EFFECT OF HIGH FAT DIET ON ESTROUS CYCLE REGULARITY AND BODYWEIGHT IN FEMALE RATS

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ABSTRACT

The current study was conducted to assess the effect of HFD (40%) on estrous cycle regularity and body weight in female rats. Fourteen female Wistar albino rats were randomly divided into two groups. The control group (I) received the standard chow diet, and the HFD group (II) received the HFD (55% basal diet, 3% sesame oil, 25% beef tallow, 5% milk powder, 5% roasted peanuts, 5% egg, and 2% NaCl) daily for 10 successive weeks. Bodyweight was recorded every week from the beginning of the study to the end, where, at the 8th week after dietary exposure, the assessment of the estrous cycle was performed daily for ten successive days using visual assessment and vaginal cytology procedures. The HFD group revealed a statistically higher proportion of rats with an irregular estrous cycle (p = 0.031) and a significantly increased diestrus index vs. the control group (p = 0.025). The HFD group revealed a nonsignificant decrease in the cycle frequency, a significant decrease in the total days of the proestrus stage (p ≤ 0.001), and a nonsignificant shortening in the estrus and metestrus stages vs. control. In contrast, the HFD group revealed a significantly longer diestrus stage than the control group (p = 0.025). Weight gain and body weight were significantly increased throughout the experiment in the HFD group when compared with the control group (p < 0.001). In conclusion, HFD results in increased body weight and increased estrous cycle irregularities, which may impair the female reproductive function.

Keywords: High-fat diet, estrous cycle, body weight, rats.

INTRODUCTION

Obesity is a widespread epidemic problem that threatens public health worldwide. In recent decades, the number of overweight and obese people has reached awesome levels; now, approximately 40% of people are overweight or obese globally (Yong et al., 2023). Obesity rates and adult overweight could worsen to 65.3% by 2030 (Hu, 2021). Obesity is related to a variety of

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health problems and medical obstacles, such as hypertension, heart disease, stroke, type 2 diabetes, and cancer (Bray, 2004). Furthermore, obesity induces plenty of ailments that interfere with reproductive function (Chakraborty et al., 2016). As observed previously, obesity has a detrimental effect on fertility, as it has resulted in increased rates of irregular menstruation, delayed spontaneous conception, natural abortions, infertility, a poorer response to infertility therapies (Pasquali et al., 2007), and impaired ovarian function (Bazzano et al., 2017).

High-fat diets (HFD) are diets that contain a high percentage of fat and more than the recommended 35% of calories from fat (Vannice and Rasmussen, 2014). Since the 1940s, HFD has been successfully used to generate models of obesity in animal experiments (Godwin, 2016). Epidemiological studies demonstrate a positive relationship between the development of obesity and high-fat diets (HFD) (Liu et al., 2019). Consuming a high-fat diet increases the risk of obesity and reproductive problems (Skaznik-Wikel et al., 2016b). Studies using animal models revealed that the increased dietary fat levels interfere with certain reproductive functions with or without the development of obesity (Skaznik-Wikel et al., 2016a). Consuming HFD has resulted in ovarian dysfunction, increased ovarian inflammation, altered ovarian gene expression, and decreased ovarian reserve (Hohos et al., 2020).

Adult females with increased abnormal fatty tissue suffer from reproductive dysfunction that is expressed in irregular menstruation, anovulation, and infertility (Koning et al., 2010). It was observed that obesity is associated with estrous cycle irregularities in mice (Patel and Shah, 2018). High-fat Diet (HFD) interferes with estrous cycle regularity (Hohos et al., 2018). Nutritional upsets-induced obesity impairs the gonadotrophin-releasing hormone (GnRH)-luteinizing hormone (LH) system, which is the core of the female reproductive axis (Volk et al., 2017). HFD consumption, without or with the development of obesity, is indicated to influence the hypothalamic-pituitary-ovarian (HPÖ) axis functionality in females (Hohos and Skaznik-Wikel, 2017a), resulting in inadequate release of critical reproductive hormones, which in turn impair the reproductive cycle (Hohos and Skaznik-Wikel, 2017a). Assessment of the estrous cycle in females can be used as an indicator of gonadotropin responsiveness. Estrous cycle length is altered by exposure to endocrine disorders (Frye, 2014). Despite a study on female mice showing that although HFD results in obesity, the regularity of the estrous cycle is not affected, and females exert a regular estrous cycle (Negrón and Radovick, 2020), while another study showed that HFD induces estrous cycle irregularity only at prolonged consumption of HFD after 20 weeks (Chakraborty et al., 2016). So, we aimed in this study to determine the exact impact of HFD consumption for ten weeks on estrous cycle regularity.

MATERIAL AND METHOD

The ethical approval number is: 17101769

Normal diet:
The normal diet (ND) contains 21% protein, 4.6% fat, and 3.45% carbohydrates (2.950 kcal/gram).

High-Fat Diet used:
The high-fat diet (40%) was composed of 55% basal diet, 3% sesame oil, 25% beef tallow, 5% milk powder, 5% roasted peanuts, 5% egg, and 2% NaCl (Ragab et al., 2015).

Experimental animals:
Fourteen female albino rats (4 weeks old) were obtained from the Laboratory Animal House, Faculty of Veterinary Medicine, Assiut University. The rats weighed about 80–85 g; they were healthy and were retained in cages at controlled humidity and temperatures (25 °C). All rats received tap
water and laboratory food ad libitum. The rats were housed in the laboratory animal house for two weeks before the experiment for acclimatization. The duration of the experiment was 10 weeks (from October to mid-December, starting at the end of the autumn and ending at the beginning of winter). The rats were randomly divided into two equal groups (n= 7 females) as the following design:

**Group (I): Control rats:**
The female rats in this group received a normal diet ad libitum for ten weeks.

**Group (II): high-fat diet rats:**
The animals in this group received a high-fat diet (ad libitum) for ten weeks.

**Assessment of the estrous cycle:**
The estrous cycle for each rat was assessed, and the estrous cycle stage was identified using both visual assessment and vaginal lavage procedures. The assessment was performed successively for ten days after the eighth week of high-fat diet consumption. It was performed between 8:00 and 9:00 a.m. daily. The assessment was performed approximately at the same time of the day over the course of the collection period.

**Visual assessment:**
The rats were gripped on the non-dominant hand and placed in the restraint with their forepaws resting on a surface. Gently, the rat’s tail was lifted, and visually, the vulva was examined and evaluated depending on the appearance and criteria of the changes to the vagina that occur during the estrous cycle. The degree of vaginal opening size, vaginal swelling, especially the dorsal lip, the moistness and color of the tissues, and the presence or absence of visible cellular debris in the vagina are all taken into account while determining the estrus stage (Ajayi and Akhigbe, 2020). The morphological characteristics of the vagina at different stages of the estrous cycle are shown in Table 1. The vulva was evaluated and examined according to Champlin et al. (Champlin et al., 1973). Digital images were taken for documentation.

**Vaginal smear/cytology:**
It seems to be the method that is most commonly used for identifying the stages of the estrous cycle. Each rat was grasped from the nape and tail and marked with a pen in the tails, according to Fig. 1(a). A plastic pipette filled with 0.2 ml of saline, is drawn into the pipette. The pipette’s tip is gently inserted into the vaginal opening at a depth of about 5–10 mm, and then the saline is flushed into the vagina and backed out 2 or 3 times. Care was taken not to insert the pipette’s tip too deep into the vaginal opening to avoid stimulation of the cervix. Excessive stimulation can result in pseudopregnancy, which is expressed as a persistent diestrus for up to 14 days (Cora et al., 2015). One drop from lavage for each rat was collected and placed on the slide Fig. 1(b), then slides were stained for evaluation as follows: air-dried smears were fixed with absolute methanol for 30 seconds, drained, and then stained for 30 minutes with 2% Giemsa stain (Nelson et al., 1982).

Vaginal cytology was analyzed to evaluate the stage of the cycle they were in, as the secretion of the vagina contains three types of cells: leucocytes, cornified epithelial cells, and nucleated epithelial cells. The determination of the stage of the estrous cycle depends on the proportion of different types of these cells in the vaginal lavage. When nucleated cells were the predominant cell type, rats were determined to be in proestrus. The estrus stage was identified when the cornified cells were the predominant type, while when cornified cells and leukocytes were predominant, the rats were determined to be in metestrus stage, and finally the diestrus stage was confirmed when the predominant cells were leukocytes (Auta and Hassan, 2016).
HFD consumption significantly increased the body weight when compared with the control group throughout the experiment (10 weeks). Also, the body weight gain in an HFD is significantly increased when compared with the control group (p< 0.001). (table 2, 3).

Table 2: The effect of a high-fat diet (HFD) on body weight throughout the experiment (10 weeks).

<table>
<thead>
<tr>
<th>Group</th>
<th>0 Week</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
<th>8th week</th>
<th>9th week</th>
<th>10th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=7)</td>
<td>83.92 ± 3.21</td>
<td>88.43 ± 3.86</td>
<td>92.35 ± 6.37</td>
<td>95.021 ± 4.71</td>
<td>120.28 ± 4.87</td>
<td>130.44 ± 3.45</td>
<td>136.50 ± 0.96</td>
<td>146.66 ± 0.37</td>
<td>156.58 ± 3.33</td>
<td>161.11 ± 3.54</td>
<td>147.06 ± 1.42</td>
</tr>
<tr>
<td>HFD (n=7)</td>
<td>84.68 ± 2.78</td>
<td>97.45 ± 3.30</td>
<td>123.95 ± 3.39</td>
<td>134.03 ± 3.72</td>
<td>146.30 ± 0.37</td>
<td>156.58 ± 3.33</td>
<td>161.11 ± 3.54</td>
<td>175.03 ± 1.00</td>
<td>177.89 ± 2.09</td>
<td>182.09 ± 2.27</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.861</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data represents the mean ± S.E. using an independent sample t-test.
Figure 2: Showing the effect of an HFD on body weight throughout the experiment (10 weeks) in different studied groups. The data represents the mean ± S.E. using an independent sample t-test. * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 3: Showing the mean body weight gain in different studied groups at the end of the experiment after 10 weeks in both the control and HFD groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.027 ± 3.32</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>HFD</td>
<td>97.41 ± 4.12</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data represents the mean ± S.E. using an independent sample t-test. * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure (3): Showing the mean body weight gain in different studied groups at the end of the experiment after 10 weeks in both the control and HFD groups. Data represents the mean ± S.E. using an independent sample t-test. * P < 0.05, ** P < 0.01, *** P < 0.001.

Estrus cycle:
As shown in Table (4) and Figure (4), the effect of HFD on the regularity of the estrus cycle, cycle frequency (number of estrus cycles), duration of the cycle stages (proestrus, estrus, metestrus, diestrus), and diestrus index for a ten-day assessment at the end of the 8th week of the experiment in both the control and HFD groups. The HFD group showed a statistically higher proportion of rats with an irregular estrous cycle and a significantly increased diestrus index (P = 0.025). HFD led to a nonsignificant decrease in the cycle frequency when compared with the control group. The effect of HFD on the total days of each stage during the observation period showed a significant decrease in the total days of the proestrus stage and a nonsignificant decline in both the estrus and metestrus stages. In contrast, the total days of the diestrus stage during the observation period were statistically significantly elevated in the HFD group in comparison with the control.
Table 4: Showing the effect of HFD on the estrus cycle regularity, cycle frequency (number of estrus cycles), the duration of the stages of the cycle (proestrus, estrus, metestrus, diestrus), and diestrus index for a ten-day assessment at the end of the 8th week of the experiment in both control and HFD groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Regularity</th>
<th>Cycle frequency</th>
<th>Total number of days for each phase</th>
<th>Diestrus index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regular</td>
<td>Irregular</td>
<td>Proestrus</td>
<td>Estrus</td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>(6)</td>
<td>(1)</td>
<td>1.78 ±</td>
<td>1.85 ±</td>
</tr>
<tr>
<td></td>
<td>85.7%</td>
<td>4.3%</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>HFD (n=7)</td>
<td>(2)</td>
<td>(5)</td>
<td>1.35 ±</td>
<td>0.28 ±</td>
</tr>
<tr>
<td></td>
<td>28.6%</td>
<td>71.4%</td>
<td>0.18</td>
<td>0.18</td>
</tr>
</tbody>
</table>

P value 0.031 0.091 < 0.001 0.801 0.735 0.025 0.025

Data represents mean ± S.E. number and percent. Using independent samples t-test for cycle frequency, duration of each phase in days, diestrus index, and chi-square test to compare the percent regularity of the cycle.

Figure (4): Showing the effect of HFD on a) the estrus cycle regularity, b) cycle frequency (number of estrus cycles), c) the duration of the stages of the cycle (proestrus, estrus, metestrus, and diestrus), and d) diestrus index for a ten-day assessment at the end of the 8th week of the experiment in both control and HFD groups. Data represents the mean ± S.E. Using independent samples t-test for cycle frequency, duration of each phase in days, diestrus index, and chi-square test to compare the regularity of the cycle * P < 0.05, ** P < 0.01, *** P < 0.001.
Figure (5) Shows the effect of an HFD on the duration of each stage of the cycle (proestrus, estrus, metestrus, and diestrus) during the assessment period.

Figure (6): Showing the appearance of the vagina in different phases of the estrous cycle. A) proestrus stage shows wide vaginal opening (arrow), and the vaginal tissues are moist, swollen, and reddish pink (curved arrow); b) estrus stage showing vaginal gaping (arrow), and the vaginal tissues are lighter pink, less moist, and less swollen (curved arrow); c) metestrus stage showing narrow vaginal opening (arrow), and the vaginal tissue is not swollen and looks pale and dry (curved arrow); while d) diestrus stage showing small vaginal openings (arrow), and the vaginal tissues are moist, with no tissue swelling, and are purple in color (curved arrow).
DISCUSSION

Obesity rates have significantly increased globally because of excessive caloric intake, a high-fat diet, decreased physical exercise, and a more sedentary lifestyle. Consequently, many studies have suggested that a rise in obesity has contributed to a variety of metabolic illnesses, ranging from systemic malfunctions to reproductive abnormalities. Reproductive function is seriously influenced by nutritional imbalance, either positively or negatively (Lie et al., 2013). High-fat diet consumption has been demonstrated to be the primary factor related to an increased risk of obesity (Kahn et al., 2006). As recorded previously, consuming an HFD might lead to a loss in fat taste sensitivity that results in excessive intake of fat, leading to weight gain (Newman et al., 2016). Information regarding how eating a high-fat diet or being obese influences one's taste sensitivity and preferences for appetizing foods is complicated (Hyde et al., 2022). Taste sensitivity and preferences may be influenced by several possible underlying gut-brain signaling pathways, the majority of which are also involved in satiety signaling. Among them are CCK, PYY, and GLP-1 signaling, which are crucial in the emergence of diet-induced obesity, particularly by causing deficiencies in the feedback loops of satiety signaling (Duca et al., 2013). In addition to that, HFD tends to induce inflammation in the brain by triggering inflammatory pathways in the hypothalamic neurons, astrocytes, and microglia (Rahman et al., 2018). Also, HFD-induced gut dysbiosis has been connected to the increased incidence of obesity via inflammatory responses, which may be another cause (Murphy et al., 2015). Diet-induced obesity doesn’t have the same response; as previously recorded, some rats were prone to resist diet-induced obesity, while others did not resist and became obese (Balasubramanian et al., 2012). In our study, HFD consumption for ten consecutive weeks was found to increase body weight and weight gain in female rats. This proposes that the current model of feeding rats, the HFD that we are using here, performs well for conditioning the animals for research on obesity. The increased weight gain in HFD groups in this study suggests that HFD offers a higher metabolic efficiency, similar to the explanation recorded before (Dharavath et al., 2019). Other
studies recorded that the increased mass gain may arise from the high dietary fats with saturated fatty acids (Iwasa et al., 2018).

Consuming a high-fat diet is a risk factor for both obesity and reproductive problems. Studies using animal models show that increased dietary fat impairs certain reproductive functions with or without the development of obesity (Skaznik-Wikel et al., 2016b). The evaluation of the estrus cycle is an important indicator of reproductive performance. In our study, HFD induced a higher proportion of rats with an irregular estrous cycle with an increased diestrus index and increased the total days in the diestrus stage while decreasing the total days in both the estrus and metestrus stages. Our data agrees with previous studies, HFD-fed mice for 10 weeks showed a higher prevalence of disrupted estrous cyclicity than the control group (Hohos et al., 2020). while Ngadjui et al., reported that all female mice fed HFD for 10 weeks showed abnormal estrous cycle some mice were missing some stages and others showed disrupted cyclicity (Ngadjui et al., 2015). Despite a study on female mice showing that although HFD results in obesity, the estrous cycle regularity is not affected, and females exert a regular estrous cycle (Negrón and Radovick, 2020), another study showed that HFD induces estrous cycle irregularity only after prolonged consumption of HFD after 20 weeks (Chakrabory et al., 2016). On the other hand, previous studies demonstrate that 40-day HFD consumption induces estrous cycle irregularities in 33% of the rats (Lie et al., 2013). This explained previously that the different methodological in these studies could skew the results and restrict the conclusions. as the duration of HFD exposure, the percentage of fat in the meals (either within HFDs or between HFDs and controls), and the kinds of fats in the diets differ significantly. These variations change the kind of HFD exposure, which may change the reaction or function of the reproductive system (Hohos and Skaznik-Wikel, 2017a). To explain the HFD effect on the estrous cycle, numerous theories have been put forth to explain these abnormalities of the estrous cycle associated with high-fat diets (HFD). The insulin/leptin pathway's involvement is one of these pathways (Farooq et al., 2014). It is possible that obesity-related hyperinsulinemia and insulin resistance, resulting from a hyperlipidemic diet, are the main causes of reproductive dysfunction in these individuals (Bermejo-Alvarez et al., 2012). Also, changes in the actions of the proliferator-activated receptor g (PPARg) may be the cause of these abnormalities, as dietary lipids are one of the ligands that can attach to PPARg, altering how it controls signaling and gene expression, which may in turn affect estrous cycle regulation (Hohos and Skaznik-Wikel, 2017b). Also, nutrition-induced obesity impairs the gonadotrophin-releasing hormone (GnRH)-luteinizing hormone (LH) system, which is the core of the female reproductive axis (Volk et al., 2017). HFD consumption, without or with obesity, is indicated to impair the hypothalamic-pituitary-ovarian (HPO) axis functionality in females (Hohos and Skaznik-Wikel, 2017a), resulting in inadequate release of critical reproductive hormones, which in turn impair the reproductive cycle (Hohos and Skaznik-Wikel, 2017a). These results in rodents match up with clinical research that links obesity to irregular estrous cycles, oligo-anovulation, and female infertility (Pasquali et al., 2007). However, the mechanisms underlying this phenomenon remain incompletely understood.

CONCLUSION

It was concluded that HFD feeding for ten weeks led to a higher proportion of rats with estrous cycle irregularities, an increased diestrus index, a decrease in the cycle frequency, a decrease in the total days of the proestrus stage and shortening in both the estrus and metestrus stages. In contrast, the total days of the diestrus stage were significantly longer in HFD. Also, body weight and weight gain increased significantly. HFD results in increased body weight and increased estrous cycle irregularity, which increases the risk of reproductive dysfunction.

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**Effect of a high-fat diet on estrous cycle and body weight in female rats**

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Aim of the study was to determine the effect of a high-fat diet on estrous cycle and body weight in female rats. The rats were divided into two groups. The groups that consumed the high-fat diet. The body weight was recorded weekly from the beginning of the study until the end. In the eighth week after dieting, the estrous cycle was determined daily for ten consecutive days, using both visual and histological methods. It was observed that the high-fat diet caused a significant increase in estrous cycle disorder (p = 0.031) and a significant increase in the total number of estrous cycle (p = 0.025). In contrast, the high-fat diet caused a significant increase in the body weight of the rats (p < 0.001). Thus, we can conclude that a high-fat diet affects the estrous cycle and body weight in female rats, which may affect reproductive function.