Adult exercise effects on oxidative stress and reproductive programming in male offspring of obese rats

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Submitted 18 September 2014; accepted in final form 9 December 2014

The incidence of obesity has increased substantially worldwide over recent years and obesity is now recognized as a major, universal health problem by organizations such as the World Health Organization (WHO) (53) with over 1.1 billion individuals throughout the world classified as obese (37). Increased prevalence of obesity is also reflected among women of reproductive age and recognized as a major complication in pregnant women (30, 55).

Maternal over nutrition and obesity during pregnancy and/or lactation affects offspring metabolic phenotype (56), cardiovascular function (46), anxiety behavior, associative learning, and motivation (36). In contrast, programming of offspring reproductive capacity by maternal obesity is poorly documented. We have reported that male offspring of pregnant rats fed a low-protein diet develop obesity and have lower sperm counts and a 50% decrease in fertility rate (58). At the other extreme of maternal nutrition, maternal over nutrition and obesity during pregnancy and lactation decreases sperm viability, motility, and concentration in adult male offspring accompanied by manifestation of increased testicular oxidative stress (35, 55). Female rat offspring from mothers fed with high-fat diet during pregnancy and lactation have earlier puberty onset (42), higher leptin and insulin serum levels (21), and altered reproductive function reflected in an increased incidence of prolonged or persistent estrus (10). Maternal obesity in humans leads to earlier onset of puberty (20) and affects semen quality in male offspring (34). In mice Founder generation F0 paternal obesity compromises F1 female pancreatic function (29). F1 females oocytes show increased oxidative stress and altered mitochondrial function and F1 males have altered sperm function with reduced motility, increased reactive oxygen species (ROS) levels, and decreased in vitro fertilization rates (13).

Human (23, 49) and animal studies (31, 38, 56) show that offspring of obese mothers are themselves predisposed to obesity. Obesity and male infertility have increased in parallel (25), which potentially explains the positive correlation between male obesity and low sperm quality and fertility (32). Human (8, 19, 45) and animal studies (33) show that spermatozoids from obese male have decreased motility, more morphological defects, increased DNA damage, and higher oxidative stress. While the etiology of male infertility is clearly multifactorial, oxidative stress is now considered to play an important role. In humans, paternal obesity is associated with increased oxidative stress in sperm (41), reduction in semen quality, and decreased fertility (6). Similarly, obese male rats have lower sperm quality and higher epididymal lipoperoxidation (48).
Given the existence of multiple negative effects of adverse programming on life-course health, there is a pressing need for development of interventions that effectively prevent or reverse unwanted outcomes. Adult exercise provides numerous health benefits (43), and we have shown that exercise in obese female rats before and during pregnancy has beneficial effects on maternal and male and female offspring metabolism (47). Lifestyle changes that induce weight loss such as dietary and exercise interventions can improve semen parameters and therefore fertility in obese men (6). From the lack of information available about the beneficial exercise effects on negative developmental programming outcomes in offspring, the present study aimed to determine the effects of maternal obesity on male offspring metabolism and fertility and determine whether developmental programming outcomes are permanent or are reversible by offspring lifestyle modification. We hypothesized that regular offspring exercise even in early adult life, would reverse the adverse effects of maternal obesity on offspring metabolism, sperm quality, and fertility.

**METHODS**

Standardization of phenotype of females (F₀) recruited to produce study F₁ offspring. All procedures were approved by the Animal Experimentation Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INNSZ), Mexico City, Mexico. Female albino Wistar rats were born and maintained in the animal facility of the INNSZ, which is accredited by and adheres to the standards of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Rats were maintained at 22–23°C under controlled lighting (lights on 07:00 to 19:00 h) and fed normal laboratory chow (Zeigler Rodent RQ22-5) containing 22.0% protein, 5.0% fat, 31.0% polysaccharide, 31.0% simple sugars, 4.0% fiber, 6.0% minerals, and 1.0% vitamins (wt/wt), energy 4.0 kcal/g. At age 14–16 wk, when they weighed 200–240 g, females were randomly assigned to either a control (C; n = 70) or maternal obesity (MO; n = 70) group that received the laboratory chow or to a maternal obesity group (MO; n = 5) fed a high-energy, obesogenic diet containing 23.5% protein, 20.0% animal lard, 5.0% fat, 20.2% polysaccharide, 20.2% simple sugars, 5.0% fiber, 5.0% mineral mix, 1.0% vitamin mix (wt/wt), energy 4.9 kcal/g. Thus each F₀ group contained one female from any litter and F₀ females in different groups, but not within groups, were sisters, providing maximal possible homogeneity in F₀ mothers’ own developmental programming and genetics.

F₁ offspring. F₀ female rats were placed with proven male breeders on day 120 and conceived during the next cycle. To minimize consumption of the obesogenic diet by the males during the mating period, males were placed with females at night and removed during the morning. Lactating mothers were maintained on their pregnancy diet. Litter size and pup weight were recorded at birth. Anogenital distance was measured to identify males and females (54). Litters with less than 10 or more than 14 pups 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 as closely as possible. Offspring were weaned at PND 21, housed 5 per cage, and fed chow diet throughout the study. Only male offspring were studied. At PND 330 five males per group from different litters were randomly selected to begin wheel-running exercise (Cex, C exercised; MOex, MO exercised) (Fig. 1).

Voluntary moderate exercise. F₁ Cex and MOex rats were trained to wheel run (15-min sessions) on 2 days in the week before PND 330. A pilot study established an optimum running schedule of 15 min, which was always completed. Throughout to ensure some recovery time as in human athletic training schedules, the study rats were allowed two nonconsecutive rest days weekly. Rats ran for only one 15-min session 5 days per week during 4 months (PND 330–PND 450). Distances run were quantified using a bicycle odometer (47). The last bout of exercise took place 24 h before tissue collection.

F₁ offspring at PND 450. At PND 450, following a 6-h fast, male rats were decapitated by trained personnel, experienced in using a rodent guillotine (Thomas Scientific) between 12:00 to 14:00 h. Testes were dissected, cleaned of fat, weighed, frozen, and stored at −70°C until analyzed. The sternal, pancreatic, perirenal, retroperitoneal, and gonadal fat pads were dissected and weighed individually. Adiposity index was calculated as total adipose tissue (g) divided by body weight (g). At PND 450 the cauda epididymis and vas deferens were rapidly removed and placed in saline at 37°C to release sperm by squeezing the vas deferens with tweezers and triturating the cauda epididymis with scissors.

Tissue and sample preparation. Frozen testes were homogenized in saline, and aliquots were frozen at −70°C for no more than 24 h for later protein quantification using the Bradford method, and for determination of antioxidant enzymes activity. Lipid peroxidation was determined at the time of homogenization of the testis. All determinations were performed in duplicate and averaged for statistical analysis.

Sperm aliquots containing 5 × 10⁶ and 1 × 10⁷ spermatozoa underwent 6 thermal shock cycles from −70°C to 45°C, sonication for 2 min with 6 intervals of 20 s each, and were then placed in ice and...
frozen at −70°C for no more than 24 h for later quantification of antioxidant enzymes.

**Lipid peroxidation assay.** Aliquots containing 5 million sperm were adjusted with saline to 100 μl. Lipid peroxidation was determined in 100-μl aliquots of either homogenized testes or 5 × 106 sperm by measurement of malondialdehyde (MDA) by the thiobarbituric acid-reactive substances assay (TBARS). All samples were read at 532 nm in a Perkin-Elmer LS50-B luminescence spectrometer. Results are expressed as either nanomoles MDA per milligram of protein or nanomoles MDA per 5 million sperm (47). Intra- and interassay coefficients of variation were <6 and <8%, respectively.

**Superoxide dismutase activity.** Superoxide dismutase (SOD) activity was determined in 10-μl aliquots of homogenized testes or sperm with a RANSOD kit (RANDOX Laboratories). A standard curve was obtained according to the manufacturer’s instructions. All samples were read at 505 nm in a Perkin-Elmer LS50-B luminescence spectrometer at 0, 30 s, and 3 min at 37°C to obtain the deltas of absorbance. Results are expressed as either units of activity per milligram of protein or percentage of activity per 5 million sperm (47).

**Glutathione peroxidase activity.** Glutathione peroxidase (GPx) activity was determined in a 10-μl aliquot of homogenized testes or sperm with the RANSEL kit (RANDOX Laboratories). All samples were read at 304 nm in a Perkin-Elmer LS50-B luminescence spectrometer at basal, 1, 2, and 3 min at 37°C to obtain the deltas of absorbance. Results are expressed as either milliunits per milligram of protein in the testes or activity units per 10 million sperm (47).

**Sperm measurements.** Sperm viability was assessed by mixing 10 μl of saline (0.90% wt/vol of NaCl) containing spermatozoa with 10 μl of eosin. Live spermatozoa were identified by absence of staining as a sign of membrane integrity. Results are expressed as the percentage of live cells. Sperm concentration and motility were evaluated with a computerized sperm analyzer (Sperm Quality Analyzer). All sperm measurements performed were according to the WHO guidelines (52).

**Evaluation of fertility rate.** At PND 440 individual experimental males were placed for 1 wk with two nonexperimental females aged 4 months. Males were then separated from the females who were kept individually until day 15 of any potential gestation. The male was considered fertile when at least one of the two females became pregnant (58). Results are expressed as percentages of fertile males and as percentages of pregnant females.

**Statistical analysis.** All data are presented as means ± SE. For differences within maternal diet and exercise intervention, data were analyzed using one-way multiple analysis of variance (ANOVA) followed by the Tukey test. Fertility rate was analyzed using the χ2 test. A P < 0.05 was considered significant. One male per litter was chosen randomly to form a group of 5.

**RESULTS**

**Voluntary exercise.** The average distance run during the first month of exercise was (Cex: 12.2 ± 2, MOex: 7.5 ± 1.06 m/15 min, P < 0.05); at the fourth month the average distance run increased 2.2 and 2 times, respectively. The average distance run per 15 min session in the 4 mo was lower in male offspring of MO mothers compared with C offspring (Fig. 2A).

**Body weight, adiposity index, and gonadal fat.** Unexercised MO male offspring had increased body weight, adiposity index, and gonadal fat compared with C and Cex offspring (Fig. 2, B–D), whereas testicular weight was similar in all groups (C: 1.6 ± 0.05, Cex: 1.5 ± 0.05, MO: 1.7 ± 0.05, MOex: 1.7 ± 0.04 g). At the end of the voluntary exercise program, none of these parameters were different between Cex and C group (Fig. 2, B–D). Exercise in the MOex group did not decrease body weight (Fig. 2B), but it partially ameliorate adiposity index and gonadal fat compared with C and Cex (Fig. 2, C and D).

**Testicular oxidative stress biomarkers.** Unexercised MO offspring increased testicular MDA levels as well as SOD and GPx activity decreased in unexercised (Fig. 3, A–C). Exercise in Cex and MOex groups decreased MDA concentration and GPx activity compared with C and MO, respectively (Fig. 3, A and C). Exercise had no effect on SOD activity (Fig. 3B).

**Sperm oxidative stress biomarkers.** Sperm MDA was increased and SOD and GPx activity decreased in unexercised...
MO compared with C offspring (Fig. 4, A–C). MDA levels were lower in C ex and MOex compared with C and MO, respectively (Fig. 4A). There were no differences in the antioxidant enzyme activity in the sperm between C and Cex (Fig. 4, B and C). Exercise in the MOex group increased SOD and GPx activity compared with the MO group but values remained lower than C and Cex values (Fig. 4, B and C).

**Sperm parameters.** Sperm quality evaluated by concentration, viability, and motility was lower in the unexercised MO compared with C offspring (Fig. 5, A–C). Sperm concentration increased in Cex and MOex compared with C and MO offspring (Fig. 5A). MOex improved viability and motility compared with MO, but values did not reach those in C and Cex (Fig. 5, A–C).

**Fertility rate.** Fertility rate reported as the percentages of fertile males was similar between groups (Fig. 6A). However, when expressed as percentage of pregnant females, the fertility rate was lower in MO offspring compared with C and Cex. MOex partially improved male fertility since it was not different from C (Fig. 6B). No differences were observed in F2 litter size among the four groups (C = 10.4 ± 0.2, Cex = 10.6 ± 0.2, MO = 10.2 ± 0.6, MOex = 11 ± 0.3 pups/litter).

**DISCUSSION**

There is growing evidence that the offspring of obese women have an increased risk of metabolic diseases in adult life. However, it has not been established if obesity during pregnancy programs the male F1 reproductive capacity. There is also a need to determine whether adverse outcomes can be reversed by later-life behavioral modification. In a previous study conducted in young adult male offspring of obese mothers at PND 110 despite differences from controls in some hormones (e.g., testosterone) and sperm quality by this age, fertility rate was not different (35). Therefore, we decided to exercise the rats after reproductive function is affected, around PND 330. The present study shows that maternal obesity impairs sperm quality and fertility in F1 male rat offspring and that these adverse outcomes can be ameliorated by increasing physical activity even in later life.

It is well known that body weight can be affected by individual’s diet and physical activity. Epidemiological and animal studies have shown that the environment experienced in utero and in early neonatal life can increase the risk not only of developing metabolic diseases but also of adult obesity (24). We have reported that male offspring from obese mothers are fatter with higher leptin and insulin serum levels than offspring of controls (47, 56). Others have reported similar observations (11, 22). In this study we report that body weight, gonadal fat and adiposity index were increased in the MO F1.

Obesity is an important factor for infertility (18). In humans, male obesity is associated with decreased serum sex hormone-binding globulin, testosterone concentrations, and increased estrogen levels (32). These changes are accompanied by higher incidence of azoospermia or oligozoospermia (39) and structural changes in germ cells and sperms (32). Changes in scrotal temperature are associated with lower fertility (40). In the present study we found that male offspring from the MO group had an increase in gonadal fat and a decrease in sperm concentration and viability. These results may be because of the higher fat accumulation around the testes in the MO group with a resulting elevation in scrotal temperature, which impairs sperm function. Scrotal lipectomy in infertile patients with scrotal lipomatosis improves sperm count and motility (40).
The improvement of sperm parameters in the MOex group might therefore be a consequence of the loss of gonadal fat.

Sperm are more susceptible to oxidative stress because they lack cytoplasmic antioxidant enzymes and have high membrane polyunsaturated fatty acid levels (3). Therefore, the lipid environment to which spermatozoa are exposed likely has an important role in sperm development and function (3). Lipid peroxidation and its products (e.g., MDA) are very toxic for sperm (2), damaging the plasma membrane, proteins, and DNA and increasing apoptosis (1). Male mice fed a high-fat diet show decreased sperm motility and capacitation as well as increased oxidative stress and DNA damage (5). In this context, we found that both the testes and sperm from the F1 MO had an increase in oxidative stress. Testes contain a sophisticated antioxidant defense system to ensure that oxidative stress does not affect spermatogenic and steroidogenic functions (4). However, sperm mitochondria have little capacity to respond to ROS attack because they are unable to respond by producing more enzymes to counteract oxidative stress and repair DNA damage. We attribute the different antioxidant responses in sperm from those in the testes to the fact that sperm are much less transcriptionally active due to the relative lack of cytoplasm (12). Thus mature sperm do not produce antioxidant enzymes de novo due to the lack of the required synthetic machinery even in the presence of increased ROS. In contrast, testicular somatic cells play an important role in antioxidant defenses to protect spermatogenic cells, and therefore respond to oxidative stress by producing new enzymes to counteract the increased ROS. As a result in our study, SOD and GPx were higher in the testes and lower in sperm from MO offspring compared with C. Paternal obesity impairs embryo development and implantation (26) and decreases the reproductive function of both male and female in the next two generations, probably through germ cell epigenetic alterations (13). However, effects of maternal obesity on offspring reproductive capacity have been generally overlooked. Female F1 offspring of rats fed a high-fat diet during pregnancy and lactation exhibit early sexual maturation and a longer proestrous phase (57). The present study provides evidence that maternal obesity negatively programs fertility in the male offspring. Human and animal studies indicate that obesity during pregnancy predisposes the offspring to chronic diseases in later life (57). Therefore, there is a need for effective interventions that can be used to prevent or reverse the adverse outcomes of maternal obesity. There is growing evidence in human (27) and animal studies (47, 56) that indicates that changes in lifestyle of obese mothers such as reducing calorie intake or increasing exercise prevents excessive weight gain during pregnancy and the subsequent adverse outcomes in offspring health. However, if these interventions cannot be applied in obese pregnant women, it is necessary to determine whether interventions in offspring are beneficial.

To date few studies in obese men have examined the effects of weight loss and exercise alone or separately on reproductive capacity. Exercise has been reported to increase serum testosterone levels and decreasing adiposity improves sperm quality (17). In young men, regular activity is associated with higher sperm concentration than men who are sedentary (16). Diet and exercise intervention in obese male rats improved sperm motility and morphology and diminished sperm DNA damage and ROS (33). In mice seminiferous tubules of lifelong regular
runners exhibited abundant Sertoli cells and well-organized stratification of spermatogenesis and a larger number of luminal sperm, which may be associated with the decrease in testicular oxidative stress (9). However, not all physical activity is beneficial for reproductive health. In humans strenuous exercise has been proposed as a risk factor for male factor infertility since long-distance runners and cyclists have lower testosterone levels and semen quality due to a negative energy balance (16). In another study in men attending a fertility clinic bicycling 5 h or more per week was associated with lower sperm concentration (51). Palmer et al. (32, 33) have reported that either diet and/or swimming exercise intervention in obese mice improves sperm motility and morphology and reduces sperm damage and ROS levels, which were correlated with the metabolic status. In the present study we found that the negative effects of maternal obesity in male reproductive capacity were ameliorated by offspring lifestyle exercise modification since regular voluntary exercise reduced body weight and fat accumulation, oxidative stress in the testes and sperm, and increased sperm quality and improved fertility rate.

Finally, an important finding in the present study in relation to its potential as an intervention is the observation that MOex F1 males exercised less than Cx. The male MO offspring weighed more and had higher adiposity index than the C offspring. In addition to the fact that exercise is more difficult in the presence of obesity and increased weight, it is also possible that MO offspring are less motivated to exercise than C F1 males. Further studies will be needed to explore this interesting question in a controlled animal model as well as to determine whether longer individual bout duration and overall period of exercise modify the outcomes we have observed.

Perspectives and Significance

In conclusion, the present study shows that maternal obesity impaired offspring reproductive capacity and that regular physical voluntary exercise even in old male offspring of obese mothers ameliorated the fertility rate that is the functional end point of reproduction. The encouraging feature of these data is the indication that it is never too late for exercise to be beneficial.

ACKNOWLEDGMENTS

M. Santos and G. L. Rodríguez-González are graduate students from Doctorado en Ciencias Biomédicas, Facultad de Medicina, Universidad Nacional Autónoma de México. C. Ibáñez is a graduate student from Doctorado en Ciencias Bioquímicas, Facultad de Química, Universidad Nacional Autónoma de México and is recipient of Consejo Nacional de Ciencias y Tecnología (CONACyT) fellowship.

GRANTS

This work was supported by CONACyT 155166.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.S., G.L.R.G., C.I., and C.C.V. performed experiments; G.L.R.G. prepared figures; P.W.N. and E.Z. conception and design of research; P.W.N. edited and revised manuscript; E.Z. interpreted results of experiments; E.Z. approved final version of manuscript.

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