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ORIGINAL ARTICLE – OPEN ACCESS

Protective Effect of Indole-3-Carbinol on Sperm Morphometric Alteration in a High-Fat Diet-Induced Obese Rat Model

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ABSTRACT

Objective: This study aimed to investigate the deleterious effect of obesity induced by high-fat diet on rat testes. Another objective was to survey the possible efficacy of indole-3-carbinol (I3C) on testicular change in obese rats.

Materials and Methods: Twenty-four rats were randomly assigned into 4 groups as follows: control, obese, indole-3-carbinol (I3C), and obese+I3C groups. Morphometric and histopathological examination was performed on sperm smears to determine the type and percentage of abnormalities in sperm morphology.

Results: We found a significant decrease in the percentage of sperms with normal morphology in the obese group in comparison with the control group. Moreover, the percentage of abnormal sperm head, neck, and tail was significantly increased in the obese group compared with that in the control group. In the obese+I3C group, the percentage of normal sperm morphology was significantly higher than that in the obese group, but the percentage of abnormal sperm heads, necks, and tails decreased. Morphological examination also exhibited various types of abnormalities in the sperm heads, necks, and tails.

Conclusion: Our finding showed an increased abnormal morphology in sperms due to the detrimental effect of obesity. Also, the administration of I3C may have improved such sperm abnormalities and, thus, infertility in obese rats.

Keywords: Indole-3-carbinol, obesity, rat, sperm

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INTRODUCTION

Obesity and its related health problems are increasing worldwide. There are potential factors such as genetics, endocrine, physical activity, and diet that can cause obesity. These factors can impair the balance between energy intake and expenditure (1). High-fat-diet (HFD)-induced obesity causes the functional and structural disruptions in the vital organs. Accordingly, this condition may result in chronic complications such as high cholesterol, cardiovascular diseases, and diabetes (2, 3). Obesity can also impact the testicular tissue, resulting in male infertility (4).

Oxidative stress, which is the major reason for male infertility, has gained importance recently (5). Oxidative imbalance occurs when the balance between oxidant and antioxidant systems is impaired. Free radicals are chemically very unstable and active, and by taking electrons from other molecules, they create new free radicals. Therefore, this creates chain reactions that can progress to cell destruction. To minimize the damage of free radicals, there is an antioxidant defense system in the body. Endogenous antioxidants protect the body from the harmful effects of free radicals by scavenging or controlling them. Free radicals are cleaned up by antioxidant enzymes through enzymatic and nonenzymatic reactions. Studies have shown that weight gain leads to a decrease in plasma antioxidant capacity (6). Obesity is thought to be one of the risk factors that cause oxidative stress by increasing free radical formation. Numerous studies have reported that obesity can cause change in the level of oxidative stress markers (7). Also, obesity as an inflammatory agent contributes to biological change in the body (8).

Diet has a significant effect on antioxidant defense system to minimize free radical damage in the body. In people prone to malnutrition, the possibility of insufficient intake of nutrient antioxidants may cause oxidative stress. Antioxidants are substances that act as free radical scavengers and prevent cellular damage by reacting with free radicals. Indole-3-carbinol (I3C) is a phytochemical substance found in the plants of Cruciferae family, which are known as therapeutic agents due to their substantial anticancer and antioxidant activities (9, 10). I3C can also exert an ameliorative effect against inflammation in obese rats (11).

Despite several studies on the male reproductive system, only few studies regarding the effect of obesity on sperm morphology and the improvement of morphological change caused by obesity have been reported. We, therefore, decided to investigate the effects of I3C on the morphological alteration of rat sperm in an HFD-induced obese model.

Table 1. Assessment of sperm morphology

Parameters	Control	p	Obese	p	I3C	p	Obese+I3C	p
Normal sperms (%)	76±3	–	29±2*	<0.01	79±4	–	67±5**	<0.05
Head abnormalities (%)	16±2	–	46±1*	<0.01	14±3	–	19±3**	<0.05
Neck abnormalities (%)	6±1	–	15±1*	<0.01	5±1	–	8±3**	<0.05
Tail abnormalities (%)	2±1	–	10±2*	<0.01	3±1	–	4±1**	<0.05

Comparisons are performed using one-way ANOVA and the Tukey's post hoc test; data are expressed as mean±SD; *: Significantly different from the control group; **: Significantly different from the obese group; I3C: Indole-3-carbinol

MATERIALS and METHODS

Adult rats (n=24, male Wistar albino) aged 8–10 weeks old with a body weight of 200 g were purchased from the Experimental Animal Research and Application Center of Medicine Faculty of Ondokuz Mayıs University. The ethical approval of the present study was granted by the Laboratory Animal Ethics Committee of Ondokuz Mayıs University (HADYEK/35). All animals then were maintained in special cages under 12:12-h day/night cycle at 22±2°C and 50±5% humidity and provided food and tap water ad libitum. Our study group was designed as follows:

1. Control group (n=6): rats were fed a standard commercial rat diet for 15 weeks (12).
2. Obese group (n=6): rats were fed an HFD for 15 weeks (12).
3. Indole-3-carbinol (I3C) group (n=6): rats were fed a standard commercial rat diet for 15 weeks and administered 5 mg/kg of I3C intraperitoneally three times a week for 6 weeks (the ninth to the fifteenth week) (13).
4. Obese+indole-3-carbinol (obese+I3C) group (n=6): rats were fed an HFD for 15 weeks, and administered 5 mg/kg of I3C intraperitoneally three times a week for six weeks (the ninth to the fifteenth week).

Body mass indexes were calculated to determine obesity conditions at the ninth week. Scarification of all rats then was performed under anesthesia by xylazine (7 mg/kg body weight) and ketamine (80 mg/kg) at the end of the fifteenth week. The left testes were immediately removed for examination of sperm morphology.

Sperm Daily Production

A quarter of the left testes were utilized for the analysis of sperm morphology. Phosphate buffer (5 mL, pH 7.4) was added to dissected parts and then centrifuged at 1.300 xg for 2 min to prepare supernatant. A drop of supernatant was gently smeared onto a glass microscope slide and then stained with hematoxylin–eosin. We used a light microscope to photograph all slides. Subsequently, all images were transferred to a private computer and then analyzed in the ImageJ program. Morphological evaluation was carried out using the procedure of Filler (14). A total of 200 normal and abnormal sperms were counted from each group of rats. Values were expressed as percentage. The percentage of sperm morphology was calculated in each group using the following formula:

$$\text{Sperm morphology (\%)} = \frac{\text{number of normal or abnormal sperms}}{\text{total number of sperms}} \times 100$$

Statistical Analysis

We used IBM SPSS (version 25.0; SPSS Inc., Chicago, IL, USA) for statistical analysis. Data was analyzed using one-way ANOVA and Tukey's post hoc test. The results were expressed as mean±standard deviation (SD). Also, p values less than 0.05 were considered statistically significant.

RESULTS

Percent Values of Normal and Abnormal Sperm Morphology

The results of normal and healthy sperm morphology are shown in Figure 1a and Table 1. The percentage of morphologically normal sperm was significantly lower in the obese group than that in the control group ($p<0.01$). A significant difference was not observed between the obese+I3C or I3C group and the control group. The normal sperm percentage was significantly higher in the obese+I3C group than that in the obese group ($p<0.05$).

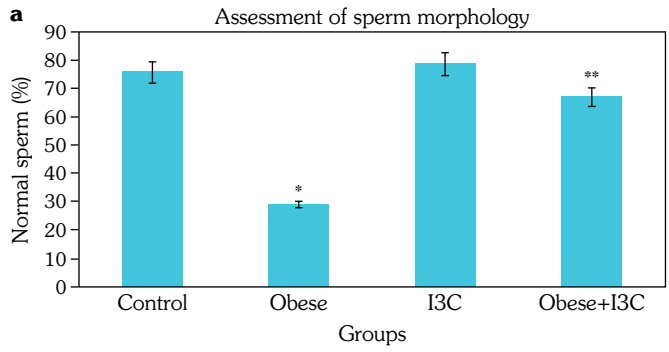
The results of sperms with abnormal head morphology are shown in Figure 1b. The percentage of sperms with abnormal head morphology was significantly higher in the obese group than that in the control group ($p<0.01$). In the obese+I3C group, a significant decrease in the percentage of sperms with abnormal heads was found compared with that in the obese group ($p<0.05$). Sperm head abnormalities did not significantly differ between the obese+I3C or I3C group and the control group.

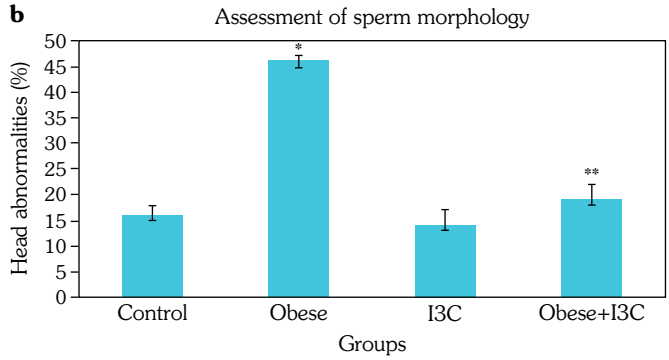
The results of sperms with abnormal neck morphology are shown in Figure 1c. The percentage of sperms with abnormal neck morphology was significantly lower in the obese group than that in the control group ($p<0.01$). A significant difference was not detected between the obese+I3C or I3C group and the control group. Sperm neck abnormalities was significantly higher in the in the obese+I3C group than that in the obese group ($p<0.05$).

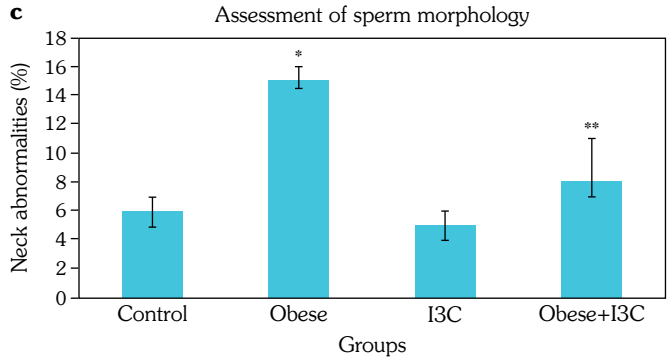
The results of sperms with abnormal tail morphology are shown in Figure 1d. The percentage of sperms with abnormal tail morphology was significantly higher in the obese group than that in the control group ($p<0.01$). On the contrary, a significantly decreased number of abnormal tails were found in the obese+I3C group compared with those in the obese group ($p<0.05$).

Assessment of Different Types of Normal/Abnormal Sperm Morphology

Figure 2 shows normal sperm morphology. The evaluation of sperm morphology exhibited the sperm with a normal head, neck, and tail. Theses normal sperms possessed a hooked head and long tail morphology.







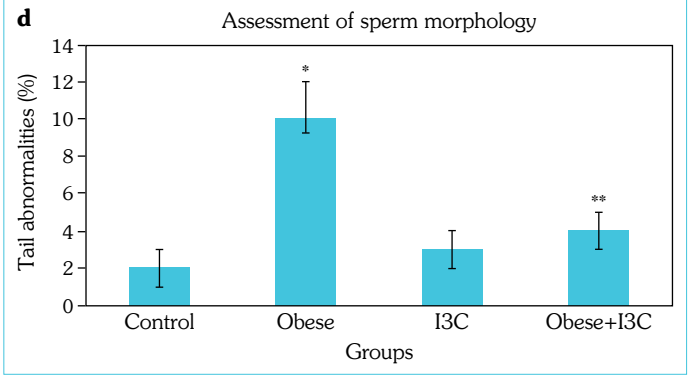


Figure 1. Representative graph of sperm morphology in all groups. (a), the percentage of normal sperms; (b), the percentage of sperms with abnormal heads; (c), the percentage of sperms with abnormal necks; (d), the percentage of sperms with abnormal tails; *, the obese group is significantly different from the control group; **, the obese+I3C group is significantly different from the obese group

Figure 2. Light micrographs of normal sperm morphology. Scale bars, 25 μm

Figure 3 shows abnormal sperm head morphology. We observed the sperms with amorphous, headless, large, small, round, and tapered heads, which were higher particularly in the obese group.

Figure 4 shows abnormal sperm neck morphology. Bent and thick neck sperm were detected as neck abnormalities in the sperm smears.

Figure 5 shows abnormal sperm tail morphology. Sperm smears exhibited marked broken and short tails.

Assessment of Different Types of Normal/Abnormal Sperm Morphology in Each Group

Healthy and intact shaped sperms appeared in the I3C and control groups. Although very rare, sperm abnormalities were also found (Fig. 6a, c). In the obese group, we found all types of abnormal sperm morphology, but tailless and dag (coiled tail) defect sperms were more evident (Fig. 6b). Although we found sperms with abnormal morphology in the obese+I3C group, these were less than those in the obese group (Fig. 6d).

DISCUSSION

Obesity has increased rapidly over the last two decades and has become a chronic disease, which affects health conditions worldwide. This pathological condition associated with several diseases is defined as the excessive fat accumulation in the body (15). Although the importance of dietary balance on obesity is controversial, feeding with an HFD is very closely related to obesity. The importance of obesity for public health cannot be denied. Recently, numerous studies have been conducted intensively on male infertility (16). Despite further reports about female infertility, the effect of obesity on male infertility has not been fully clarified. The relationship between obesity and infertility has also been surveyed more in clinical research, but experimental studies on this subject are limited. Moreover, the present study was a continuation of our previous study.

To the best of our knowledge, the present study is the first to examine the effect of I3C on morphological changes of sperms in obese rats. Our morphological finding that obesity significantly decreased the normal sperm percentage in the obese group compared with that in the control group is parallel to earlier reports. Håkonsen et al. (17) suggested decreased sperm quality and healthy sperm morphology in obese subjects. In the obese group, obesity significantly increased the percentage of sperms with head, neck, and tail abnormalities compared with that in the control group. Studies have reported that there have been a serious decrease in sperm count

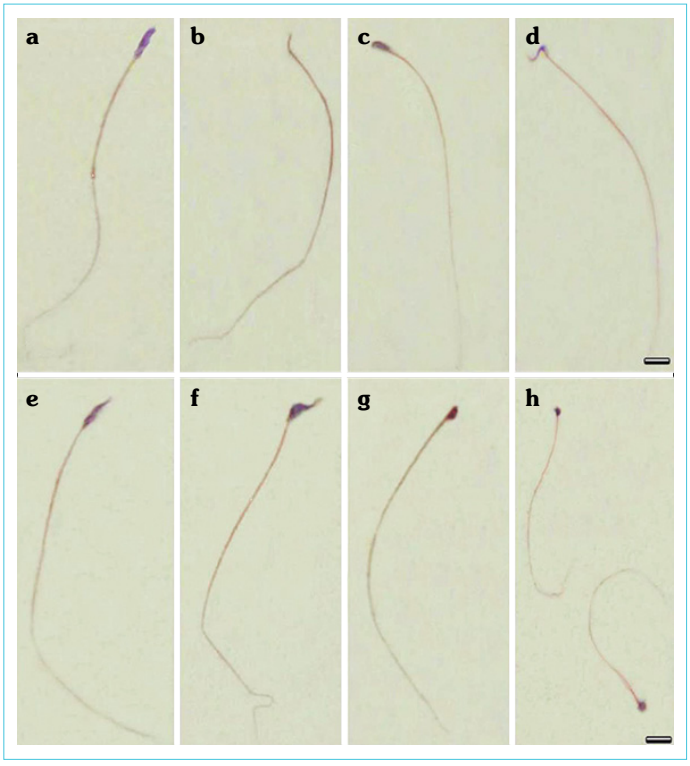


Figure 3. Light micrographs of sperms with abnormal head morphology. (a), large head; (c), headless; (c), small head; (d), amorphous head; (e, f), tapered head; (g, h), round head. Scale bars, 25 μm

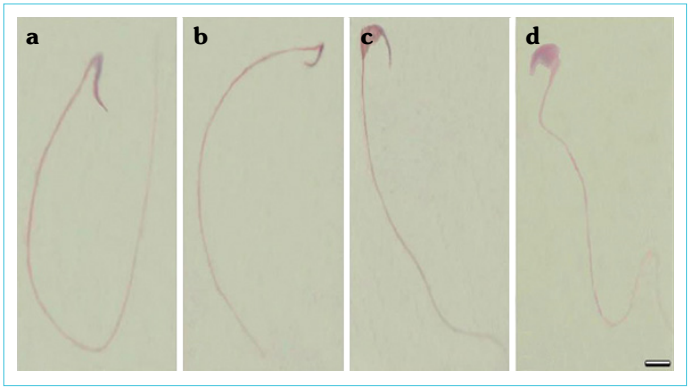


Figure 4. Light micrographs of sperms with abnormal neck morphology. (a, b), bent neck; (c, d), thick neck. Scale bars, 25 μm

and increase in abnormal sperm morphology in the obese rats (18). Ramlau-Hansen et al. (19) reported that the risk of infertility in obese men was higher than that of men with normal weights.

Oxidative stress formation is thought to be major cause of complications arising from long-term obesity (20). Sperms are highly vulnerable to oxidative stress because of the low level of the cytoplasmic antioxidant defense system. Inflammation is also considered an inevitable consequence of obesity resulting in damage to the testicular tissues (11). Obesity can increase oxidative stress in association with conditions such as insulin resistance and dyslipidemia (21). Oxidative stress that derives from overproduction of free oxygen radicals may be one of the mechanisms for histopathological alteration. Oxidative stress impacts the cellular biomolecules such

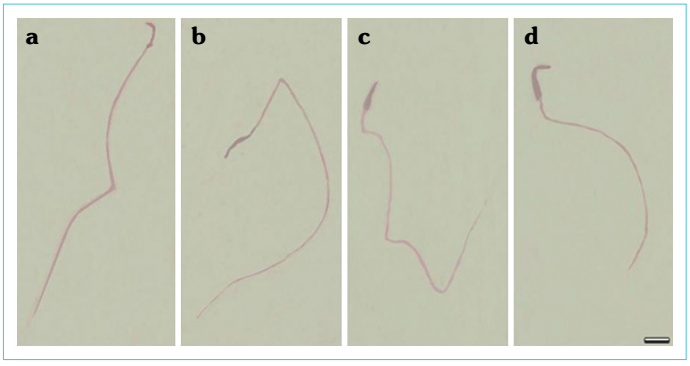


Figure 5. Light micrographs of sperms with abnormal tail morphology. (a, b), bent tails; (c, d), thick tails. Scale bars, 25 μm

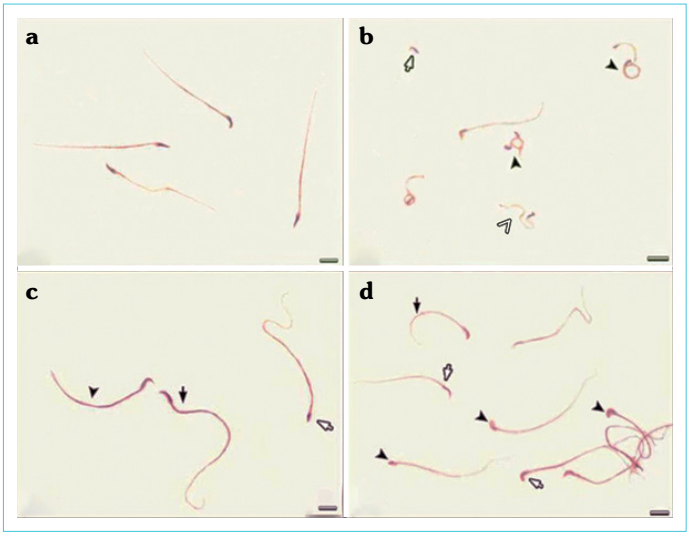


Figure 6. Light micrographs of sperms with abnormal sperm morphology in the control (a), obese (b), I3C (c), and obese+I3C (d) groups. Black arrow, normal sperm; black arrowheads, sperm head abnormalities; white arrowhead, tailless sperm; double black arrowhead, dag defect sperm; double white arrowhead, broken tail sperm; white arrow, short tail sperm. I3C, indole-3-carbinol. Scale bars, 25 μm

as the lipid, carbohydrate, protein, and enzyme and, thus, causes cell damage and apoptosis (22). The present study was a continuation of our previous study. In our previous study, HFD-induced obesity caused a significant increase in the catalase and myeloperoxidase levels in the rat testicular tissues (23). Ye et al. (7) reported a relationship between obesity and increased oxidative stress in testicular tissues. They documented that the superoxide dismutase and malondialdehyde levels increased in obese men. An important consequence of oxidative stress is damages to DNA single- and double-strand breaks, sugars, and bases (24). Moreover, oxidative stress is thought to be a potential causative agent of sperm DNA damage. Another study also documented that obesity contributed to cellular DNA damage and DNA repair impairment, resulting in alteration of gene expression (25). Therefore, damage to DNA can result in morphological abnormalities of sperm. Obesity can impair spermatogenesis and decrease the viability and motility of sperm (26). Ramaraju et al. (27) reported that obesity induced abnormal sperm morphology such as thin and pyriform heads.

The administration of I3C in the obese+I3C group significantly improved the percentage of abnormal sperm architectures compared with that in the control group. Therefore, the number of sperms with healthy architecture increased. This was possibly due to the ameliorative effect of I3C on morphological changes caused by obesity. It has been reported that impairment of endogenous antioxidant defense mechanisms by obesity contributes to various complications (28). Therefore, antioxidant therapy is a potential treatment method for several diseases. Earlier studies have reported the antioxidant and anti-inflammatory efficacy of I3C that plays a substantial role in the reduction of inflammatory cytokines and oxidative stress (9, 11).

In conclusion, our findings suggested that obesity contributed to head, neck, and tail abnormalities in sperm morphology. Also, the administration of I3C ameliorated such morphological changes caused by the deleterious effect of obesity. The possibility that I3C could be beneficial in diminishing morphological changes induced by obesity in human sperm should be evaluated.

Ethics Committee Approval: The Laboratory Animal Ethics Committee of Ondokuz Mayıs University granted approval for this study (date: 27.04.2011, number: HADYEK/35).

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Author Contributions: Concept – NHT; Design – NHT; Supervision – NHT; Resource – NHT; Materials – NHT; Data Collection and/or Processing – NHT, AY; Analysis and/or Interpretation – NHT, AY; Literature Search – AY; Writing – AY; Critical Reviews – AY.

Conflict of Interest: The authors have no conflict of interest to declare.

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