ORIGINAL ARTICLE

The effect of diet induced obesity on testicular tissue and serum oxidative stress parameters

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KEYWORDS
Obesity; Male infertility; Testosterone; Oxidative stress; Anastrazole

Abstract
Objective: The aim of this study was to evaluate the effects of diet induced obesity on semen parameters and serum antioxidant enzyme levels.

Material and methods: Six-week-old male rats were randomized into three groups as follows: group 1 (n=10) received a control diet, group 2 (n=9) received a high-fat diet and group 3 (n=11) received high-fat diet plus anastrozole. At the completion of a 10-week period, testicular tissues were obtained and spermatogenesis was evaluated with Johnsen Score System. The normal Johnsen Score was accepted as >9.39. In addition, serum antioxidant enzyme levels, triglyceride, cholesterol, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol levels were measured in serum.

Results: Body weights were significantly increased in mice fed with a high-fat diet compared to normal diet (P<.05). The mean triglyceride levels were 64.00 ± 20.48 mg/dl, 98.89 ± 27.80 mg/dl and 95.27 ± 15.02 mg/dl in group 1, group 2 and group 3, respectively (P<.05). Male rats fed with a high-fat diet had significantly lower levels of testosterone compared with the control diet male rats (P=.005). Testicular pathology revealed that Johnsen score were 9.60 ± 0.15, 8.72 ± 1.81 and 9.29 in group 1, group 2 and group 3, respectively (P=.169). In addition serum nitric oxide (NO) levels were higher in group 2 and group 3 compared to group 1 (P<.05).

Conclusion: As a result it may be concluded that obesity may induce oxidative stress and decrease testosterone levels. These changes may alter testicular functions and consequently it may be speculated that obesity can be an important causative factor in the etiology of the male infertility.

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PALABRAS CLAVE
Obesidad; Infertilidad masculina; Testosterona; Estrés oxidativo; Anastrozol

Efecto de la obesidad inducida por dieta en el tejido testicular y parámetros de estrés oxidativo en el suero

Resumen
Objetivo: El objetivo de este estudio fue evaluar los efectos de la obesidad inducida por dieta en los parámetros de semen y los valores séricos de enzimas antioxidantes.

Material y métodos: Ratas macho de 6 semanas fueron distribuidas aleatoriamente en tres grupos: el grupo 1 (n = 10) recibió una dieta controlada; el grupo 2 (n = 9), una dieta alta en grasas, y el grupo 3 (n = 11), una dieta alta en grasas junto con anastrozol. A las 10 semanas se obtuvieron los tejidos testiculares y se evaluó la espermatogénesis con el sistema de puntuación de Johnson. Se aceptó > 9,39 como puntuación normal. Además, se midieron los valores séricos de enzimas antioxidantes, los triglicéridos, el colesterol, la testosterona, la hormona luteína-zante (HL), la hormona estimulante del foliculo (HEF) y el estradiol en el suero.

Resultados: El peso corporal aumentó considerablemente en los ratones alimentados con una dieta alta en grasas en comparación con los que recibieron una dieta normal (p < 0,05). Los valores medios de triglicéridos fueron 64,00 ± 20,48, 98,89 ± 27,80 y 95,27 ± 15,02 mg/dl en los grupos 1, 2 y 3, respectivamente (p < 0,05). Las ratas macho alimentadas con una dieta alta en grasas presentaban valores de testosterona considerablemente más bajos en comparación con las que recibieron una dieta controlada (p = 0,005). La patología testicular reveló que la puntuación de Johnson fue 9,60 ± 0,15, 8,72 ± 1,81 y 9,29 en los grupos 1, 2 y 3, respectivamente (p = 0,169). Además, los valores de óxido nítrico (NO) en el suero fueron mayores en los grupos 2 y 3 que en el 1 (p < 0,05).

Conclusión: Como resultado, se puede concluir que la obesidad puede provocar estrés oxidativo y la disminución de los valores de testosterona. Estos cambios pueden alterar las funciones testiculares y, por lo tanto, se puede especular que la obesidad puede ser un factor causante importante en la etiología de la infertilidad masculina.

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Introduction

Infertility is defined as a couple’s inability to achieve pregnancy following one year of unprotected intercourse, and it is considered one of the main public health issues. A male factor is contributory in more than 50% of couples showing fertility evaluation. Although the most common etiologic factor of male infertility is idiopathic, other causes are varicocele, urogenital infections, sexual and immunologic factors, cryptorchidism, testicular torsion or trauma, gonadal dysgenesis, and obstruction of the reproductive channels. Many studies revealed that there is an increased likelihood of abnormal semen parameters among overweight and obese men and an elevated risk for subfertility among couples in which the male partner is overweight or obese.

In studies reporting the relation between obesity and male infertility, several mechanisms have been proposed to explain this connection. The aromatization of testosterone is the key step in estrogen synthesis and is catalyzed by the aromatase enzyme system. Aromatase enzyme, which converts testosterone to estradiol, is highly expressed in peripheral fat tissue. It is thought that the increase in estrogens in obese males is due to increased conversion of testosterone owing to the increase in the available aromatase enzyme in the fatty tissue. This peripheral conversion may result in decreased testosterone levels and increased estradiol levels. Increased estradiol production may inhibit secretion of the gonadotropin-releasing hormone (GnRH), LH, and FSH from the hypothalamus and pituitary glands. The main strategy in usage of aromatase inhibitors is to increase testosterone and reduce estrogen levels. Although it has been reported that body weight correlates negatively with blood testosterone levels and testosterone/estradiol ratio in some previous studies, this fact has not been understood clearly. Apart from these endocrinologic changes, it has been emphasized that metabolic syndrome or one of its components (hyperlipidemia) may cause oxidative stress in obesity cases.

Oxidative stress is the condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants commonly known as reactive oxygen species (ROS). The major targets of ROS are membrane lipids and this process is called lipid peroxidation. It is also known that, the testicular tissues and spermatozoa are very sensitive to ROS attack and lipid peroxidation. Susceptibility of testicular tissues to oxidation was attributed to the highly rich polyunsaturated fatty acid content of sperm membranes. In this context, although the majority of previous related studies confirmed a negative relation between sperm concentration, motility, and male obesity, some others reported different findings. So, in this study, we aimed to evaluate the effects of obesity on testicular histology and oxidative stress parameters in diet induced rat model.

Material and methods

The study was approved by the local ethical committee (Ethical approval number 2009-HADYEK-025). A total of 30
male Wistar albino rats, 5.5–6 months old, were used in the study. The experimental animals were housed at 18–22 °C and had free access to diet for rats and to tap water ad libitum throughout a 10-week study period. All surgical procedures were performed under xylazine/ketamine anesthesia in sterile conditions. The rats were randomly divided into three groups as follows: group 1 (n = 10) received a standard controlled diet, group 2 (n = 9) received a high-fat diet, and group 3 (n = 11) received a high-fat diet plus an orally aromatase inhibitor (anastrozole). The high-fat diet in groups 2 and 3 was prepared with high-fat and caloric index cafeteria foods such as sausages, chips, butter, candy, biscuits, chocolate, salted peanuts, and cheese, which were provided ad libitum as previously described.\(^\text{10,11}\)

Both testes of all rats were harvested for pathologic examination and each testis was cut into two halves and placed in a 10% formalin solution, processed by routine histological methods and embedded in paraffin blocks. The sections were cut by a rotary microtome and stained with hematoxylin and eosin. The stained sections were studied under the light microscope to evaluate spermatogenesis. Johnsen’s Criteria were used to categorize the spermatogenesis. Johnsen’s method applies a score of 1–10 for each tubule cross section examined, according to the criteria in Table 1. The germinal epithelium of at least 50 tubules was assessed for each testis and the mean Johnsen’s Score per rat was calculated subsequently.

Blood samples from the inferior vena cava were stored in heparin-free tubes for biochemical analyses. After centrifugation (2000 x g for 15 min at -4 °C), serum samples were stored frozen at -70 °C. Determinations of the triglyceride and total cholesterol levels, calorimetric enzyme method parameters were made in the serum samples using Roche Cobas c 501 device. Serum testosterone and estradiol levels were measured with the ECLIA (Electrochemiluminescence immunoassay) method (Roche Cobas e 601).

Biochemical analysis

Serum antioxidant enzyme analysis

The total SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al.\(^\text{12}\). The principle of the method is based on inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml of ethanol–chloroform mixture (5:3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount causing 50% inhibition in the NBT reduction rate. The SOD activity is expressed as U mg\(^{-1}\) protein.\(^\text{13}\) The glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine.\(^\text{14}\) The enzymatic reaction in the tube—containing NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase was initiated by the addition of H\(_2\)O\(_2\) and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity is expressed as U g\(^{-1}\) protein. The catalase (CAT) activity was determined according to Aebi’s method.\(^\text{15}\) The principle of the method was based on determination of the rate constant \(k (s^{-1})\) of the H\(_2\)O\(_2\) decomposition at 240 nm. The results are expressed as k g\(^{-1}\) protein. All samples were assayed in duplicate.

The carbonyl contents were determined spectrophotometrically (Cintra 10 E, Austria) based on the reaction of carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone. 2,4-Dinitrophenylhydrazine was the reagent originally used for proteins subjected to metal-catalyzed oxidation. The results were given as nanomoles of carbonyl per milligram of protein.\(^\text{16}\)

NO measurement is very difficult in biological specimens, therefore tissue nitrite (NO\(^2^-\)) and nitrate (NO\(^3^-\)) were estimated as an index of NO production. The samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite + nitrate) was measured after the conversion of nitrate to nitrite by copperized cadmium granules by a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10\(^{-8}\) to 10\(^{-3}\) mol/L) of sodium nitrite. Linear regression was carried out using the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. The results were expressed as nmol/g wet tissue.\(^\text{17}\)

The thiolbarbituric acid-reactive substance (TBARS) level was determined by a method based on reaction with thiobarbituric acid (TBA) at 90–100 °C. In the TBA test reaction, malondialdehyde (MDA) or MDA-like substances and TBA react to produce a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2–3 and 90 °C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water-bath for 10 min. After cooling, the absorbance was read at 532 nm. The results were expressed as nmol wet tissue, according to the standard graphic prepared from measurements with a standard solution (1,1,3,3-tetramethoxypropane).\(^\text{18}\)

Statistical analysis

The continuous variables were in normal distribution according to the Kolmogorov–Smirnov normality test. Therefore, one-way ANOVA was used to compare the biochemical parameters among the three groups. For pairwise comparisons LSD test (Fisher’s Least Significant Difference Test) was
Results

The mean body weights were 111 ± 12.86 g, 301.11 ± 18.33 g and 334.55 ± 25.83 g in groups 1, 2 and 3, respectively. Body weights were significantly increased in mice fed with a high-fat diet compared with those given a normal diet (P < 0.001) (Figs. 1 and 2). The mean triglyceride levels were 64.00 ± 20.48 mg/dl, 98.89 ± 27.80 mg/dl and 95.27 ± 15.02 mg/dl in group 1, group 2 and group 3, respectively. The triglyceride levels were significantly higher in group 2 and group 3 compared with group 1 (P < 0.05). However, cholesterol levels were not statistically different among the groups. The total testosterone levels were significantly lower in group 2 compared with controls (2.30 vs. 0.73; P < 0.01) (Table 2). While the testosterone levels decreased in group 2 compared with group 1, anastrozole treatment increased the levels of testosterone. After anastrozole treatment, testosterone levels were detected as 0.86 ± 0.53. The LH, FSH and estradiol levels were not statistically different among the groups (P > 0.05). The mean NO levels were 329.46 ± 38.29 nmol/ml, 373.89 ± 32.08 nmol/ml and 350.42 ± 38.89 nmol/ml in groups 1, 2 and 3, respectively. Serum MDA, SOD, GSH-Px and CAT levels were not statistically different (692 ± 83 vs. 409 ± 22 units; P > 0.01) in the controlled group compared with group 2 and group 3. In the pathologic evaluation, Johnsen Score decreased in group 2 in comparison to group 1 (9.60 ± 0.15 vs. 8.72 ± 1.81) (Figs. 3 and 4). However, anastrozole treatment increased Johnsen score in group 3 (9.29 ± 0.22) (P = 0.169).

Discussion

Male factor infertility is the sole cause of infertility in approximately 20% of infertile couples, with an additional 30–40% secondary to both male and female factors. Thus, male factor infertility is present in approximately half of all infertile couples. The incidence of obesity is reaching epidemic levels in the Western World. In a recent study, the prevalence of male obesity in the United States was reported...
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Table 2  Comparison results of the biochemical parameters among groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Obese (n = 9)</th>
<th>Obese + Anastrozole (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mL)</td>
<td>7.34 ± 0.78</td>
<td>7.11 ± 0.64</td>
<td>7.36 ± 0.66</td>
<td>0.689</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>2.06 ± 0.66</td>
<td>2.16 ± 0.22</td>
<td>2.04 ± 0.11</td>
<td>0.755</td>
</tr>
<tr>
<td>GSH-Px (U/L)</td>
<td>2289.50 ± 314.23</td>
<td>2035.11 ± 255.37</td>
<td>2120.80 ± 15.44</td>
<td>0.090</td>
</tr>
<tr>
<td>NO (mmol/L)</td>
<td>329.46 ± 38.29</td>
<td>373.89 ± 32.08</td>
<td>350.42 ± 38.89</td>
<td>2.04*</td>
</tr>
<tr>
<td>PC (nmol/ml)</td>
<td>1034.40 ± 320.79</td>
<td>1119.27 ± 346.89</td>
<td>706.31 ± 297.13</td>
<td>0.017**</td>
</tr>
<tr>
<td>CAT (k/mg protein)</td>
<td>8.34 ± 3.61</td>
<td>7.35 ± 2.75</td>
<td>8.89 ± 4.39</td>
<td>0.654</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>72.50 ± 7.98</td>
<td>69.67 ± 18.20</td>
<td>68.91 ± 14.17</td>
<td>0.829</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>64.00 ± 20.48</td>
<td>98.89 ± 27.80</td>
<td>95.27 ± 15.02</td>
<td>0.002***</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>14.67 ± 3.15</td>
<td>13.20 ± 3.74</td>
<td>14.39 ± 2.83</td>
<td>0.584</td>
</tr>
<tr>
<td>Total testosterone (ng/ml)</td>
<td>2.30 ± 1.54</td>
<td>0.73 ± 0.94</td>
<td>0.86 ± 0.53</td>
<td>0.005***</td>
</tr>
</tbody>
</table>

GSH-Px: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde; SOD: superoxide dismutase; NO: nitric oxide; PC: protein carbonyl.

Data are shown as mean ± SD.

* There was a statistically significant difference between control and obese groups.
** There was a statistically significant difference between obese + anastrozole and the other groups.
*** There was a statistically significant difference between control and the other groups.

to be 33.8%. It is well known that the higher the body mass index (BMI), the greater the risks for a person to develop chronic diseases. Recently, the relationship between the obesity and semen parameters has been evaluated largely in the etiology of the male infertility. In this context, many authors have addressed a relationship between increased BMI and decreased sperm concentrations, sperm motility, and fertility rates. For example, in a study, it has been stated that 40% of men presenting to one infertility clinic were overweight. A recent study of a large cohort of Danish men showed that a high BMI is negatively associated with reduced semen quality. Similarly to the above studies, Kort and colleagues analyzed the correlations between BMI and semen parameters in 520 male partners in infertile couples. The authors found a significant negative correlation between BMI and semen parameters. As a result, there is an extremely limited number of studies about this subject. The relation between obesity and male infertility has not been understood clearly, yet.

Adverse effects of obesity on male fertility are postulated to occur through several mechanisms. As estrogen receptors are present in hypothalamic nuclei and in pituitary gonadotropes, it is thought that estrogen acts on the hypothalamus to affect GnRH pulses and at the pituitary level to regulate FSH and LH secretion. Peripheral conversion of testosterone to estrogen in excess of peripheral adipose tissue may lead to secondary hypogonadism through hypothalamic-pituitary-gonadal axis inhibition as well. Estrogens may also affect spermatogenesis directly within the testis as well as by alterations in gonadotropin secretion by the pituitary gland. Obese men have been shown to exhibit higher levels of circulating estradiol and/or elevated estradiol/testosterone ratios in many studies already. Several studies revealed the direct correlation between the rise in BMI and a decline in both free and total blood testosterone levels. Further, some studies have correlated this decline in testosterone levels with a rise in infertility rates. In the present study, the serum testosterone level decreased in mice fed with the high-fat diet compared to the normal diet. In relation to this, Johnsen Score in pathologic examinations of the testes also decreased in mice fed with the high-fat diet.

The hypothesis that elevated estrogen plays an important role in the androgen abnormalities in obesity was strongly supported by observations of the effect of the aromatase inhibitor letrozole in obese men. In a study which was carried out on 10 severely obese men with markedly low testosterone levels and clinical symptoms of hypogonadism, letrozole administration for 6 weeks increased testosterone levels more than 3-fold from mean baseline. Unfortunately, this study did not include data on measures of semen quality. In another report, a patient with hypogonadism due to obesity was treated with the aromatase inhibitor anastrozole. This treatment normalized his serum testosterone levels and spermatogenesis, and his wife was pregnant after 6 months of therapy. In this study, although there was no statistically significant difference between group 2 and group 3, it was detected that anastrozole (an aromatase inhibitor), increased the levels of testosterone in mice fed with the high-fat diet. In addition, Johnsen Score improved in the same group. To date, the literature lacks controlled animal model studies, where the effect of diet-induced obesity could directly be studied in relation to male fertility. In a recent study, the authors have demonstrated a significant reduction in male fertility in association with diet-induced obesity in mice. There are some reports indicating that paternal obesity may negatively affect basic sperm parameters such as concentration and motility. However, as in our study, there is considerable contradiction with several other studies demonstrating that semen and hormone parameters are not affected by paternal obesity. Furthermore, a recently published meta-analysis concluded that paternal obesity is not associated with standard sperm function parameters. Because there were no statistically significant changes in semen parameters, it can be argued that the described hypogonadotropic hypogonadism is mild. In a study, Strain et al. reached this conclusion, noting that their study population showed no changes in semen volume, sperm count and sperm motility, libido, and potency with increasing obesity. Similarly, in
an experimental study, Bakos et al. reported that although testosterone levels decreased in the high-fat diet group, this did not reach statistical significance and, hence, testosterone was an unlikely cause especially given that sperm concentration did not significantly decrease. Nevertheless, it was emphasized that their study exposed the mice to the high-fat diet group only for 9 weeks. It is very likely that testosterone levels may significantly fall in the event of exposure for a longer period of time. In this study, the levels of FSH and LH were within normal ranges in groups 2 and 3 compared with the controlled diet group. In the aforementioned studies, FSH and LH levels were normal or low in obese men. Strain et al. argued that even normal levels of gonadotropins in the context of low free testosterone signify suppression of the hypothalamic-pituitary axis, resulting in subclinical hypogonadotropic hypogonadism. In addition, it has been reported that spermatogenesis may be related to the degree of the BMI. In multiple studies, authors have revealed that spermatogenesis is affected in higher BMIs.

Another possible mechanism related to obesity is oxidative stress. Oxidative stress is a pathological process common in a number of disease states, including autoimmune, cardiovascular, and infectious processes, atherosclerosis, cancers, diabetes mellitus, liver damage, rheumatoid arthritis, cataracts, inflammatory bowel disease, and central nervous system disorders. With regard to male reproductive health, several studies have revealed that oxidative stress resulted in sperm membrane lipid peroxidation with impairment in sperm motility and sperm-oocyte interaction. These studies have also noted that dyslipidemia is associated with systemic proinflammatory states and increased oxidative stress with lipid peroxidation. In the present study, we detected a positive correlation between obesity and serum NO levels. The relation between oxidative stress and sperm damage has been reported in an experimental study. In a clinical study which evaluated the relation between obesity and oxidative stress, the authors revealed that oxidative stress increased and so did BMI, primarily due to a rise in seminal macrophage activation. However, the magnitude of this increase was small and only of minor clinical significance as there was no associated decline in sperm DNA integrity or sperm motility with increasing ROS production. Compared with healthy controls, obese children had significantly increased concentrations of markers of NO synthesis and oxidative stress that were correlated with each other.

Conflict of interest

The authors declare that they have no conflict of interest.

References


