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Effect of obesity on rat reproduction and on the development of their adult offspring

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The aim of the present study was to assess the reproductive parameters of obese Wistar rats and to determine the frequency of their obese adult offspring. Neonatal rats were divided into two groups: F_1 generation, induced to obesity by monosodium glutamate (MSG; F_1MSG, N = 30), and rats given saline (F_1CON, N = 13). At 90 days of age all animals were mated, producing the F_2 offspring (F_2CON, N = 28; F_2MSG, N = 15). Reproductive parameters (fertility, pregnancy, and delivery indexes) were evaluated in F_1 rats. F_2 newborns were weighed, and the obesity parameter for F_1 and F_2 generations was determined from months 5 to 7 of life. At month 7, periovarian fat was weighed and no differences were found. Mean newborn weight also did not differ. The F_1 and F_2MSG groups presented approximately 90% of obese rats since month 5 of life, whereas F_1 and F_2CON groups presented only 33%. There was no difference in periovarian weight among groups. Although obesity did not affect reproductive parameters, obese dams (F_1MSG) were responsible for the appearance of obesity in the subsequent generation. Thus, obesity induced by neonatal MSG administration did not interfere with reproduction, but did provide a viable model for obesity in second-generation adult Wistar rats. This model might contribute to a better understanding of the pathophysiological mechanisms involved in transgenerational obesity.

Key words: Obesity; Offspring; Fertility; Wistar rats; Monosodium glutamate; Transgeneration

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Although the negative effects of obesity on reproductive function were first documented more than 2000 years ago by Hippocrates, few scientific investigations of women have dealt with the etiology of this unfavorable condition (1). Obesity is rapidly growing among women in general and women of fertile age (2). Clinical investigations show that obese women have a greater prevalence of amenorrhea and infertility. It is common to find greater risks of complications during pregnancy among these women such as hypertension, gestational diabetes and greater susceptibility to complications during delivery, which result in unplanned cesarean surgeries. Maternal obesity can have a negative effect on children's health (3).

Since it is difficult to determine obesity-induced reproductive complications in women, experimental studies are carried out to induce obesity. The model of neonatally monosodium glutamate (MSG)-treated rats is of special interest regarding the development of obesity (4-6). Neonatal treatment with MSG has been shown to destroy hypothalamic arcuate nucleus neurons, resulting in several endocrine disturbances (7), stunted growth, and obesity (8).

In the context of the existing relationship between obesity and complications of female reproduction and considering the interest in hereditary consequences (transgenerational effect), the objective of the present study aims to evaluate the reproductive parameters of Wistar rats treated with MSG during the neonatal period and to determine the frequency of their obese offspring.

Wistar rats were kept in collective cages under con-

trolled conditions of temperature (22 ± 3°C), light (12-h light/dark cycle) and relative humidity (60 ± 5%). The animals were fed laboratory chow (Purina®) and had free access to tap water. The animals were cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals. The local Ethics Committee of Animal Experimentation approved all the experimental procedures of the present study.

Female newborns were divided into two groups: rats receiving subcutaneously (*sc*) saline solution (2.0% NaCl, control) at days 2, 4, 6, 8, and 10 of life (F_1CON group, N = 13), and rats receiving a solution of MSG (4.0 mg/g body weight, *sc*) on the same days (F_1MSG group, N = 30) (4,5,9). After weaning, rats were housed 4 to a cage and kept under controlled conditions.

Obesity was determined by the Lee index at months 3, 5, 6, and 7 of life for each rat, calculated by the cube root of body weight (g) x 10 / naso-anal length (mm), for which a value equal to or lower than 0.300 was classified as normal at month 3 of life. For values higher than 0.300, the rats were classified as obese (9). In the 3rd month, rats classified as obese were included in the experiment and mated. Each female (F_1CON group, N = 8; F_1MSG group, N = 5, total = 13 female rats) was mated with 1 normal male rat (total males used = 13). Several indexes were evaluated to estimate female reproductive performance: fertility index was calculated by the number of pregnant females x 100 / number of mated females; delivery index was calculated by the number of delivering females x 100 / number of pregnant females, and pregnancy index was determined by the number of dams with live newborns x 100 / number of pregnant females (10). At month 7 all rats were anesthetized and killed and total periovarian fat was weighed (4,11).

The female offspring of F_1 generation was kept with their dams until day 21 of life. After weaning all F_2 pups were separated from their dams and denominated according to maternal experimental group: F_2CON (N = 28, obtained from F_1CON dams) and F_2MSG (N = 15, obtained from F_1MSG rats; Figure 1). Food and tap water were given *ad libitum*. F_2 rats were kept until month 7 of life. The Lee index was evaluated monthly from the 5th to 7th month of life (9). In month 7, F_2 rats were anesthetized and killed to collect and weigh periovarian fat. Data regarding the reproductive parameters were analyzed statistically by the z-test, with $z > 1.96$ being considered significant (12). Periovarian fat weight data were analyzed by analysis of variance (ANOVA) followed by the multiple comparisons Tukey test (13). The frequency of obese animals was calculated by the χ^2 test. The level of statistical significance was set at 5% ($P < 0.05$) (12).

The negative effects of obesity on reproductive physiology have been studied for many years. In the present study, all 13 F_1CON rats that started the experiment reached adult age and were submitted to mating and 84.6% of them presented a positive diagnosis of pregnancy. Twenty-one of the 30 F_1MSG rats (70%) reached adult age and 9 (30%) died before this period. We suggest that death in the MSG group occurred due to the fact that blood brain barrier was not fully formed during the first 10 days of life and, therefore, permitted MSG passage, leading to its pharmacological action in the central nervous system (6,7). This fact caused an exhaustion of energy stores and cell death, which impaired other vital functions, affecting neonatal development.

Obesity may interfere with many ovarian and extra-ovarian functions, thereby reducing both ovulatory and fertility rates in otherwise healthy women (14). In the present study, pregnancy was confirmed in 14 of 21 obese rats mated (66.7%), but no difference was found compared to F_1CON ($z < 1.96$). Only 5 of 14 pregnant obese rats (35.7%) delivered when compared to F_1CON (72.7%), but the difference was non-significant ($z < 1.96$; Table 1). These results did not agree with literature data because obesity affects female fertility, with a greater risk of ovulatory infertility, that is increased in women with a high body mass index (3,9).

In the present study, the mean weight of the F_2MSG group did not differ significantly from that of F_2CON ($P > 0.05$; Table 1). All F_1MSG rats were classified as obese (100%) according to the Lee index. The periovarian fat of obese F_1 and F_2 rats did not differ significantly from control ($P > 0.05$; Table 1). There was an increased percentage of obese rats in the F_2MSG group compared to the F_2CON group ($P < 0.05$). This result confirms that maternal obesity caused obesity in the subsequent generation and demonstrates that the experimental model employed was viable for obtaining a similar clinical diagnosis of obese patients based on body mass index. Children of obese parents have an increased risk of becoming obese compared to children of parents of normal weight (15). The Lee index values of F_1 and F_2MSG were higher, a fact probably justified by lower body length because obesity disorders contribute to a reduction of body protein by the presence of high levels of circulating corticosterone (16). There is considerable experimental and clinical evidence that an altered body composition before and during pregnancy produces altered metabolism in the offspring; unbalanced maternal nutrition or overweight and gestational diabetes are all associated with changes in metabolic control in the offspring, which then have a greater propensity to develop diabetes and/or obesity (17). However, there is little infor-

Table 1. Reproductive performance, newborn mean weight, obesity month frequency (%), and periovarian fat weight of controls (CON, saline-treated rats) and obese females (MSG, monosodium glutamate-treated rats) in both generations (F_1 and F_2).

	F_1 generation		F_2 generation	
	F_1CON (N = 13)	F_1MSG (N = 30)	F_2CON (N = 28)	F_2MSG (N = 15)
Mated rats	13/13 (100%)	21/30 (70%)	–	–
Fertility index	11/13 (84.6%)	14/21 (66.7%)	–	–
Pregnancy index	8/11 (72.7%)	5/14 (35.7%)	–	–
Delivery index	8/11 (72.7%)	5/14 (35.7)	–	–
Mean weight of newborn (g)	3.50 ± 0.6	4.31 ± 1.3	3.72 ± 1.2	4.64 ± 1.0
Obesity month 5	12.5%	100%*	28.6%	93.3%*
Obesity month 6	12.5%	100%*	48.4%	80%*
Obesity month 7	50%**	100%	48.4%	100%*
Periovarian fat weight (g)	3.07 ± 0.5	3.57 ± 0.3	2.57 ± 0.9	3.35 ± 2.31

Data are reported as means ± SD, number or percent.
*Significant difference compared to control group ($P < 0.05$ - χ^2 test). **Significant difference compared to 5th and 6th months ($P < 0.05$, χ^2 test).

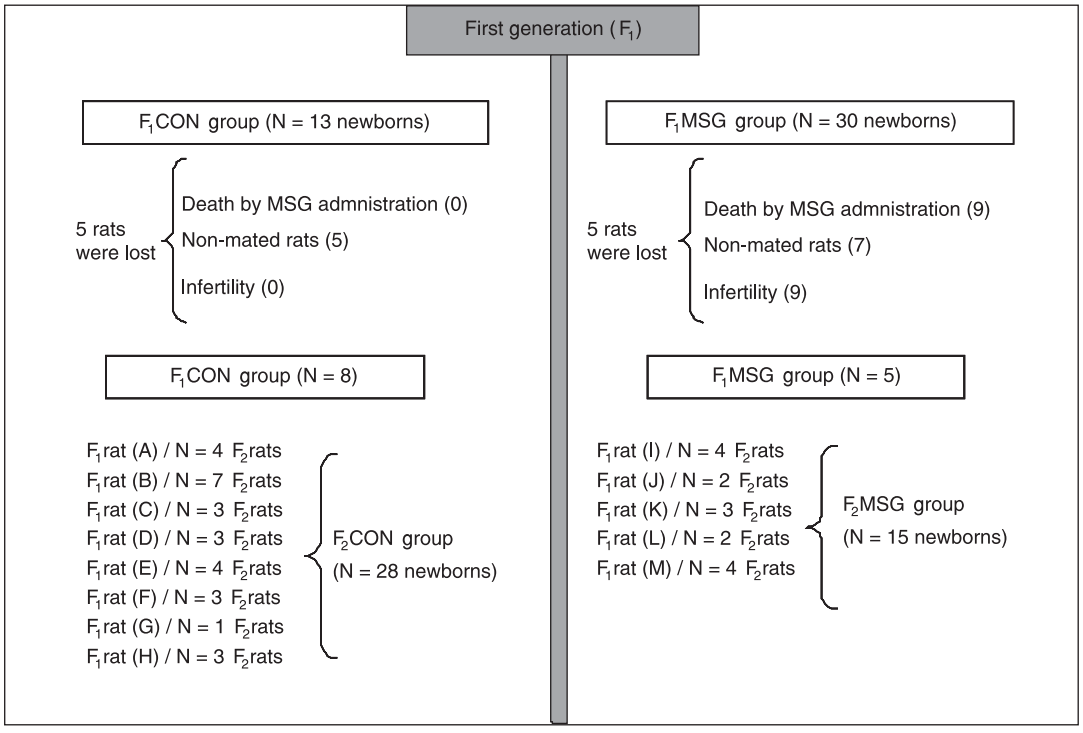

Figure 1. Rat generation scheme (F_1 and F_2) of obese rats neonatally treated with monosodium glutamate (F_1MSG group) and of the control group (F_1CON) and their respective offspring (F_2 generation). F_2 rat distribution was almost similar in both groups (approximately N = 3 F_2 female rat/dam).

mation about the pathological mechanisms of transgenerational obesity.

In the 7th month of life, there was a significant increase in number of obese animals (50%) in the F_1CON group compared to other months ($P < 0.05$). The presence of obesity in F_1 and F_2CON rats at month 7 of life might be justified by the aging process. In rats, this process starts at the end of month 6 of life (18). Similarly, in month 7 of life, F_1 and F_2MSG rats presented a higher rate of obesity compared to their respective control groups. There is evidence that obesity is present in the human population and its prevalence increases as the population ages (19).

The most interesting result of the present study is that, although obesity did not influence the reproductive parameters, obese dams (F_1MSG) were responsible for the appearance of obesity in the subsequent generation. Thus, obesity induced by neonatal MSG administration was not a worsening factor for reproduction. However, we conclude that this is a viable model for obtaining obesity in adult Wistar rats of the second generation. This might contribute to a better understanding of the pathophysiological mechanisms involved in transgenerational obesity.

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