

Effects on a hypercaloric diet ingested from weaning on sexual behavior of adult male rats

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Abstract

Obesity is a nutritional disorder which has spread across the world mostly due to a more sedentary life style together with an increase in dietary fat and carbohydrate. The scientific literature in the field of obesity shows an extensive amount of clinical and experimental studies dealing with etiopathogeny, intervening problems, prevention and potential intervention. Among them, many studies have examined the impact of obesity in the reproductive field, mainly in the female sphere. The aim of the present study was to investigate whether a hypercaloric diet ingested from weaning interferes with the sexual behavior of adult male rats. Male *Wistar* rats (at 100 days of age) from the hypercaloric diet group (HD group, n=9) received a hypercaloric diet, containing 4,79 kcal/g and Control animals (n=7) were fed a normocaloric laboratory chow containing 3.78 Kcal/g. For analysis of sexual behavior, one adult male was placed in the observation cage 4 hours after the beginning of the dark phase and 10 min before (for adaptation to the environment) an adult receptive female in natural estrus was introduced. The parameters of sexual behavior (latency and quantity of mounts, intromission and ejaculation), were tape-recorded and registered simultaneously by two investigators, during a 30-min session, with light provided by a 40-watt red lamp. Concerning sexual behavior, the HD animals presented (i) a 70% increase in the amount of incomplete mounts (mounts without intromission), (ii) a 3-fold increase in the latency for the first intromission, (iii) a 27% decrease in the latency for the first pos-ejaculatory intromission, and (iv) a 26% decrease in the frequency of ejaculation. The lower reproductive performance and the tendency of fertility decrease in animals fed a hypercaloric diet from weaning to adulthood, allows us to suggest a negative interference between the ingestion of a hypercaloric diet and these reproductive parameters. Although results obtained from animals should not be directly extrapolated to humans, they may add evidence concerning the deleterious effects of a hypercaloric diet on humans and the species.

Keywords: obesity, hypercaloric diet, sexual behavior

Resumo

A obesidade é um distúrbio nutricional que tem se espalhado pelo mundo principalmente devido a um estilo de vida mais sedentário, aliado ao aumento da ingestão de gorduras e carboidratos na dieta. A literatura científica na área da obesidade apresenta uma extensa quantidade de estudos clínicos e experimentais que tratam da etiopatogenia, problemas intervenientes, prevenção e possíveis intervenções. Dentre eles, muitos estudos examinaram o impacto da obesidade no campo reprodutivo, principalmente na esfera feminina. O objetivo do presente estudo foi investigar se uma dieta hipercalórica ingerida desde o desmame interfere no comportamento sexual de machos adultos. Ratos *Wistar* (100 dias de idade) do grupo dieta hipercalórica (grupo HD, n=9) receberam dieta hipercalórica, contendo 4,79 kcal/g Os animais Controle (n=7) foram alimentados com ração laboratorial normo calórica contendo 3,78 Kcal/g. Para análise do comportamento sexual, um macho adulto foi colocado na gaiola de observação, 4 horas após o início da fase escura e 10 minutos antes do início do teste (para adaptação ao ambiente) uma fêmea adulta receptiva em estro natural ser introduzida. Os parâmetros do comportamento sexual (latência e quantidade de: montas, intromissão e ejaculação), foram gravados e registrados simultaneamente por dois investigadores, durante uma sessão de 30 minutos, com luz fornecida por uma lâmpada vermelha de 40 watts. Em relação ao comportamento sexual, os animais HD apresentaram (i) um

aumento de 70% na quantidade de montas incompletas (montas sem intromissão), (ii) um aumento de 3 vezes na latência para a primeira intromissão, (iii) uma diminuição de 27% na latência para a primeira intromissão pós-ejaculatória e (iv) diminuição de 26% na frequência de ejaculação. O menor desempenho reprodutivo e a tendência de diminuição da fertilidade em animais alimentados com dieta hipercalórica desde o desmame até a idade adulta, permite sugerir uma interferência negativa entre a ingestão de uma dieta hipercalórica e esses parâmetros reprodutivos. Embora os resultados obtidos em animais não devam ser extrapolados diretamente para humanos, eles podem acrescentar evidências sobre os efeitos deletérios de uma dieta hipercalórica em humanos e na espécie.

Palavras-chave: obesidade, dieta hipercalórica, comportamento sexual

Resumen

La obesidad es un trastorno nutricional que se ha extendido por todo el mundo debido principalmente a un estilo de vida más sedentario, combinado con una mayor ingesta de grasas y carbohidratos en la dieta. La literatura científica en el área de la obesidad presenta una extensa cantidad de estudios clínicos y experimentales que tratan sobre la etiopatogenia, problemas intervinientes, prevención y posibles intervenciones. Entre ellos, muchos estudios han examinado el impacto de la obesidad en el campo reproductivo, especialmente en el ámbito femenino. El objetivo del presente estudio fue investigar si una dieta hipercalórica ingerida desde el destete interfiere en el comportamiento sexual de machos adultos. Las ratas Wistar (100 días de edad) del grupo de dieta hipercalórica (grupo HD, n=9) recibieron una dieta hipercalórica que contenía 4,79 kcal/g. Los animales control (n=7) recibieron comida de laboratorio normo calórica que contenía 3,78 kcal/g. Para el análisis del comportamiento sexual se colocó un macho adulto en la jaula de observación, 4 horas después del inicio de la fase de oscuridad y 10 minutos antes del inicio de la prueba (para adaptarse al medio) se introdujo una hembra adulta receptiva en estro natural. Los parámetros de conducta sexual (latencia y cantidad de: montas, intromisión y eyaculación) fueron registrados y registrados simultáneamente por dos investigadores, durante una sesión de 30 minutos, con luz proporcionada por una lámpara roja de 40 watts. En cuanto al comportamiento sexual, los animales HD mostraron (i) un aumento del 70 % en la cantidad de montas incompletas (montas sin intromisión), (ii) un aumento de 3 veces en la latencia a la primera intromisión, (iii) una disminución del 27 % en la latencia a la primera intromisión post-eyaculatoria y (iv) disminución del 26% en la frecuencia de la eyaculación. El menor rendimiento reproductivo y la tendencia a disminuir la fertilidad en animales alimentados con dieta hipercalórica desde el destete hasta la edad adulta sugiere una interferencia negativa entre la ingesta de una dieta hipercalórica y estos parámetros reproductivos. Aunque los resultados obtenidos en animales no deben extrapolarse directamente a humanos, pueden añadir evidencia sobre los efectos deletéreos de una dieta hipercalórica en humanos y la especie.

Palabras clave: obesidad, dieta hipercalórica, conducta sexual

1. Introduction

Obesity is a nutritional disorder which has spread across the world mostly due to a more sedentary life style together with an increase in dietary fat and carbohydrate (Lopez et al., 2003; Pozza; Isidori, 2018). The scientific literature in the field of obesity shows an extensive amount of clinical and experimental studies dealing with etiopathogeny, intervening problems, prevention and potential intervention. Among them, many studies have examined the impact of obesity in the reproductive field, mainly in the female sphere, focusing in problems related to menopause, ovulatory dysfunction, fertility and risks through pregnancy (Nelson; Fleming, 2007; Sagae, 2012).

A considerably smaller amount of papers refer to the effects of obesity on male reproduction and report more often alterations in the androgenic status and semen quality (Du Plessis, 2010; Fernandez et al., 2011; Deeh Defo, 2017). A reduced number of investigations reveal a sexual dissatisfaction associated with human obesity (Adolfsson et al. 2004; Nagelkerke et al. 2006; Kolotkin et al. 2006; Sarwer et al., 2012; Sarwer DB; Steffen, 2015).

The prevailing experimental models of obesity are accomplished by (i) genetic manipulation, (ii) lesion of the ventromedial hypothalamic nucleus, achieved by administration of monosodium glutamate or by direct electrical lesion, or (iii) feeding on hypercaloric diets (Diemen et al., 2006). Very few experimental studies were conducted to examine the sexual behavior of obese males, using genetically modified (Edmonds & Withyachumnarnkul

1980; Young et al., 1986) or monosodium glutamate-treated rats (Sun et al., 1991), both protocols interfering directly with the shared neuroendocrine control of the dietary and sexual behaviors.

To the best of our knowledge, the only study conducted to investigate the sexual behavior of hypercaloric diet fed rats, an experimental model that most closely resembles human obesity, reports a decrease in the proportion of ejaculators and in the number of ejaculations per hour (Mandenoff et al., 1986).

The aim of the present study was to investigate whether a hypercaloric diet ingested from weaning interferes with the sexual behavior of adult male rats.

2. Materials and Methods

Male *Rattus norvegicus Wistar* kept in the bioterium of the discipline of Physiology at the Federal University of Triângulo Mineiro, Uberaba-MG, rats were housed in a controlled environment (temperature $23 \pm 2^\circ\text{C}$, 12 h dark period starting at 10:00 a.m.), had free access to laboratory chow and tap water and were regularly weighed since weaning to adulthood (at 100 days of age). The animals from the hypercaloric diet group (HD group, n = 9) received a hypercaloric diet as described by Estadella et al. (2004), containing 4,79 kcal/g, whose ingredients (15g of a normocaloric laboratory chow having 3.78 kcal/g, 10 g of toasted peanuts having 5.95 kcal/g, 10g of milk chocolate having 5.40 kcal/g, 5g of maize cracker having 4.25 kcal/g) were minced, mixed and molded as pellets, resulting in a mixture containing 19% protein, 47% carbohydrate, 16% lipids, 3% fibers (cellulose) and 5% vitamins and mineral salts. Control animals (n=7) were fed a normocaloric laboratory chow (NUVILAB, Curitiba, PR, Brasil) containing 3.78 Kcal/g.

For analysis of sexual behavior, one adult male with no previous sexual experience was placed in the observation cage (56 x 35 x 31 cm with a glass front wall) 4 hours after the beginning of the dark phase and 10 min before (for adaptation to the environment) an adult receptive female in natural estrus was introduced. The parameters of sexual behavior (latency and quantity of mounts, intromission and ejaculation), identified as described in Ahlenius; Larson (1984) and Agmo (1997), were tape-recorded and registered simultaneously by two investigators, during a 30-min session, with light provided by a 40-watt red lamp. In the day after, the males were dead and the epididymal fat was removed and weighed. Differences among groups were analyzed by the Mann-Whitney test ($p=0.05$ for statistical significance), (Jamovi 1.6.23). The project was conducted in compliance with ethical principles as approved by institutional guidelines (CEUA-UFTM: 54/2007).

3. Results

The HD rats failed to present an increase in body weight gain throughout the experimental period and reached maturity with a body weight similar to that of the controls (controls = $386,6 \pm 9,7$ g; HD = $387,8 \pm 8,8$ g; mean \pm SEM) but exhibited a 44% increase in the epididymal fat (controls = $1,8 \pm 0,1$ g; HD = $2,6 \pm 0,3$ g; mean \pm SEM; $p < 0,05$; Mann-Whitney test).

Concerning sexual behavior, the HD animals presented (i) a 70% increase in the amount of incomplete mounts (mounts without intromission), (ii) a 3-fold increase in the latency for the first intromission, (iii) a 27% decrease in the latency for the first post-ejaculatory intromission, and (iv) a 26% decrease in the frequency of ejaculation (Table 1).

Table 1. Sexual behavior of HD animals in the control and hypercaloric groups.

Parameters	Control Group (n = 7-9)	Hypercaloric group (n = 8-9)
Latency to first mount (s)	$21,8 \pm 3,6$	$16,8 \pm 2,6$
Number of mounts without intromission	$2,3 \pm 1,0$	$3,9 \pm 0,4^*$
Latency to first intromission (s)	$27,3 \pm 3,8$	$79,3 \pm 23,5^*$
Number of intromissions	$19,6 \pm 3,7$	$12,4 \pm 2,1$
Latency to first ejaculation (s)	$740,6 \pm 146,3$	$522,0 \pm 94,8$
Latency to post-ejaculatory intromission (s)	$1150,8 \pm 83,7$	$842,2 \pm 103,0^*$
Number of ejaculations	$3,4 \pm 0,2$	$2,5 \pm 0,4^*$

Data are mean \pm S.E.M. Statistically significant difference: * $p<0,05$ (Mann-Whitney test). Source: Authors, (2022).

4. Discussion

Among the experimental models carried out to induce obesity, ingestion of hypercaloric diets is one that most closely resembles human obesity (Diemen et al., 2006) with the advantage of avoiding neurologic damage or genetic manipulation that could directly compromise the reproductive axis.

Even though being fed with a hypercaloric diet, the HD rats in the present study did not present an increase in body weight gain. The hypercaloric diet had an increased amount of fat, as described by Estadella et al. (2004). Bruneiko et al. (2006) reported that rats fed this kind of high-fat diet consume significantly less amount of food than the controls but, despite of the decrease in food consumption, their energy intake is higher. However, the hypercaloric diet reduces feed efficiency (mean body weight gain/mean energy intake) and this may explain why the animals have no increase on body weight gain. In spite of the maintenance of body weight gain, the animals fed a high-fat diet exhibit a dyslipidemic profile, characterized by increased concentrations of triacylglycerol and very low-density lipoprotein (Bruneiko et al., 2006). In the present study, the HD animals had a 44% increase in the weight of epididymal fat, compared to the controls. With regards to ingestion of hypercaloric diets, the visceral white adipose tissue (WAT) has been considered physiologically equivalent to the subcutaneous WAT and, in males, the epididymal fat is a source of visceral WAT whose gene expression is altered and the mass increases following a long period of high-fat feeding (Lopez et al., 2003). The male sexual behavior has a complex neuroendocrine control involving testicular androgens which, in conjunction with chemosensory inputs from the olfactory and genitosensory systems, activate the hypothalamic medial preoptic area and the amygdala; several neurotransmitters such as dopamine, serotonin, norepinephrine, opiates and neuropeptide Y (NPY), depending on the doses and on receptor subtypes, can inhibit or facilitate specific aspects of mating, in rodents (Hull; Dominguez, 2007).

There is increasing evidence that many aspects of the reproductive axis and body weight regulation share genetic and neural controls. Leptin is the adipocyte-specific hormone which signals to the hypothalamus to regulate body weight homeostasis and has also emerged as a neuroendocrine mediator in the hypothalamic-pituitary-testicular axis, acting also as a direct inhibitory signal for testicular steroidogenesis (Tena-Sempere; Barreiro, (2002); Vendramini et al., (2014); Kahn; Brannigan (2017)). Consequently, obese men are characterized by a progressive decrease of testosterone levels, reduced spermatogenesis and erectile dysfunction (Pasquali, 2006; Pasquali et al., 2007).

Appetite and reproductive function seems to share neuroendocrine control also via central NPY, which is a potent stimulator of food intake and suppressive of sexual behavior in male Zucker rats, a genetic model of obesity (Beck et al., 1990). Chronic consumption of a high-fat diet leads to increased leptin expression, which down-regulates NPY in hypothalamic neurons (Stutz et al., 2007). Additionally, prolonged administration of NPY in the hypothalamus causes the obesity syndrome characterized by hyperphagia, hyperinsulinism, a marked increase in plasma leptin and collapse of reproductive functions (Raposinho et al., 2001).

Focusing specifically on male sexual behavior, a higher body mass index has been associated with greater impairment in sexual quality of life, recognized by lack of enjoyment of sexual activity, lack of sexual desire, difficulties in sexual performance and avoidance of sexual encounters, mostly in women but also in men (Kolotkin et al., 2006). Obese and overweight men self reported also fewer sex partners than individuals of normal weight, although the authors informed evidence of substantial misreporting (Nagelkerke et al., 2006). Older (50 years) obese men interviewed about sexual satisfaction reported a greater decrease of sexual desire (Adolfsson et al., 2004). Male Wistar rats were fed either with a 15 % palm oil diet as evidenced by the significant delay recorded in the latencies of mount, intromission and post-ejaculatory interval (Deeh Defo et al., 2017).

Few experimental studies focused on the sexual behavior of obese animals. Hardly any of male Zucker rats placed with receptive females were able to ejaculate (Edmonds; Withyachumnarnkul, 1980), in spite of normal serum levels of testosterone (Young et al., 1986). Chemical lesion of the hypothalamus, produced by neonatal administration of monosodium glutamate to male rats, caused, at adulthood, reduced copulatory behavior characterized by reduction of attractivity and sexual approach to females (Sun et al., 1991); the authors suggested that a decline in sexual hormones would be responsible for that.

The HD animals in the present study showed signs of a decrease in the sexual performance presenting a marked

increase in the amount of incomplete mounts and in the latency for the first intromission, in addition to a decrease in the frequency of ejaculation. They presented also a decrease in the latency for the first post-ejaculatory intromission, suggesting as well an alteration in the complex regulatory mechanism of sexual behavior. It is possible that the prolonged ingestion of a high-fat diet led to increased levels of leptin, which signaled to the hypothalamus to regulate food intake and interfered with the hypothalamus-pituitary-testicular axis, the NPY expression or other neuroendocrine mediators to impair the reproductive function. This hypothesis, however, deserves confirmation.

5. Conclusions

The lower reproductive performance and the tendency of fertility decrease in animals fed a hypercaloric diet from weaning to adulthood, allows us to suggest a negative interference between the ingestion of a hypercaloric diet and these reproductive parameters.

Although results obtained from animals should not be directly extrapolated to humans, they may add evidence concerning the deleterious effects of a hypercaloric diet on humans and the species. The relevance of this fact is based on the increase in the incidence of obesity worldwide and the decrease of fertility. Furthermore, it deals with a comprehensive and worrying topic that has increased and become, more and more, a public health issue in the recent years.

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