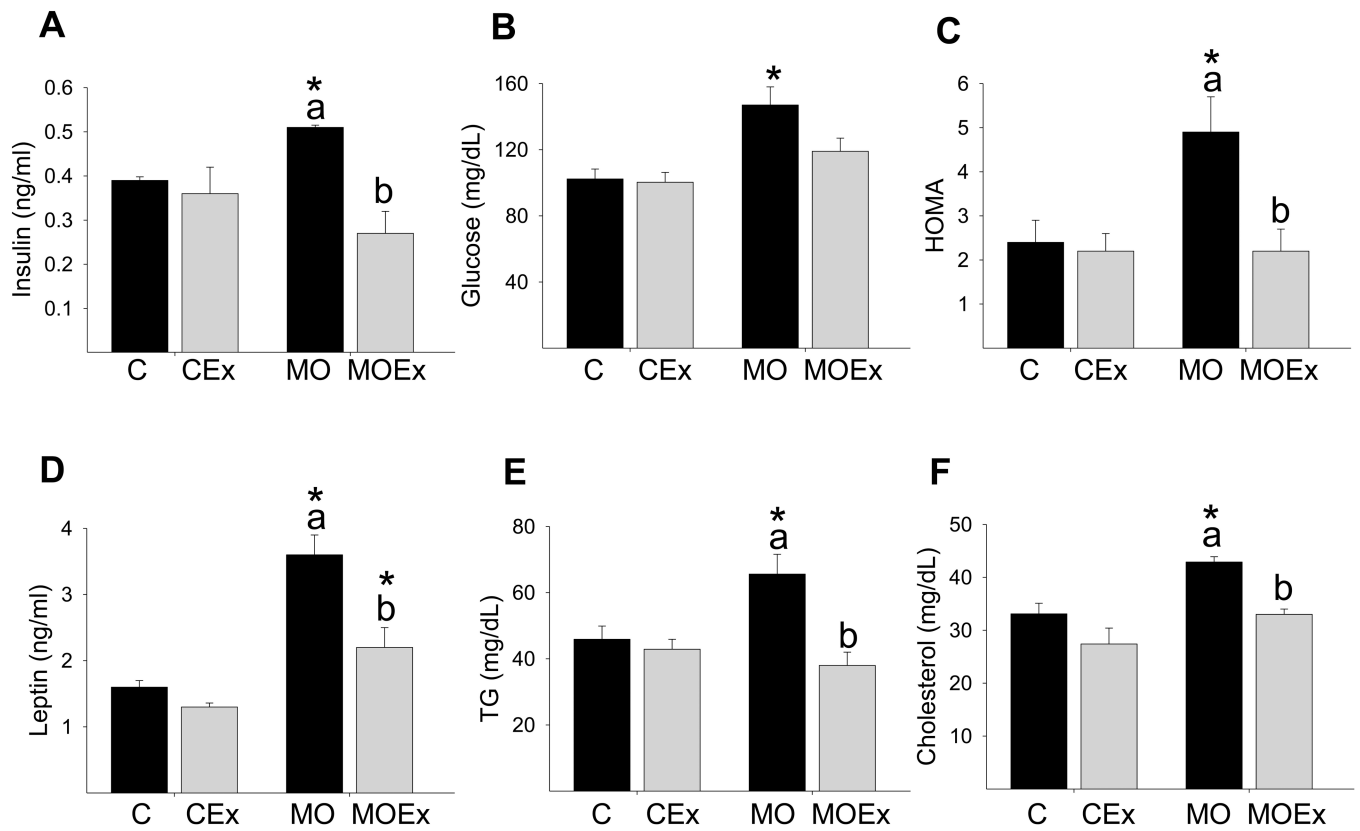
Fig. 3. F0 Maternal metabolism at the end of lactation when offspring are weaned
A) Insulin, **B)** glucose, **C)** HOMA, **D)** leptin, **E)** triglycerides and **F)** cholesterol. C = control, CEx= Control + exercise, MO = Maternal obesity, MOEx = Maternal obesity + exercise. Data are mean ± SEM; n=8; * p< 0.05 vs effect of diet between C and MO or CEx and MOEx groups; p<0.05 for different letters between C and CEx or MO and MOEx.
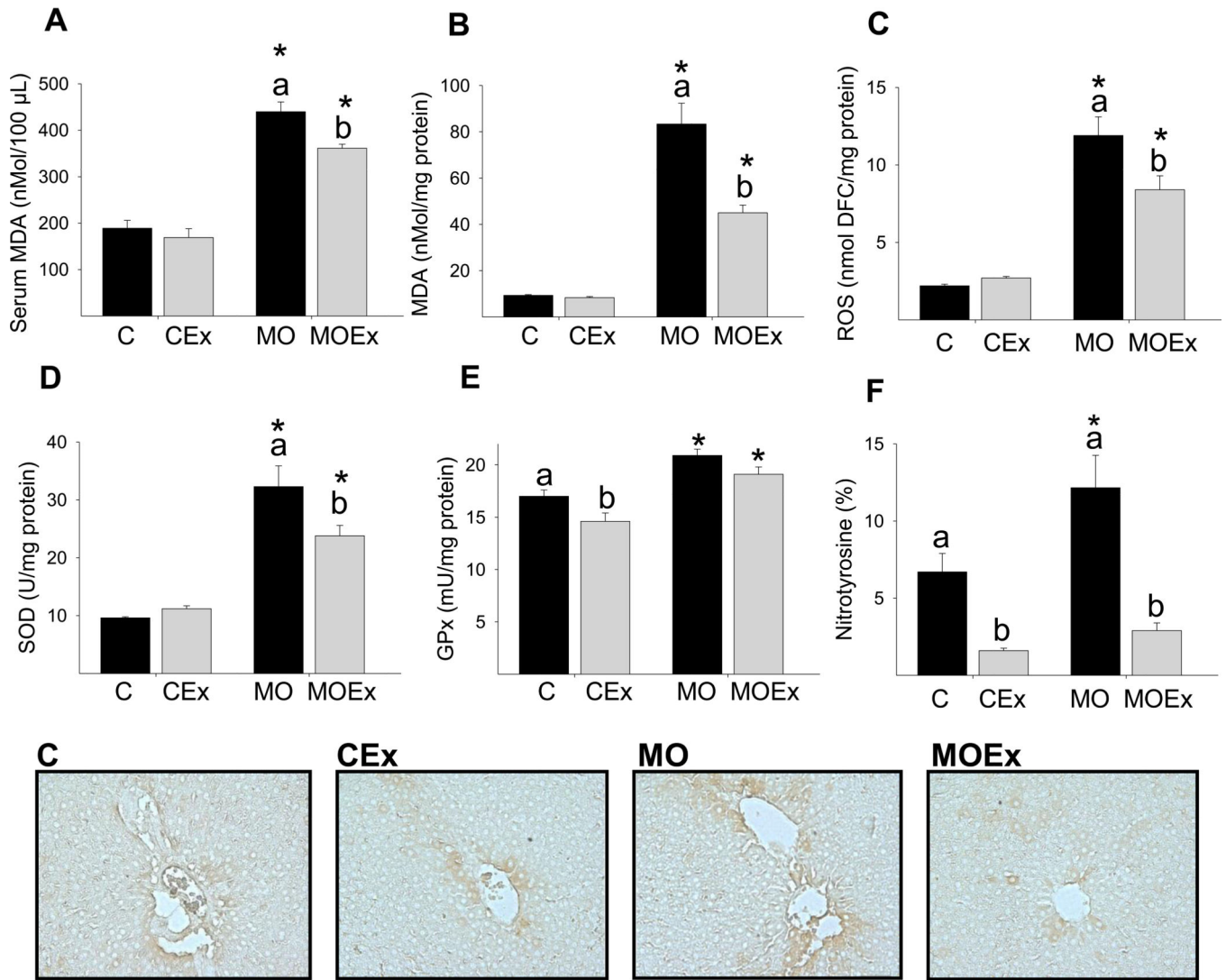
**Fig. 4. F0 reactive oxygen and nitrogen stress at breeding at day 120**
A) serum malondialdehyde (MDA), **B)** maternal liver MDA, **C)** maternal liver reactive oxygen species (ROS), **D)** maternal liver superoxide dismutase (SOD) (U/mg protein), **E)** maternal liver glutathione peroxidase (GPx) (MU/mg protein) and **F)** maternal liver nitrotyrosine fraction stained. Histology shows immunoreactive maternal liver nitrotyrosine. C = control, CEx = Control + exercise, MO = Maternal obesity, MOEx = Maternal obesity + exercise. Data are mean ± SEM; n = 6 (A to E) and n=5 (F) per group; * $p < 0.05$ vs effect of diet between C and MO or CEx and MOEx groups; $p < 0.05$ for different letters between C and CEx or MO and MOEx.
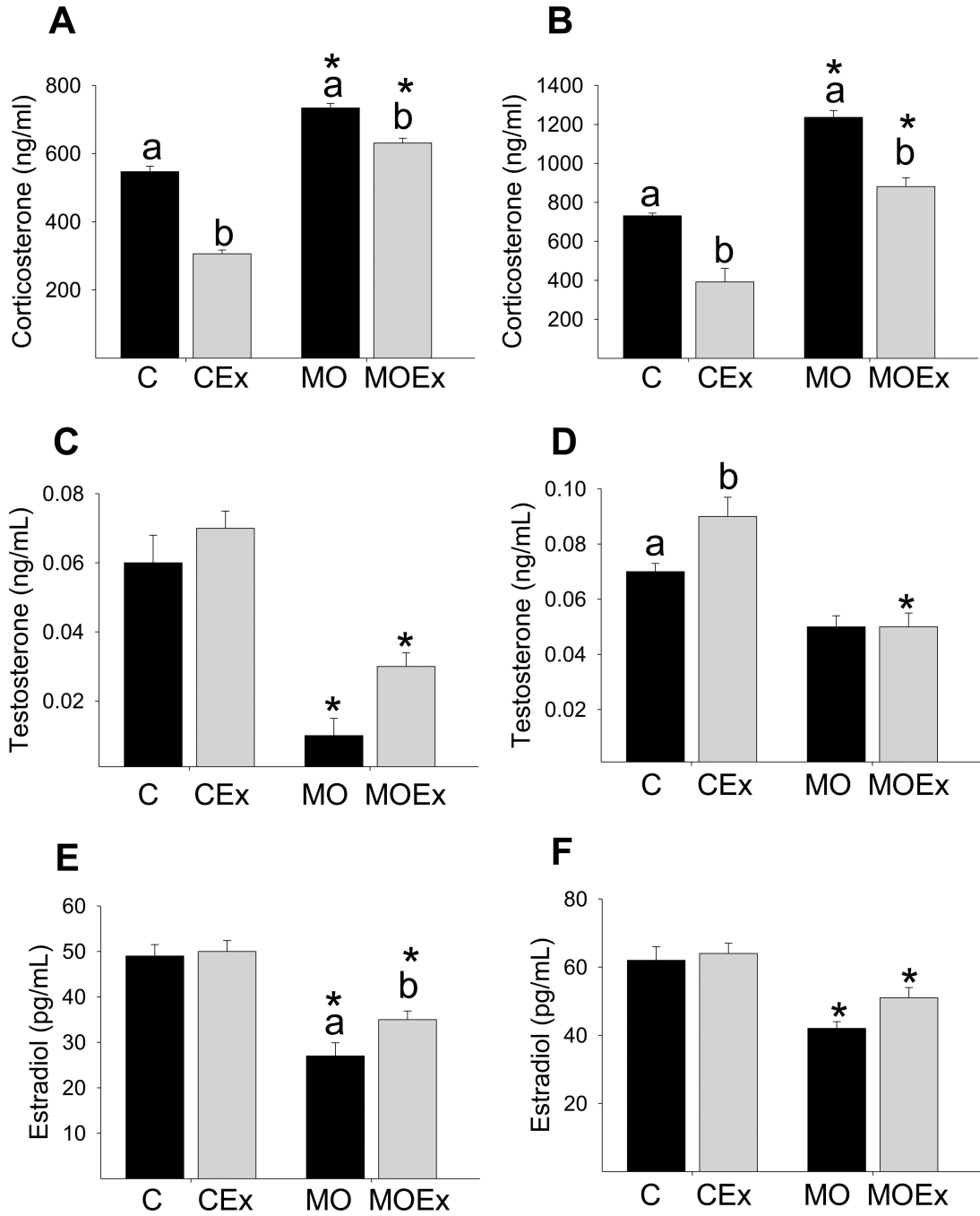
Fig. 5. F0 steroid hormones: Pre-pregnancy hormones
A) corticosterone, **C)** testosterone, **E)** estradiol serum. **F0 Maternal hormones at the end of lactation B)** corticosterone, **D)** testosterone and **F)** estradiol. C = control, CEx = Control + exercise, MO = Maternal obesity, MOEx = Maternal obesity + exercise. Data are mean ± SEM; pre-pregnant n = 6, end of lactation n=8 per group; * p< 0.05 vs effect of diet between C and MO or CEx and MOEx groups; p<0.05 for different letters between C and CEx or MO and MOEx.

MALE
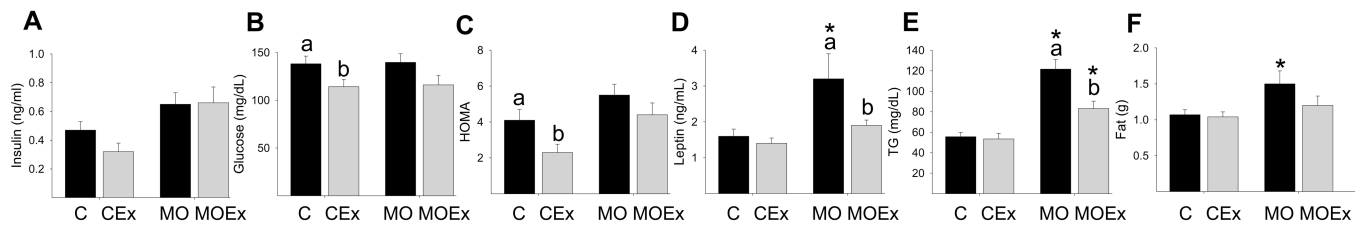
FEMALE
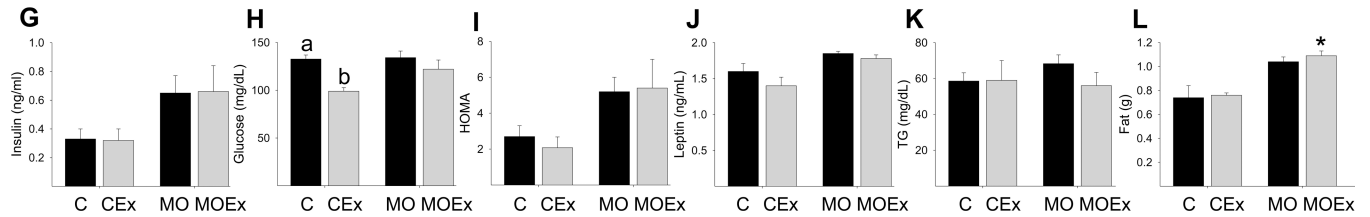
Fig. 6. F1 Offspring metabolism at postnatal day (PND)36
Male offspring A) insulin, **B)** glucose, **C)** HOMA, **D)** leptin, **E)** TG and **F)** fat. **Female offspring G)** insulin, **H)** glucose, **I)** HOMA, **J)** leptin, **K)** TG and **L)** fat. C = control, CEx = Control + exercise, MO = Maternal obesity, MOEx = Maternal obesity + exercise; for all groups n=8; Data are mean ± SEM. * p< 0.05 vs effect of diet between C and MO or CEx and MOEx groups; p<0.05 for different letters between C and CEx or MO and MOEx.