High-fat Diet Alone or Combined with Stress
Impaired Glucose Tolerance in Female Rats

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Authors’ contributions
This work was carried out in collaboration between all authors. Author HZ designed the study, wrote the protocol and interpreted the data. Author HV did the experiments and gathered the initial data. Authors HV and MS managed the literature searches and produced the initial draft. Authors HZ and FR wrote the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: According to the role of estrogens in metabolic responses to high-fat diet and/or stress, this study aimed to investigate the effect of high-cow intra-abdominal fat diet either alone or combined with acute foot-shock stress on glucose metabolism at proestrus and diestrus phases of estrous cycle.

Material and Methods: Female rats were divided into high-fat and normal diet groups. The diet groups recruited into control and stressed and finally subdivided into proestrus and diestrus groups. Stress was applied by a communication box. Blood samples were taken after stress exposure to

¹ This paper is part of a MSc thesis by Hamid Vasfi.

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1. INTRODUCTION

High-fat diet and/or stress as two life threatening environmental factors separately or in combination often impair insulin secretion, increase insulin resistance and predispose the organism to metabolic disorders [1,2]. However, considering the studies conducted in males and females, it seems that females are protected against these metabolic derangements [3,4]. Estrogens (especially estradiol, as the main sex hormone secreted by the gonads of females) have been assumed to be determining factor in hormonal and metabolic responses to high-fat diet and/or stress in females [5,6]. Most studies conducted in rodents have shown that females are resistant against insulin resistance induced by a high-fat diet [7,8]. Corsetti et al. [3] showed that among the obese diabetic fatty (ZDF) rats, which are genetically susceptible to develop type 2 diabetes, females rarely develop diabetes in response to high-fat diets compared to males. Moreover, Hevener et al. [9] showed that intravenous lipid emulsion infusion decreased insulin stimulated glucose disposal rate and insulin stimulated skeletal muscle insulin substrate receptor-1 phosphorelation in male rats. However, they found that female rats did not show such lipid induced reduction in insulin action [9]. Also female rats are protected against impaired glucose tolerance induced by a high-fat diet and in this issue estrogens might also play an important role [10,11]. In this regard, studies have shown that bilateral ovariectomized monkeys and rats have reduced insulin sensitivity and impaired glucose metabolism, while chronic use of estrogens could resolve these problems [12,13]. Moreover, following high-fat diet consumption, female rats did not show energy imbalance, which could be related to the presence of estradiol in females [6], and the ability of this hormone in increasing leptin sensitivity and decreasing free radicals as well as inflammatory signals [14]. On the other hand, females show a greater stress response than males. For example, glucocorticoids concentration in response to acute electric shock and swim stress showed a greater increase in female than male rats [4]. In this case, the role of estrogens is also critical [15]. Due to the changes in plasma estradiol levels during estrous cycle the metabolic responses to the above mentioned environmental factors might show cyclical changes. As Viau and Meaney observed in female rats, plasma ACTH and corticosterone concentrations were higher in proestrous phase (high estradiol level) than diestrus phase (low estradiol level) in response to 20 min restraint stress [16]. In addition, cyclical changes were observed in food intake, body weight, serum concentration and mRNA expression of leptin in females [17].

According to the role of estrogens in metabolic responses to high-fat diet and/or stress and their cyclic changes in estrous cycle, and considering that few studies investigated the effect of these important factors in female rats; this study was designed to examine possible effects of high-fat diet containing cow intra-abdominal fat (66.67% energy from fat) combined with acute electrical foot-shock stress on glucose metabolism, in female rats at proestrus (high estradiol concentration) and diestrus (low estradiol concentration) phases [18]. To achieve this purpose, plasma glucose, insulin, corticosterone, leptin and estradiol (dominant estrogen of female's reproductive cycle) concentrations were measured. Also glucose tolerance, homeostasis model assessment of insulin resistance (HOMA-
2.2 Stress Procedure

A communication box consisting of 9 chambers (16 × 16) was used to induce stress. The device is designed so that the animals in the chambers can have visual, auditory and olfactory communication with each other. The floor of 5 chambers is from metal wire made of stainless steel which is connected to electricity to put the animals in the chambers under electrical shock [21]. The animals of the stressed groups received electrical shocks (1 mA, 1 Hz) for a 10 second duration every 60 second [21]. The stress was applied to the animals for 1 h, once, between 8.00 and 10.00 AM. However, the controls were kept in the box for the same time without receiving any stress. The stressed groups were kept under normal or high-fat diet for 30 days, then after confirmation of diestrus or proestrus phase (on day 31 of the experiment) the animals received electric foot-shock.

The control animals were also kept under normal or high-fat diet for 30 days, then after confirmation of diestrus or proestrus phase (on day 31 of the experiment) were transported to communication box apparatus (Borje Sanat, Iran) without receiving electric foot-shock stress. In order to adapt the animals to the communication box, all groups of animals from day 25 were placed in communication box one hour daily without receiving any stress.

2.3 Method of Determining the Estrous Cycle Phases in Female Rats

Reproductive cycle of female rats is called the estrous cycle [22]. The cycle includes proestrus, estrus, metestrus and diestrus phases. The duration of the cycle in female rats is about 4 days. Estradiol concentration begins to increase at metestrus phase and peaked at proestrus phase and returns to the basal value at estrus phase [22]. In the vaginal smear three cell types (epithelial, cornified and leukocytes) are observed. At proestrus phase the percentage of nucleated epithelial cells are prominent. Whereas, the percentage of un nucleated cornified cells is higher at estrus phase. At metestrus phase, the three types of cells are present in the same percentage. At diestrus leukocytes are seen predominantly [22]. In this study to determine normal sequence and timing of estrous cycle, vaginal secretions between 8-8:30 AM by a plastic pipette containing 10 μl saline (salt solution 0.9%) were removed and placed on glass slides. The slides, without staining, were examined to determine the different phases of the cycle by using a light microscope (objective lenses 10 and 40) [22]. Before entering the animals into the groups, at least 3 consecutive cycles were checked for each animal. Also on the day of stress induction (31±1) the cycle phase was determined and electrical foot-shock stress was applied when the animal was in the intended phase (proestrus or diestrus).
2.4 Blood Sampling and Assays
In all groups, blood sampling was performed in fasting status (16 hours) at the end of the experimental period (day 31±1) after removing the animals from communication box (between 8-10 AM). Retro-orbital puncture method was used for blood sampling with isoflurane (Nicholas Primal, UK) as an anesthetic agent [23]. The blood samples were collected in Eppendorf tubes containing 5 μl heparin (5000 IU/ml) per one ml blood. Then the tubes were centrifuged at 3000 round per minute at 4ºC for 5 minutes; the separated plasma was preserved at -80ºC.

Plasma glucose concentration was determined using the glucose oxidase method (Pars Azmoon, Iran). Cholesterol and triglyceride concentrations were measured by enzymatic colorimetric method (Pars Azmoon, Iran). Plasma estradiol was determined by estradiol ELISA kit (DBC, Canada). Rat insulin ELISA kit (Mercodia, Sweden), corticosterone ELISA kit (DRG, Germany) and rat leptin ELISA kit (CUSABIO, China) were used to measure plasma insulin, corticosterone and leptin concentrations.

2.5 Intra Peritoneal Glucose Tolerance Test (IPGTT)
An IPGTT was performed at the end of experimental period (day 31±1) in overnight fasted (16 h) animals by IP injection of 2 g/kg glucose using 20% glucose solution [24]. Blood samples were taken 15, 60 and 90 min after IPGTT start to measure plasma glucose and insulin concentrations.

2.6 Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) Index
Fasting plasma glucose and insulin concentrations were used to determine HOMA-IR, as a method of assessing insulin resistance. In all of the study groups, the HOMA-IR index was calculated as follows: HOMA-IR= (ci×cg)/22.5, where ci is fasting insulin level (μU/ml) and cg is fasting glucose level (mmol/L) [25].

2.7 Adrenal Gland and Abdominal Fat Weights
In each of the study groups, the animals were decapitated at the end of the experimental period (day 31±1) after IPGTT blood sampling. Then the adrenals and left side abdominal fat [26] were removed and weighed.

The protocol of the experiment has been shown briefly in Fig. 1.

2.8 Statistical Analysis
All data are expressed as the Mean ± SEM. To compare the diets in different groups Three-way analyses of variance (ANOVA) was performed by SPSS Version 21 program package and followed by Tukey test by considering diet, stress and estrous cycle phase as factors. Moreover, to compare diets in different days a mixed ANOVA with repeated measures within the high-fat diet and normal diet groups (day was considered as a repeated factor) and independent measures between the two groups (diet was considered as an independent factor) was performed. A P-value below 0.05 was considered to be statistically significant.

3. RESULTS
3.1 The Effect of High-fat Diet on Body Weight
The body weight of both high-fat and normal diet groups increased significantly during thirty days of the experiment. So that, the body weights of each diet group showed a significant difference on days 6, 12, 18, 24 and 30 as compared to day 1 (P<0.001) (Fig. 2). However, during thirty days of high-fat diet any significant difference was observed between the body weights of high-fat and normal diet groups.

3.2 The Effect of High-fat Diet on Food and Energy Intake
In high-fat diet group the food intake showed a decreasing trend during 30 days of the experiment and a significant difference except for day 2, compared to normal diet group (day 1 P<0.01, days 3 to 30 P<0.001) (Fig. 3a). Moreover, the high-fat diet rats showed a significant decrease in food intake on days 5 to 30 as compared to day 1. On the other hand, in normal diet group only on day 2 of the experiment the food intake decreased (P<0.001) whereas, on days 4 and 20 increased (P<0.001) significantly as compared to day 1 (Fig. 3a).

In high-fat diet group the food intake was significantly higher than normal diet one from the beginning of experiment till day 11 (days 1 to 9 P<0.001, days 10 and 11 P<0.01) (Fig. 3b). However, after that the energy intake of this group approached to normal diet group and no significant difference was observed between the groups except for day 17 (P<0.001) (Fig. 3b).
Table 1. Composition of the diets

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>High-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g%</td>
<td>Kcal%</td>
</tr>
<tr>
<td>Protein</td>
<td>17.5</td>
<td>18.48</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>72.72</td>
<td>76.77</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2</td>
<td>4.75</td>
</tr>
<tr>
<td>Cow intra-abdominal fat</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fiber</td>
<td>6.6</td>
<td>0</td>
</tr>
<tr>
<td>Total phosphate</td>
<td>0.62</td>
<td>0</td>
</tr>
<tr>
<td>Total calcium</td>
<td>0.56</td>
<td>0</td>
</tr>
<tr>
<td>Caloric density (kcal/g)</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

Moreover, high-fat diet increased plasma leptin concentration in non STR-Pro and non STR-Di groups as compared to respective normal diet groups (P<0.05) and in STR-Pro group as compared to respective normal diet group (P<0.01). However, in HF- STR-Di group the plasma leptin increment was not significant as compared to respective normal diet group.

No significant change was observed in any of the study groups in relation to plasma cholesterol and triglyceride concentrations (Table 4).

3.4 The Effect of High-fat Diet and/or Acute Foot-shock Stress on Plasma Estradiol Concentration

The statistical analysis showed a significant reduction of plasma estradiol concentration in normal diet of both STR and non STR diestrus groups as compared to respective proestrus groups (P<0.01) (Table 5). There was a decreasing trend of plasma estradiol concentration in both STR and non STR high-fat diet groups compared to normal diet one, although only in proestrus phase of the groups the reduction was significant (P<0.01 for non STR group, P<0.001 for STR group) (Table 5). On the other hand, any significant difference was observed between the high-fat diet groups.

3.5 The Effect of High-fat Diet and/or Acute Foot-shock Stress on Plasma Corticosterone Concentration and Adrenal Glands Weight

The high-fat diet in non STR groups did not induce any significant alteration of plasma corticosterone concentration compared to respective normal diet ones. Whereas, in the presence of stress significant increase in plasma corticosterone concentration was observed in HF-STR-Pro as compared to both ND-STR-Pro (P<0.01) and HF-STR-Di (P<0.05) (Table 5). In

Moreover, the high-fat diet rats showed a significant decrease in energy intake on days 5 to 30 as compared to day 1 (P<0.001) (Fig. 3b).

On the other hand, in normal diet group only on days 2 of the experiment the energy intake decreased (P<0.001) whereas on days 4 and 20 increased (P<0.001) significantly as compared to day 1 (Fig. 3b).

Table 2. The fatty acid composition of the cow intra-abdominal fat

<table>
<thead>
<tr>
<th>Type of fatty acid</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>5.15</td>
</tr>
<tr>
<td>C16:0</td>
<td>18.46</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.1</td>
</tr>
<tr>
<td>C18:0</td>
<td>29.9</td>
</tr>
<tr>
<td>C18:1t</td>
<td>1.2</td>
</tr>
<tr>
<td>C18:1c</td>
<td>32.19</td>
</tr>
<tr>
<td>C18:2t</td>
<td>0.5</td>
</tr>
<tr>
<td>C18:2c</td>
<td>9.8</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.1</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.5</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.2</td>
</tr>
<tr>
<td>other</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Three way ANOVA showed that the abdominal fat weight as a percentage of body weight in high-fat diet rats was higher than normal diet ones. However, only the difference between HF-non STR-Pro and ND-non STR-Pro groups was statistically significant (P<0.001) (Table 4).

On the other hand, acute foot-shock stress did not significantly affect the abdominal fat weight of any study groups.
addition, stress exposure caused a significant increase of plasma corticosterone concentration in both diet groups as compared to non STR condition (P<0.01 for ND-STR-Pro; P<0.001 for ND-STR-Di, HF-STR-Pro and Di) (Table 5).

High-fat diet and stress either alone or in combination did not significantly affect the adrenal glands weights of the study groups (Table 5).

3.6 The Effect of High-fat Diet and/or Acute Foot-shock Stress on HOMA-IR Index and Plasma Levels of Glucose and Insulin before (fasting state) and after IPGTT Performance

Fasting plasma glucose and insulin concentrations (0 min) did not show any significant difference among the study groups. However, Three-way ANOVA showed a significant increase of HOMA-IR index in normal diet STR-Pro compared to non STR-Pro (P<0.05) and STR-Di (P<0.01) of the same diet (Fig. 4).

At 15 and 60 minutes after performing IPGTT the plasma glucose concentrations of all study groups were significantly higher than 0 min (before performing IPGTT) (P<0.001 for all study groups excluding HF-STR-Di group which was P<0.01) (Fig 5a). However, after 60 min in ND-STR-Di and HF-STR-Pro groups the increment was not significant. In all normal diet groups plasma glucose concentration at 60 min showed a decreasing tendency but the decrement was not significant as compared to 15 min. On the other hand, in non STR-Pro and STR-Pro groups of high-fat diet rats the levels of plasma glucose at 15 and 60 min were almost the same (Fig. 5a). Whereas, in non STR-Die and STR die groups of high-fat diet rats a non-significant reduction was observed (Fig. 5a).

Table 3. Grouping of the animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet (ND)</td>
<td>Non-stress (ND-non STR)</td>
</tr>
<tr>
<td></td>
<td>Stress (ND-STR)</td>
</tr>
<tr>
<td>High-fat diet (HF)</td>
<td>Non-stress (HF-non STR)</td>
</tr>
<tr>
<td></td>
<td>Stress (HF-STR)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measurement days</th>
<th>Food intake measurement days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>1 6 12 18 24 31</td>
</tr>
<tr>
<td>Food intake</td>
<td>2 3 4 5 6 7 8 9 10 11 12 13 14 15</td>
</tr>
<tr>
<td></td>
<td>… … 25 26 27 28 29 30 31</td>
</tr>
</tbody>
</table>

Fig. 1. Experimental protocol
Table 4. The effect of high-fat diet and/or acute foot-shock stress at proestrus and diestrus phases on abdominal fat, plasma triglyceride, cholesterol and leptin concentrations

<table>
<thead>
<tr>
<th></th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Leptin (ng/ml)</th>
<th>Abdominal fat weight /body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non STR-Pro</td>
<td>77.63±3.11</td>
<td>78.88±3.14</td>
<td>2.46±0.1</td>
<td>0.48±0.1</td>
</tr>
<tr>
<td>Non STR-Di</td>
<td>89.71±5.34</td>
<td>78.14±3.92</td>
<td>2.39±0.25</td>
<td>0.59±0.06</td>
</tr>
<tr>
<td>STR-Pro</td>
<td>72.14±2.96</td>
<td>72.25±2.88</td>
<td>2.36±0.28</td>
<td>0.54±0.05</td>
</tr>
<tr>
<td>STR-Di</td>
<td>80.25±6.34</td>
<td>71.57±3.15</td>
<td>2.42±0.14</td>
<td>0.46±0.04</td>
</tr>
<tr>
<td>High-fat diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non STR-Pro</td>
<td>82.13±6.5</td>
<td>76.83±3.98</td>
<td>3.72±0.62</td>
<td>0.8±0.07c</td>
</tr>
<tr>
<td>Non STR-Di</td>
<td>85.25±4.57</td>
<td>74.13±1.85</td>
<td>3.68±0.57</td>
<td>0.82±0.1</td>
</tr>
<tr>
<td>STR-Pro</td>
<td>80.63±4.81</td>
<td>69.63±1.96</td>
<td>4.05±0.53a</td>
<td>0.65±0.06</td>
</tr>
<tr>
<td>STR-Di</td>
<td>76.5±9.5</td>
<td>70.5±2.18</td>
<td>3.08±0.16</td>
<td>0.74±0.07</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM of 8 rats; aP<0.05, bP<0.01, cP<0.001 significant difference versus normal diet of the same group; STR: stress, Pro: proestrus, Di: diestrus

Table 5. The effect of high-fat diet and/or acute foot-shock stress at proestrus and diestrus phases on plasma corticosterone and estradiol concentrations and adrenal glands weight

<table>
<thead>
<tr>
<th></th>
<th>Corticosterone (nmol/ml)</th>
<th>Adrenal glands weight (mg)</th>
<th>Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non STR-Pro</td>
<td>0.34±0.05</td>
<td>45.0±5.00</td>
<td>173.29±7.55</td>
</tr>
<tr>
<td>Non STR-Di</td>
<td>0.24±0.02</td>
<td>38.75±5.49</td>
<td>130.43±11.15f</td>
</tr>
<tr>
<td>STR-Pro</td>
<td>0.47±0.02a</td>
<td>32.5±2.50</td>
<td>163.29±19.71</td>
</tr>
<tr>
<td>STR-Di</td>
<td>0.53±0.03a</td>
<td>37.5±4.12</td>
<td>125.86±10.64f</td>
</tr>
<tr>
<td>High-fat diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non STR-Pro</td>
<td>0.3±0.05</td>
<td>46.25±4.20</td>
<td>125.43±11.93a</td>
</tr>
<tr>
<td>Non STR-Di</td>
<td>0.25±0.02</td>
<td>35.71±6.49</td>
<td>105.43±7.44</td>
</tr>
<tr>
<td>STR-Pro</td>
<td>0.61±0.03ac</td>
<td>41.25±4.79</td>
<td>103±11.47b</td>
</tr>
<tr>
<td>STR-Di</td>
<td>0.49±0.02a</td>
<td>43.75±3.75</td>
<td>105.33±11.19</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM of 8 rats; aP<0.01, bP<0.001 significant difference versus normal diet of the same group; cP<0.05 significant difference versus STR-Di of the same diet; dP<0.01, eP<0.001 significant difference versus non STR of the same group; fP<0.01 significant difference versus proestrus group of the same diet; STR: stress, Pro: proestrus, Di: diestrus

Fig. 2. The effect of high-fat diet on body weight during 30 days of experimental procedure
Values are presented as Mean±SEM of 32 rats. *P<0.001 significant difference versus the first day in high fat diet group; †P<0.001 significant difference versus the first day in normal diet group
Fig. 3. The effect of high-fat diet on food intake (a) and energy intake (b) during 30 days of experimental procedure. Values are expressed as Mean±SEM of 32 rats.

Fig. 4. The effect of high-fat diet and/or acute foot-shock stress at proestrus and diestrus phases on HOMA-IR index. Each column is presented as Mean±SEM of 7 rats; *P<0.05 significant difference versus non STR-Pro of the same diet; **P<0.01 significant difference versus STR-de of the same diet; STR: stress, Pro: proestrus, Di: diestrus.
In normal and high-fat diet groups at 15 and 60 min after performing IPGTT the plasma insulin concentrations were higher than 0 min (before performing IPGTT) (Fig. 5b). However, in non STR-Pro and Di groups of normal diet rats (P<0.001) and non STR-Pro group of high-fat diet rats (P<0.01) at 15 min as compared with 0 min the increment was significant (Fig. 5b). Moreover, at 60 min in non STR-Pro of normal diet group and non STR-Pro and Di of high-fat diet group compared to 0 min the increment was significant (P<0.001) (Fig. 5b). In all study groups except for STR-Pro of high-fat diet rats a reducing tendency for plasma insulin level was observed at 60 min after IPGTT. At 15 and 60 min following IPGTT the plasma insulin levels of the stress groups were lower than non-stress ones (P<0.001 for ND-STR-Di at 15 min and HF-STR-Pro at 60 min, P<0.01 for HF-STR-Pro at 15 min) (Fig. 5b).

4. DISCUSSION

The data of the present study did not show any significant difference between high-fat and normal diet groups in relation to body weight. However, food intake decreased and energy intake increased in high-fat diet groups. High-fat diet increased intra-abdominal fat weight as a percentage of body weight and plasma leptin concentration as compared to normal diet, whereas did not affect plasma triglyceride and cholesterol concentrations. On the other hand, high-fat diet decreased plasma estradiol concentration, whereas had no significant effect on plasma corticosterone by itself, however in the presence of acute foot-shock stress increased plasma corticosterone concentration in STR-Pro group. High-fat diet either alone or in combination with stress impaired glucose tolerance, however did not affect HOMA-IR index.

In agreement with the present results 14 weeks high-fat diet (55.2% energy from fat) [27] and 2 weeks cafeteria diet (containing 23.4% lipid) [28] in female Wistar rats did not significantly affect body weight but increased energy intake. In the former the food intake was also decreased and in the latter plasma leptin level and white fat weight showed a significant increase. On the other hand, in another study 14 weeks high-fat diet (36% energy from fat containing mostly soy bean oil) increased the body weight of female Wistar rats which was associated with increased energy intake and reduced plasma leptin concentration [26]. However, in the present study the increment of the plasma leptin concentration, which is almost proportional to the increase of intra-abdominal fat, could be the cause of non-significant change of body weight in high-fat fed rats. Because, leptin (a hormone which is produced by fat tissue) is able to regulate body weight by reducing food intake and increasing energy consumption [29].

In this regard, by continual decreasing of food intake, the energy intake of the high-fat fed rats got closer to those of normal diet fed ones. This finding confirms the suggestion that healthy rats are sensitive to the caloric properties of their diet and adjust their food intake with constant daily energy intake [30].

Moreover in this study, neither high-fat diet nor acute stress changed the plasma cholesterol and triglyceride concentrations. In agreement with the present results, 8 weeks high-fat diet (42% energy from lipids) [31] did not change, whereas 10% lard fat for 55 days increased plasma cholesterol and triglyceride concentrations in male rats [32]. Moreover, after exposure to acute restraint stress (10 h) the plasma cholesterol remained unchanged, whereas plasma triglyceride level increased in female rats [33]. On the other hand, acute exposure to cold accompanied with starvation stress increased both cholesterol and triglyceride levels of female rats [34]. Thus obtaining different results could be attributed to the usage of different type or duration of fat and/or stress [33].

The increased plasma leptin concentration, which was observed in the present study, might be the cause of the reduction of plasma estradiol concentration in high fat diet groups. Because besides energy balance regulation, leptin has an important role in reproduction and is able to interfere with estradiol synthesis by inhibiting the synthesis of pregnenolone (the estradiol precursor) [35,36]. Nevertheless, a positive relationship between the amount of fat and plasma estradiol level [37] and also a negative relationship between type of the fat (polyunsaturated fat) and the level of estradiol has been suggested [38]. However, in the present study regarding to the type of fat (containing relatively high percentage of monounsaturated fat) possibly leptin would be the cause of estradiol reduction in high-fat fed rats.
Fig. 5. The effect of high-fat diet and/or acute foot-shock stress at proestrus and diestrus phases on plasma glucose (a) and insulin (b) concentrations in fasting state (0 min), 15 and 60 min after performing IPGTT.

Each column is presented as Mean±SEM of 7 rats. $P<0.01$, $P<0.001$ significant difference versus fasting state (0 min) of the same group; $P<0.01$, $P<0.001$ significant difference versus non STR of the same group.

In this study, plasma estradiol concentration of normal diet groups was significantly lower in diestrous than proestrus phase, which is consistent with the results of previous studies [18]. On the other hand, the results of the present study did not show any significant difference of plasma leptin concentration between proestrus and diestrous phases. Consistent with the present study, some studies did not report any significant difference between leptin levels during estrous cycle [39,40]. Whereas, some other studies reported that plasma leptin level is higher in proestrus than diestrous [41,42]. Possibly, the differences between results could be attributed to the various blood sampling time [17,41,42].

In this study, foot-shock stress exposure resulted in non-significant changes of plasma estradiol concentration in all of the study groups. However, in HF-STR-Pro a greater reduction of plasma estradiol concentration was observed as compared to its respective normal diet.

Several studies on female rats, showed decreased [43,44], or increased plasma estradiol concentration [45] following stress exposure. Changes in plasma estradiol concentration subsequent to stress induction may be caused by the inhibitory effect of stress hormones on reproductive hormones secretion [46,47].

As shown in table 5, stress caused plasma corticosterone concentration increment in both diet groups and both proestrus and diestrous phases, however compared with normal diet group only HF-STR-Pro showed a significant increase. On the other hand, this group (HF-STR-Pro) in the presence of stress showed higher plasma leptin and lower plasma estradiol levels as compared to the respective normal diet group. Therefore, considering the interaction between these three parameters (corticosterone, estradiol and leptin) the results seem reasonable. So that, stress increased the corticosterone concentration, which in turn led to increased plasma leptin [48], and the leptin increment ultimately caused further reduction of plasma estradiol level in the above mentioned group [48,49]. The higher significant change which was observed in the proestrus phase in relation to plasma corticosterone, estradiol and leptin concentrations reflects the greater sensitivity of this phase compared to the diestrous phase against environmental interventions. In this regard Antunes et al. also showed that each of the estrous cycle phases has a different sensitivity to stress [50].

On the other hand, in the present study high-fat diet alone did not cause any changes in plasma corticosterone concentration. However, Kamara et al. showed that high-fat diet consumption for less than three weeks activated the HPA axis, whereas its usage for longer term did not make significant changes in the HPA activity [30], so in this study, the likely reason for the lack of significant change in plasma corticosterone concentration and also the adrenal gland weights after consumption of high-fat diet is for relatively long application of the diet (i.e. 4 weeks).

The findings of the present study did not show any significant changes in fasting plasma glucose and insulin concentrations either in the presence of a high-fat diet or the presence of stress. HOMA-IR index also showed no significant change following high-fat diet consumption alone or combined with stress, while showed a significant increment in ND-STR-Pro group.

Considering that estrogens significantly increase glucocorticoid responses to acute stress in females [15] and with regard to the point that estradiol concentration is higher in proestrus phase compared to other phases of the rat estrous cycle [16] increased HOMA-IR index caused by stress which was observed in ND-STR-Pro group with high estradiol concentration in comparison with ND-STR-Di group, with lower estradiol concentration, seems reasonable.

On the other hand, despite a significant increase in plasma corticosterone concentration the HOMA-IR index did not change significantly in HF-STR-Pro group. In this regard the protective role of the fat used in this study, against insulin resistance might be suggested [51].

Changes in plasma glucose and insulin concentrations during the glucose tolerance test in HF-non STR-Pro and HF-STR-Pro groups show a degree of glucose tolerance impairment. Nevertheless, studies have shown that female rats are protected against insulin resistance and impaired glucose tolerance induced by high-fat diet, because of the presence of estrogens [11].

However, in the present study high-fat diet reduced plasma estradiol concentration, so animals seem not to be protected against high fat diet-induced glucose tolerance impairment.

In addition, the glucose intolerance which was observed in the presence of stress might be due
to the decreased plasma insulin concentration caused by stress exposure. Interestingly, in the proestrus phase the changes were more significant than diestrus phase.

5. CONCLUSION

The results of the present study showed that the high-cow intra-abdominal fat diet alone or combined with acute stress impaired glucose homeostasis in female rats. In this regard, the proestrus phase compared with diestrus phase was more sensitive to environmental interventions (such as high-fat diet and/or stress), which is reflected as glucose metabolism impairment in that phase.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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